Polytetrahydrofuran-and Dendrimer-Based Novel Sol-Gel Coatings for Capillary Microextraction (CME) Providing Parts Per Trillion (ppt) and Parts Per Quadrillion (ppq) Level Detection Limits in Conjunction With Gas Chromatography and Flame Ionization Detection (FID)

by

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy Department of Chemistry College of Arts and Sciences University of South Florida

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Keywords: SPME, In-tube SPME, PAHs, Aldehydes, Ketones, Phenols, Alcohols

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Dedication

To my deceased parents, Moqbulur Rahman and Suraiya Rahman, who brought me in this beautiful world and provided their endless inspiration, love and support.
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Table of Contents

List of Tables vii
List of Figures xii
List of Schemes xvii
List of Abbreviations, Symbols, and Acronyms xviii
Abstract xx

Chapter One: Solid Phase Microextraction-A Solvent-Free Sample Preparation Technique 1
1.1 Introduction 1
1.2 Evolution of solid phase microextraction: a historical perspective 2
1.3 Working principles of SPME 8
1.4 Modes of extraction 10
1.4.1 Extraction modes with a coated fiber 10
1.4.2 Extraction modes with in-tube SPME 13
1.5 Preparation of coating on fibers 14
1.6 Experimental parameters affecting extraction efficiency 14
1.6.1 pH adjustment of the matrix 15
1.6.2 Agitation of the matrix 15
1.6.3 Heating the matrix 16
1.6.4 Addition of salt to the matrix 19
1.6.5 Addition of organic solvents 19
1.7 Derivatization 20
1.8 References for Chapter One 23

Chapter Two: An Overview on Stationary Phases Used in Solid Phase Microextraction (SPME) 26
2.1 Introduction 26
2.2 Commercially available sorbents for fiber SPME 32
2.2.1 Homogeneous polymeric sorbents 33
2.2.2 Polymeric composite sorbents 34
2.2.2.1 Polydimethylsiloxane/DVB (PDMS/DVB) 34
2.2.2.2 Carboxen/Polydimethylsiloxane (CAR/PDMS) 35
2.2.2.3 Carbowax/Divinylbenzene (CW/DVB) 35
2.2.2.4 Carbowax/Templated resin (CW/TPR) 36
3.3.2 Non-polysiloxane based sorbents
   3.3.2.1 Polyethylene glycol-based sol-gel sorbents
   3.3.2.2 Non-polysiloxane sol-gel sorbents with alkyl ligands
3.3.3 Cyclodextrin-based non-polysiloxane sol-gel sorbents
3.4 Miscellaneous sorbents
3.5 References for Chapter Three

Chapter Four: Capillary Microextraction on Sol-Gel Benzyl Terminated Dendrimer Coating

4.1 Introduction
4.2 Experimental
   4.2.1 Equipment
   4.2.2 Chemicals and materials
   4.2.3 Preparation of sol-gel dendrimer coated extraction capillaries
      4.2.3.1 Cleaning and hydrothermal treatment of the fused silica capillary
      4.2.3.2 Preparation of the sol solution
      4.2.3.3 Coating of the fused silica capillary with sol solution
      4.2.3.4 Thermal conditioning of the coated capillary
      4.2.3.5 Rinsing the capillary with organic solvents to remove unbonded material
   4.2.4 Preparation of sol-gel PDMS coated capillary GC column
   4.2.5 Preparation of sol-gel PEG coated capillary GC column
   4.2.6 Gravity-fed sample dispenser for capillary microextraction
   4.2.7 Deactivation of glassware
   4.2.8 Preparation of standard sample solution for sol-gel dendrimer CME
   4.2.9 Extraction of analytes on sol-gel dendrimer coated capillaries
   4.2.10 Transferring the extracted analytes to the GC column and Gas chromatographic analysis of the extracted analytes
   4.2.11 Calculation of the limit of detection (LOD) for individual analyte
4.3 Results and discussion
   4.3.1 Sol-gel dendrimer coating and chemical aspects of its preparation
   4.3.2 Characterization of surface morphology and determination of coating thickness using scanning electron microscopy
4.3.3 Determination of extraction kinetics for both polar and nonpolar analytes 206
4.3.4 Applications of sol-gel dendrimer coated microextraction capillaries 208
   4.3.4.1 Polycyclic aromatic hydrocarbons (PAHs) 208
   4.3.4.2 Aldehydes and ketones 215
   4.3.4.3 Phenols 227
   4.3.4.4 CME of butylatedhydroxytoluene (BHT) 233
   4.3.4.5 Alcohols 234
4.4 Conclusion 244
4.5 References for Chapter Four 245

Chapter Five: Capillary Microextraction on Sol-Gel Polytetrahydrofuran 250
5.1 Introduction 250
5.2 Experimental 256
   5.2.1 Equipments 256
   5.2.2 Chemicals and materials 259
   5.2.3 Preparation of sol-gel poly-THF coated Microextraction capillaries 259
      5.2.3.1 Cleaning and hydrothermal treatment of the fused silica capillary 260
      5.2.3.2 Preparation of the sol solution 262
      5.2.3.3 Coating fused silica capillary with sol solution 264
      5.2.3.4 Thermal conditioning of the coated capillary 264
      5.2.3.5 Rinsing the capillary with organic solvents to remove unbonded materials 265
   5.2.4 Preparation of sol-gel PDMS coated capillary GC columns 265
   5.2.5 Preparation of sol-gel PEG coated capillary column 267
   5.2.6 Cleaning and deactivation of glassware 268
   5.2.7 Preparation of standard solutions for CME on sol-gel Poly-THF coated capillaries 269
   5.2.8 Gravity-fed sample dispenser for capillary microextraction 270
   5.2.9 Extraction of analytes on sol-gel poly-THF coated capillaries 270
   5.2.10 Transferring the extracted analytes to the GC column and gas chromatographic analysis of the extracted analytes 271
5.3 Results and Discussion 273
   5.3.1 Sol-gel chemistry of the coating process 273
   5.3.2 Characterization of sol-gel poly-THF sorbent 283
5.3.2.1 Fourier-transform infrared spectroscopic characterization of the sol-gel poly-THF coating 283
5.3.2.2 Scanning electron microscopy 285
5.3.2.3 Thermogravimetric analysis 288
5.3.3 Determination of the extraction kinetics 291
5.3.4 Determination of the analyte enrichment factors of sol-gel poly-THF sorbent 296
5.3.5 Determination of thermal stability of the sol-gel poly-THF coating 300
5.3.6 Applications of sol-gel poly-THF coating 302
   5.3.6.1 Polycyclic aromatic hydrocarbons 302
   5.3.6.2 Aldehydes 309
   5.3.6.3 Ketones 315
   5.3.6.4 Chlorophenols 321
   5.3.6.5 Alcohols 327
   5.3.6.6 Mixture of polar, moderately polar, and nonpolar compounds 332
5.3.7 Possibility of automation 334
5.4 Conclusion 335
5.5 References for Chapter Five 337

Appendices 342
Appendix A: Capillary Electrophoresis and Fluorescence Anisotropy for Quantitative Analysis of Peptide-Protein Interactions Using JAK2 and SH2-Bβ as a Model System 343
Appendix B: Sol-Gel Approach to In Situ Creation of High pH-Resistant Surface-Bonded Organic-Inorganic Hybrid Zirconia Coating for Capillary Microextraction (In-Tube SPME) 351
Appendix C: High pH-Resistant, Surface-Bonded Sol-Gel Titania Hybrid Organic-Inorganic Coating for Effective On-Line Hyphenation of Capillary Microextraction (In-Tube SPME) with High-Performance Liquid Chromatography 364
Appendix D: Parts Per Quadrillion Level Ultra-Trace Determination of Polar and Nonpolar Compounds via Solvent-free Capillary Microextraction on Surface-bonded Sol-Gel Polytetrahydrofuran Coating and Gas Chromatography-Flame Ionization Detection 375
Appendix E: Capillary Microextraction on Sol-gel Dendrimer Coatings 
Appendix F: Sol-Gel Poly (ethylene glycol) Stationary Phase for High-Resolution Capillary Gas Chromatography 
Appendix G: Sol-Gel Capillary Microextraction 
Appendix H: Synthesis of Benzyl-Terminated Dendron for Use in High-Resolution Capillary Gas Chromatography 

About the Author
List of Tables

Table 2.1 Commercially available SPME sorbents 32
Table 2.2 Commercial GC columns used in in-tube SPME 38
Table 2.3 Tailor-made sorbents on SPME fibers and their applications 42
Table 2.4 Tailor made sorbents used in in-tube SPME 62
Table 2.5 Coating used in electrochemically controlled SPME 70
Table 3.1 Homogeneous polysiloxane sorbents 98
Table 3.2 Important features of sol-gel hydroxyfullerene sorbent 107
Table 3.3 Sol-gel crown ether sorbents used in SPME 111
Table 3.4 Comparison of partition coefficients of fibers with different compositions 113
Table 3.5 Sol-gel calixarene sorbents used in SPME 133
Table 3.6 Salient features of sol-gel PVA, polyphenylmethylsiloxane, divinylbenzene, and polymethylphenylvinylsiloxane SPME sorbents 148
Table 3.7 Non-polysiloxane based sorbent used in SPME 150
Table 4.1 Name, function and chemical structure of sol-gel dendrimer coating solution ingredients 183
Table 4.2 Chemical structures and pertinent physical properties of polyaromatic hydrocarbons (PAHs) analyzed using sol-gel dendrimer coating 210
Table 4.3 Run-to-run peak area reproducibility for PAHs in capillary microextraction using sol-gel dendrimer coatings 212
| Table 4.4 | Capillary-to-capillary peak area reproducibility for PAHs in capillary microextraction using sol-gel dendrimer coatings |
| Table 4.5 | Limit of detection (LOD) data for PAHs in CME-GC-FID using sol-gel dendrimer microextraction capillaries |
| Table 4.6 | Chemical structures and pertinent physical properties of aldehydes analyzed using sol-gel dendrimer coating |
| Table 4.7 | Run-to-run peak area reproducibility for aldehydes in capillary microextraction using sol-gel dendrimer coatings |
| Table 4.8 | Capillary-to-capillary peak area reproducibility for aldehydes in capillary microextraction using sol-gel dendrimer coatings |
| Table 4.9 | Limit of detection (LOD) data for aldehydes in CME–GC-FID using sol-gel dendrimer microextraction capillaries |
| Table 4.10 | Chemical structures and pertinent physical properties of ketones analyzed using sol-gel dendrimer coating |
| Table 4.11 | Run-to-run peak area reproducibility for ketones in capillary microextraction using sol-gel dendrimer coatings |
| Table 4.12 | Capillary-to-capillary peak area reproducibility for ketones in capillary microextraction using sol-gel dendrimer coatings |
| Table 4.13 | Limit of detection (LOD) data for ketones in CME-GC-FID sol-gel dendrimer microextraction capillaries |
| Table 4.14 | Chemical structures and pertinent physical properties of phenols analyzed using sol-gel dendrimer coating |
| Table 4.15 | Run-to-run peak area reproducibility for phenols in capillary microextraction using sol-gel dendrimer coatings |
| Table 4.16 | Capillary-to-capillary peak area reproducibility for phenols in capillary microextraction using sol-gel dendrimer coatings |
| Table 4.17 | Capillary-to-capillary peak area reproducibility for phenols in capillary microextraction using sol-gel dendrimer coatings |
Table 4.18 Chemical structures and pertinent physical properties of butylated hydroxy toluene (BHT) analyzed using sol-gel dendrimer coating

Table 4.19 Run-to-run peak area reproducibility for BHT in capillary microextraction using sol-gel dendrimer coatings

Table 4.20 Capillary-to-capillary peak area reproducibility for BHT in capillary microextraction using sol-gel dendrimer coatings

Table 4.21 Limit of detection (LOD) data for BHT in CME-GC-FID using sol-gel dendrimer microextraction capillaries

Table 4.22 Chemical structures and pertinent physical properties of alcohols analyzed using sol-gel dendrimer coating

Table 4.23 Run-to-run peak area reproducibility for alcohols in capillary microextraction using sol-gel dendrimer coatings

Table 4.24 Capillary-to-capillary peak area reproducibility for alcohols in capillary microextraction using sol-gel dendrimer coatings

Table 4.25 Limit of detection (LOD) data for alcohols in CME-GC-FID using sol-gel dendrimer microextraction capillaries

Table 5.1 Names, functions and chemical structures of sol-gel poly-THF coating solution ingredients

Table 5.2 Analyte enrichment factors of commercial PDMS (100 µm), commercial PA (85 µm) and sol-gel poly-THF sorbents for polar and nonpolar compounds

Table 5.3 Thermal stability data for sol-gel poly-THF coated microextraction capillaries

Table 5.4 Chemical structures and pertinent physical properties of polyaromatic hydrocarbons (PAHs) analyzed using sol-gel poly-THF coated capillaries

Table 5.5 Run-to-run peak area reproducibility for PAHs in capillary microextraction using sol-gel Poly-THF coating

Table 5.6 Capillary-to-capillary peak area for PAHs in capillary microextraction using sol-gel poly-THF coated capillaries
| Table 5.7 | Limit of detection (LOD) data for PAHs in CME-GC-FID using sol-gel Poly-THF microextraction capillaries | 309 |
| Table 5.8 | Chemical structures and pertinent physical properties of aldehydes analyzed using sol-gel poly-THF coated capillaries | 310 |
| Table 5.9 | Run-to-run peak area reproducibility for aldehydes in capillary microextraction using sol-gel poly-THF coating | 311 |
| Table 5.10 | Capillary-to-capillary peak area reproducibility for aldehydes in capillary microextraction using sol-gel poly-THF coating | 312 |
| Table 5.11 | Limit of detection (LOD) data for aldehydes in CME-GC-FID using sol-gel poly-THF microextraction capillaries | 315 |
| Table 5.12 | Chemical structures and pertinent physical properties of ketones analyzed using sol-gel poly-THF coating | 316 |
| Table 5.13 | Run-to-run peak area reproducibility for ketones in capillary microextraction using sol-gel Poly-THF coating | 317 |
| Table 5.14 | Capillary-to-capillary peak area reproducibility for ketones in capillary microextraction using sol-gel poly-THF coating | 318 |
| Table 5.15 | Limit of detection (LOD) data for ketones in CME-GC-FID using sol-gel poly-THF microextraction capillaries | 321 |
| Table 5.16 | Chemical structures and pertinent physical properties of chlorophenols (CPs) analyzed using sol-gel poly-THF coating | 322 |
| Table 5.17 | Run-to-run peak area reproducibility for chlorophenols in capillary microextraction using sol-gel poly-THF coating | 323 |
| Table 5.18 | Capillary-to-capillary peak area reproducibility for chlorophenols in capillary microextraction using sol-gel poly-THF coating | 324 |
| Table 5.19 | Limit of detection (LOD) data for chlorophenols in CME-GC-FID using sol-gel poly-THF microextraction capillaries | 327 |
| Table 5.20 | Run-to-run peak area reproducibility for alcohols in capillary microextraction using sol-gel poly-THF coating | 328 |
Table 5.21 Capillary-to-capillary peak Area ($A \times 10^{-4}$) reproducibility for alcohols in capillary microextraction using sol-gel poly-THF coating 329

Table 5.22 Limit of detection (LOD) data for alcohols in CME-GC-FID using sol-gel poly-THF microextraction capillaries 330
List of Figures

Figure 1.1  Design of the first commercial SPME device  5
Figure 1.2  (a) Schematic representation of a PDMS coated stir bar  7
(b) Head space stir bar extraction device  7
Figure 1.3  Different modes of SPME operation  11
(a) direct extraction  11
(b) headspace SPME  11
(c) membrane-protected SPME  11
Figure 1.4  Schematic of an internally cooled SPME device  18
Figure 1.5  Derivatization techniques used in solid-phase microextraction  21
Figure 2.1  Graphical representation of number of articles published on SPME since its inception in 1989  28
Figure 2.2A  Different formats of SPME (a) fiber SPME where extracting phase resides outside of a fiber, (b) in-tube SPME where extracting phase resides inside the capillary  29
Figure 2.2B  Comparison of two major formats of SPME  30
Figure 2.3  Wire-in-tube SPME extraction capillary  41
Figure 2.4  Reactions involved in antibody immobilization  46
(a) silanization of silica surface with APTES  46
(b) surface modification with  46
(c) immobilization of antibody  46
Figure 2.5  Scanning electron micrograph of the surface of an anodized aluminum wire  49
Figure 2.6  Scanning electron micrographs of  (A) porous layer  55
(B) PDMS coated SPME fibers  55
Figure 2.7  Drawing of the headspace membrane SPME system 58
Figure 2.8  Different steps involved in MIP synthesis 64
Figure 2.9  SEM image of ADS particles immobilized on a fused silica fiber 68
Figure 2.10  Scanning electron micrographs of
(a) uncoated metal surface 75
(b) PPY-coated metal surface 75
(c) PPPY-coated metal surface 75
Figure 2.11  Impact of storage on extracted sample using SPME sampler for long-term storage 78
Figure 2.12  Fully automated solid-phase dynamic extraction (SPDE) process 81
Figure 2.13  Schematic representation of a needle trap device 83
Figure 3.1  Classification of sol-gel sorbents used in fiber-SPME/in-tube SPME (CME) 96
Figure 3.2  Scanning electron microscopic image of sol-gel PDMS fiber at 3600-fold magnification 100
Figure 3.3  SPME-GC analysis of aliphatic alcohols (C\textsubscript{10} -, C\textsubscript{12} -, C\textsubscript{14} -, C\textsubscript{16} -, C\textsubscript{18} -)-using sol-gel PDMS fiber and GC/FID system 102
Figure 3.4  Scanning electron micrograph of sol-gel fullerol fiber at 2000-fold magnification 108
Figure 3.5  Relative extraction efficiencies of commercial PDMS fiber (100µm), commercial CW-DVB (65 µm) and OH-DB14C4/OH-TSO (65 µm) in amine extraction 117
Figure 3.6  Scanning electron micrograph of the DOH-B15C5 fiber at 800-fold magnification 118
Figure 3.7  Comparison of extraction efficiencies of commercial PDMS (100 µm), commercial CW/DVB (65 µm) and DATEG/OH-TSO in extracting BTEX from aqueous solution 131
Figure 3.8  Comparison of mass absorbed in unit volume of sorbent using five different fibers for 10 ng/mL BTEX solution  

Figure 3.9  Electron scanning micrograph (600 x magnification of a sol-gel Carbowax 20M ormosil fiber  

Figure 4.1  Schematic of a homemade capillary filling/purging device for preparation of capillary microextraction capillaries and open-tubular sol-gel GC columns  

Figure 4.2  Schematic of a gravity-fed sample dispensing unit used in sol-gel dendrimer capillary microextraction  

Figure 4.3  Schematic representation of the connection of the extraction capillary with the analysis column inside the GC oven using a press-fit quartz capillary  

Figure 4.4  Scanning electron microscopic image of a 250 µm i.d. sol-gel dendrimer coated microextraction capillary illustrating the coating thickness. Magnification: 10,000x  

Figure 4.5  Scanning electron microscopic image of a 250 µm i.d. sol-gel dendrimer coated microextraction capillary illustrating the typical roughened surface obtained by sol-gel coating process. Magnification: 10,000x  

Figure 4.6  Illustration of the extraction kinetics of a non-polar compound (phenanthrene) and a polar compound (2,4,6-trichlorophenol) obtained on a 13 cm x 250 µm i.d. sol-gel dendrimer coated microextraction capillary using 100 ppb aqueous solution  

Figure 4.7  CME- GC analysis of PAHs at 10 ppb concentration using sol-gel dendrimer coated microextraction capillary  

Figure 4.8  Capillary Microextraction- GC analysis of Aldehydes at 100 ppb concentration using sol-gel dendrimer coated microextraction capillary  

Figure 4.9  Capillary Microextraction- GC analysis of ketones at 100 ppb concentration using sol-gel dendrimer coated microextraction capillary
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 4.10</td>
<td>Capillary Microextraction- GC analysis of Phenols at 10 ppb concentration using sol-gel dendrimer coated microextraction capillary</td>
</tr>
<tr>
<td>Figure 4.11</td>
<td>Capillary Microextraction- GC analysis of BHT at 10 ppb concentration using sol-gel dendrimer coated microextraction capillary</td>
</tr>
<tr>
<td>Figure 4.12</td>
<td>Capillary Microextraction- GC analysis of alcohols at ppb level concentrations using sol-gel dendrimer coated microextraction capillary</td>
</tr>
<tr>
<td>Figure 5.1</td>
<td>Schematic of a gravity-fed sample dispensing unit in capillary microextraction with a sol-gel poly-THF coated capillary</td>
</tr>
<tr>
<td>Figure 5.2</td>
<td>Schematic of a homemade capillary filling/purging device for preparation of capillary microextraction capillaries and open-tubular sol-gel GC columns</td>
</tr>
<tr>
<td>Figure 5.3</td>
<td>Schematic representation of the connection of the extraction capillary with the analysis column inside the GC oven using a press-fit quartz capillary</td>
</tr>
<tr>
<td>Figure 5.4</td>
<td>IR spectra of pure polytetrahydrofuran (left), sol solution having all ingredients except polytetrahydrofuran (middle), sol-gel polytetrahydrofuran coating (right)</td>
</tr>
<tr>
<td>Figure 5.5a</td>
<td>Scanning electron microscopic image of a 320 µm i.d. sol-gel poly-THF coated fused silica capillary used in CME</td>
</tr>
<tr>
<td>Figure 5.5b</td>
<td>Scanning electron microscopic image of a 320 µm i.d. sol-gel poly-THF coated fused silica capillary used in CME</td>
</tr>
<tr>
<td>Figure 5.6a</td>
<td>TGA curve of pure poly-THF for programmed heating (10 ºC/min) under N₂</td>
</tr>
<tr>
<td>Figure 5.6b</td>
<td>TGA curve of sol-gel poly-THF for programmed heating (10 ºC/min) under N₂</td>
</tr>
<tr>
<td>Figure 5.7</td>
<td>Extraction time profiles of PAHs for direct-SPME using a commercial PDMS (30 µm) fiber</td>
</tr>
<tr>
<td>Figure 5.8</td>
<td>Illustration of the extraction kinetics of nonpolar fluoranthene and phenanthrene and moderately polar (heptanophenone and dodecanal) compounds extracted on a 12.5 cm x 320 µm i.d. sol-gel gel poly-THF coated capillary using 10 ppb aqueous solution of each analyte in a mixture</td>
</tr>
<tr>
<td>Figure 5.9</td>
<td>Comparison of extraction efficiencies of commercial PDMS (100 µm), PA (85 µm) and sol-gel poly-THF (0.5 µm) coatings</td>
</tr>
<tr>
<td>Figure 5.10</td>
<td>Capillary microextraction- GC analysis of PAHs (20 ppb each using sol-gel poly-THF coated capillary</td>
</tr>
<tr>
<td>Figure 5.11</td>
<td>Capillary microextraction- GC analysis of aldehydes at 20 ppb concentration using poly-THF coated capillary</td>
</tr>
<tr>
<td>Figure 5.12</td>
<td>Capillary microextraction- GC analysis of ketones at (20 ppb) using sol-gel poly-THF coated capillary</td>
</tr>
<tr>
<td>Figure 5.13</td>
<td>Capillary microextraction- GC analysis of chlorophenols using poly-THF coated capillary</td>
</tr>
<tr>
<td>Figure 5.14</td>
<td>Capillary microextraction- GC analysis of alcohols (100 ppb each) using sol-gel poly-THF coated capillary</td>
</tr>
<tr>
<td>Figure 5.15</td>
<td>Capillary microextraction- GC analysis of a mixture of nonpolar, moderately polar and polar compounds using poly-THF coated</td>
</tr>
</tbody>
</table>
## List of Schemes

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Hydrolysis reaction of (a) methyltrimethoxysilane (MTMOS) and (b) phenyl terminated dendrimer with a trimethoxysilane derivatized root</td>
</tr>
<tr>
<td>4.2</td>
<td>Polycondensation of hydrolyzed methyltrimethoxysilane</td>
</tr>
<tr>
<td>4.3</td>
<td>Condensation of phenyl-terminated sol-gel-active dendron with a growing sol-gel network formed from MTMOS</td>
</tr>
<tr>
<td>4.4</td>
<td>Chemical anchoring of sol-gel dendrimer stationary phase</td>
</tr>
<tr>
<td>4.5a</td>
<td>Deactivation of residual silanol group using HMDS</td>
</tr>
<tr>
<td>4.5b</td>
<td>Deactivation of fused silica capillary inner surface with PMHS</td>
</tr>
<tr>
<td>5.1</td>
<td>Cationic ring opening polymerization of tetrahydrofuran</td>
</tr>
<tr>
<td>5.2</td>
<td>Hydrolysis of the sol-gel precursor, MTMOS</td>
</tr>
<tr>
<td>5.3</td>
<td>Polycondensation of hydrolyzed MTMOS</td>
</tr>
<tr>
<td>5.4</td>
<td>Chemical incorporation of poly-THF into the sol-gel network</td>
</tr>
<tr>
<td>5.5</td>
<td>Chemical anchoring of the sol-gel hybrid organic-inorganic polymer to the silanol groups on the fused silica capillary inner walls</td>
</tr>
<tr>
<td>5.6</td>
<td>Deactivation of residual silanol groups by derivatization with hexamethyldisilazane (HMDS)</td>
</tr>
</tbody>
</table>
# List of Abbreviations, Symbols, and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>Alabama</td>
</tr>
<tr>
<td>AZ</td>
<td>Arizona</td>
</tr>
<tr>
<td>CA</td>
<td>California</td>
</tr>
<tr>
<td>CE</td>
<td>Capillary Electrophoresis</td>
</tr>
<tr>
<td>CEC</td>
<td>Capillary Electrochromatography</td>
</tr>
<tr>
<td>CME</td>
<td>Capillary Microextraction</td>
</tr>
<tr>
<td>CME-GC</td>
<td>Capillary Microextraction-Gas Chromatography</td>
</tr>
<tr>
<td>CW/DVB</td>
<td>Carbowax/Divinylbenzene</td>
</tr>
<tr>
<td>CW/TPR</td>
<td>Carbowax/Templated Resin</td>
</tr>
<tr>
<td>CAR/PDMS</td>
<td>Carboxen/Polydimethylsiloxane</td>
</tr>
<tr>
<td>DVB/CAR/PDMS</td>
<td>Divinylbenzene/Carboxen/Polydimethylsiloxane</td>
</tr>
<tr>
<td>FID</td>
<td>Flame Ionization Detector</td>
</tr>
<tr>
<td>GC-FID</td>
<td>Gas Chromatography with Flame Ionization Detector</td>
</tr>
<tr>
<td>GC</td>
<td>Gas Chromatography</td>
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<tr>
<td>HMDS</td>
<td>Hexamethyldisilazane</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HPLC-MS</td>
<td>High-Performance Liquid Chromatography-Mass Spectrometry</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>Inductively Coupled Plasma-Mass Spectrometry</td>
</tr>
</tbody>
</table>
INCAT  Inside Capillary Adsorption Trap
LLE  Liquid-Liquid Extraction
LOD  Limit of Detection
MEKC  Micellar Electro Kinetic Chromatography
MTMOS  Methyltrimethoxysilane
PA  Pennsylvania
PAH  Polycyclic Aromatic Hydrocarbon
PEEK  Polyetheretherketone
PEG  Polyethylene Glycol
PMHS  Poly(methylhydrosiloxane)
PPY  Polypyrrole
PTV  Programmed Temperature Vaporizer
RMS  Root Mean Square
RPM  Revolutions per Minute
RSD  Relative Standard Deviation
SEM  Scanning Electron Micrograph
S/N  Signal-to-Noise Ratio
SPE  Solid Phase Extraction
SPME  Solid Phase Microextraction
TFA  Trifluoroacetic Acid
THF  Tetrahydrofuran
USEPA  United States Environmental Protection Agency
VOC  Volatile Organic Compound
Polytetrahydrofuran-and Dendrimer-Based Novel Sol-Gel Coatings for Capillary Microextraction (CME) Providing Parts Per Trillion (ppt) and Parts Per Quadrillion (ppq) Level Detection Limits in Conjunction With Gas Chromatography and Flame Ionization Detection (FID)

Abuzar Kabir

ABSTRACT

Sol-gel capillary microextraction (CME) is a new direction in solvent-free extraction and preconcentration of trace analytes. CME presents significant interest in environmental, pharmaceutical, petrochemical, biomedical, agricultural, food, flavor, and a host of other important areas. Sol-gel CME utilizes advanced material properties of organic-inorganic hybrid sol-gel polymers to perform efficient extraction and enrichment of target analytes from a variety of matrices. In this dissertation, two novel sol-gel coatings were developed for CME: (a) sol-gel benzyl-terminated dendrimer coating, and (b) sol-gel polytetrahydrofuran (poly-THF) coating. A detailed investigation was conducted to evaluate the performance of the newly developed sol-gel coatings in solvent-free extraction of a wide range of polar and nonpolar analytes.

Sol-gel chemistry was used to chemically immobilize dendrimer- and poly-THF-based hybrid organic-inorganic coatings on fused silica capillary inner surface. The sol-gel coatings were created using a coating solution containing a sol-gel active organic component (dendrimer or poly-THF), a sol-gel precursor (methyltrimethoxysilane, MTMOS), a sol-gel catalyst (trifluoroacetic acid, TFA, 5% water) and a deactivating
reagent (hexamethyldisilazane, HMDS). Sol-gel reactions were conducted inside a hydrothermally treated fused silica capillary for 60 min. A wall-bonded sol-gel coating was formed via condensation of silanol groups residing on the capillary inner surface with those on the sol-gel network fragments evolving in close vicinity of the capillary walls. Due to the strong chemical bonding with capillary inner walls, these sol-gel coatings showed excellent thermal and solvent stability in CME in hyphenation with gas chromatography (GC). Using a Flame ionization detector (FID), low parts per trillion (ppt) and parts per quadrillion (ppq) level detection limits were achieved in CME-GC for both polar and nonpolar analytes including polycyclic aromatic hydrocarbons (PAHs), aldehydes, ketones, phenols, and alcohols. The sol-gel coatings were found to be effective in carrying out simultaneous extraction of both polar and nonpolar analytes from the same sample.

1.1 Introduction

An analytical procedure consists of several individual but equally important steps: sampling, sample preparation, separation, quantitation, and statistical interpretation of analytical data, often used in important socio-economical decision making. Since the overall success of an analytical procedure depends on the performance of each individual step, and because the overall speed of the analytical procedure is determined by the speed of the slowest step, it is very important to pay necessary attention to each step. Sampling and sample preparation are considered to be the slowest among all the steps involved in an analytical procedure. Surveys show that more than 80% of the analysis time is spent on sampling and sample preparation [1]. Therefore, if we are to expedite the analytical procedure, much attention needs to be paid to sampling and sample preparation steps. Although the last couple of decades have witnessed significant improvement in analytical instrumentation, inadequate attention has been paid to improvement of the sample-preparation step. This is paradoxical since the sample preparation is an unequivocally necessary step to isolate the target analyte(s) from the sample matrix as well as to purify and concentrate the analyte(s) to reach the detection limit of the analytical instrument involved.
Conventional sample preparation procedures include Soxhlet extraction [2], liquid-liquid extraction (LLE) [3], accelerated solvent extraction (ASE) [4], microwave-assisted solvent extraction (MASE) [5], solid-phase extraction (SPE) [6], supercritical fluid extraction [7], and purge and trap [8]. These conventional sample preparation techniques are time-consuming, labor-intensive and involve multi-step operation. Moreover, they often employ large volumes of hazardous organic solvents. Most of these techniques are not suitable for field application. Therefore, sample collection and transportation to the laboratory is required prior to further processing. As a consequence, incorrect sample handling during collection, transportation, and preservation may lead to significant variability in analysis results putting the validity and authenticity of the method in question.

In an attempt to eliminate these sampling and sample preparation related problems, Belardi and Pawliszyn [9] developed solid-phase microextraction in 1989 which not only is a solvent-free technique but also incorporates sample extraction and preconcentration in a single step. Solid-phase microextraction provides analysts the flexibility in sample preparation both in the laboratory and in the field where the material system under investigation is located.

1.2 Evolution of solid-phase microextraction: a historical perspective

The history of solid-phase microextraction dates back to late 1980s when Pawliszyn and co-workers[10] were involved in laser desorption/gas chromatography experiments to accomplish rapid speed in separation, even for high molecular mass
species. A small piece of optical fiber was used in the experiment. One end of the optical fiber was dipped into the concentrated analyte solution to generate a coating of the analyte(s) on the surface of it by evaporating the solvent. Then the fiber was inserted into the GC injection port to transfer the analyte(s) from the fiber into the injection port through desorption. Desorption was accomplished by transmitting laser light energy through the optical fiber. The desorbed analytes then got separated as they moved through the GC analysis column. It was observed that using laser pulse, analytes could be quantitatively transferred from the optical fiber probe to GC injection port for further separation, but the sample preparation step took hours. As a result, the total time for the sample analysis still remained fairly long. This led to a strong recognition of the need for a rapid sample preparation technique that might assist in retaining the time efficiency advantages accomplished by using laser pulse and a high-speed separation instrument. To address the challenge, similar optical fibers having polymeric coating on the outer surface were utilized. The purpose of the polymeric coating (10-100 µm) was to protect the fibers from breakage.

In the preliminary work on SPME [9, 11], sections of fused silica optical fibers, both uncoated (obtained by burning off the polyimide coating), and coated with liquid and solid polymeric phases were used to verify the conceived idea of SPME. Extraction was carried out by inserting both the coated and uncoated fibers into the aqueous solution of the analyte(s) and then placed the fibers into the GC injection port for the thermal desorption of the extracted analyte(s). Preliminary data indicated the future potential of the new technique by extracting both polar and nonpolar analyte(s) rapidly and reproducibly from aqueous solution.
The success in preliminary investigation on solid phase microextraction accelerated its rapid development leading to the invention of a rather simple device by attaching the coated fiber to the plunger of a microsyringe. This, in fact, was the first SPME device [11]. In this first SPME device, the extraction fiber was housed inside a Hamilton™ 7000 series microsyringe. The plunger of the syringe was glued to the fiber with epoxy glue. This simple mechanism allowed facile movement of the coated end of the fiber in and out of the syringe needle by pushing the plunger forward or by retracting it back. A forward movement of the plunger exposed the fiber coating to the sample, and extraction of the target analyte(s) was accomplished because of higher affinity of the analyte(s) for the coated sorbent than the sample matrix. After carrying out the extraction, the extracted analyte(s) were released from the coating into the injection port of a gas chromatograph by thermal desorption. For this, the exposed fiber was first retracted back into the syringe needle to protect it from mechanical damage, and then the needle was used to vertically pierce the septum of the injection port. Following this, the plunger was pushed forward to expose the fiber to the space in the glass liner of the hot injection port. As a result, the analyte(s) extracted on the fiber were desorbed and entered into the GC analysis column for separation. Figure 1.1 illustrates the configuration of the first commercial SPME device [12]. This configuration has drawn wide acceptance among the researchers and proved suitable particularly for gas phase separation, although several other modifications of the SPME devices were also reported [13, 14].
Figure 1.1 Design of the first commercial SPME device (Reproduced from Ref. [12] with permission from Elsevier)
The sorbent coating on the external surface of the fiber, however, is not well-suited for hyphenation with liquid phase separation (e.g., HPLC, CE, CEC, etc.) because in this case the extracted analyte(s) needs to be desorbed using an organic sorbent which later would carry the analyte(s) to the liquid mobile phase for separation. The format-related incompatibility of fiber-based SPME has prompted researchers to develop so-called in-tube SPME. In this format, the extraction sorbent is located on the inner surface of a fused silica capillary. In most cases, a piece of open tubular GC column is used for this purpose. The coating inside the capillary acts as the sorbent. The aqueous sample containing the analytes is passed through the capillary and the target analyte(s) get extracted by the sorbent. Once the extraction system reaches equilibrium, the analytes are washed into the HPLC column using the liquid organo-aqueous mobile phase or any organic solvent which is compatible with the liquid mobile phase. This format has opened the window for automated sample preparation and preconcentration in liquid phase separation (HPLC, CE, CEC, etc.)

There are several other formats of SPME worth mentioning. Nickerson et al. [15] proposed a sorbent coated vial sealed with a rubber septum. Piercing the septum with a sampling syringe can collect analytes from the headspace inside the sealed vessel. For extracting less volatile compounds, the vessel can be even heated. Another recently popular format is organic polymer coated stir bar[16] in which sorbent materials are coated on a small stir bar using either static coating technology[17] or sol-gel coating technology[18] originally reported by Malik and co-workers, and subsequently adapted by Liu et al.[19] for stir bar SPME. Figure 1.2 demonstrates a coated stir bar and the device for headspace stir bar extraction [20].

6
**Figure 1.2** (a) Schematic representation of a PDMS coated stir bar and (b) Head space stir bar extraction device (Reproduced from Ref. [20] with permission from American Chemical Society)
1.3 Working principle of solid phase microextraction

In solid phase microextraction, the extracting phase coated either on the outer surface of a fused silica fiber (fiber-SPME) or inside a fused silica capillary (in-tube SPME, Capillary microextraction) plays a vital role in the extraction process. Unlike solid phase extraction (SPE), solid phase microextraction is an equilibrium extraction process. When the extracting phase comes in contact with the analyte(s) to be extracted, the analyte concentration equilibrium is gradually established between the sample matrix and the extracting phase. As soon as the equilibrium is reached, the extracting phase cannot accumulate the analyte(s) any more: the amount of the analyte extracted in a given time is exactly equal to the amount of analyte desorbed from the coating. The time required for establishing the extraction equilibrium depends on various factors: coating thickness, partition co-efficient between the analyte and extracting phase, agitation of the sample matrix, extraction temperature, solute diffusion rates in the extracting phase and the sample matrix, viscosity of the sample matrix, physical state of the matrix, etc. The equilibrium condition can be described by the following equation:

\[ n_f = \frac{K_{fs} V_f V_s C_0}{K_{fs} V_f + V_s} \]

Equation 1.1

Where \( n_f \) = number of moles of the analyte(s) extracted by the extracting phase

\( K_{fs} \) = fiber coating/ sample matrix distribution constant

\( V_f \) = volume of the extracting phase on the fiber

\( V_s \) = volume of the sample

\( C_0 \) = Initial concentration of the analyte(s) in the sample.
This equation is valid for partitioning equilibrium involving liquid polymeric extracting phases. When solid sorbents are used, this equation is valid only when the concentration of analyte(s) is low and the sorbent is porous.

When the volume of the sample is very large compared to the volume of the extracting phase, the value of the term $K_{fs}V_f$ becomes insignificant. Therefore, Equation 1.1 can be written as

$$n_f = K_{fs} V_f C_0$$

Equation 1.2

As can be seen from the equation 1.2, once the extraction equilibrium is reached, there is a direct proportional relationship between the initial concentration of the analyte(s) and the amount (moles) of analyte extracted. This is the theoretical basis for analyte Quantification.

This equation clearly expresses the advantage of SPME for field application. The amount of extracted analyte(s) is independent of the sample volume. There is no need to collect a defined sample prior to analysis as the extracting sorbent can be exposed directly to the air, water, production stream where the target analyte is located. The amount of the analytes extracted onto the extracting sorbent will correspond directly to its concentration in the matrix. Thus SPME incorporates sampling and sample preparation into one single step and eliminates the errors associated with sample storage (adsorption on the container wall or decomposition).
1.4 Modes of extraction

1.4.1 Extraction modes with a coated fiber

When coated fibers are used in solid phase microextraction, there are three different modes of extraction that can be followed: (a) direct extraction, (b) headspace extraction, and (c) membrane protected extraction. Figure 1.3 illustrates the different modes of extraction used in fiber-SPME [12].

In the direct extraction mode, the coated fiber is inserted into the matrix containing the target analyte(s) so that the analyte(s) can be directly transported from matrix to the extracting phase on the fiber (coating). The rate of extraction can be increased in order to achieve faster extraction equilibrium by employing agitation. For gaseous samples, natural convection of air is sufficient for faster equilibrium. When extraction is carried out in aqueous solution, different means of agitation (e.g., fast flow of the aqueous solution, rapid fiber or vial shaking, stirring or sonication of the solution, etc.) may be employed. In the headspace mode, the coated fiber stays above the aqueous phase in a sealed container. The analyte(s) first reaches equilibrium between the aqueous phase and the air in the closed container. Extraction is carried out from the gaseous phase. As a result, unwanted matrix interferences (e.g., compounds having high molecular mass, humic materials, proteins, etc.) cannot disturb the performance of the coating as well as its life-span. Moreover, such an extraction mode allows the modification of the matrix such as pH change, addition of salts etc. frequently used to improve the extraction efficiency of the coating.
Figure 1.3 Different modes of SPME operation (a) direct extraction, (b) headspace SPME, (c) membrane-protected SPME (Reproduced from Ref. [12] with permission from Elsevier)
The third extraction mode is membrane protected extraction. In this case, a membrane is used which surrounds the fiber coating to give protection against unwanted sorptive components in the sample matrix. This extraction mode is particularly suitable when the matrix is very dirty. In addition to protecting the fiber from damage, using appropriate membrane material may provide additional selectivity to the extraction process. One of the major disadvantages of this mode is its substantially longer extraction equilibrium. Using thinner membrane and elevated extraction temperature may results in shorter extraction time [21].

The time needed for accomplishing extraction equilibrium greatly depends on the mode of extraction. In headspace extraction, analyte(s) to be extracted are first removed from the aqueous phase to the headspace followed by the extraction on the sorbent coating on the fiber. Overall mass transfer to the coating in this case is typically limited by mass transfer rates of the analyte(s) from the aqueous phase to the headspace. As a result, volatiles are extracted faster than semi-volatiles due to their faster mass transfer to the headspace. Temperature has a significant impact on the kinetics of the extraction process because it determines the vapor pressure of the analyte(s). As is known, diffusion coefficients in gaseous phase are typically 2-4 orders of magnitude larger than in aqueous phase and since mass transfer from aqueous phase to vapor phase is faster due to low vapor pressure of the analyte(s), equilibration time for volatiles are significantly shorter in headspace mode compared to direct mode under similar agitation conditions.
1.4.2 Extraction modes with in-tube SPME

In in-tube SPME, there are two different modes of extraction: (a) active or dynamic and (b) passive or static. In active or dynamic extraction mode, the matrix containing the analyte(s) is passed through the tube and the analytes are extracted by the sorbent coating as the sample passes by. In passive or static extraction mode, the capillary is filled with sample matrix and the high affinity of the analytes for the sorbent material serves as the driving force for their extraction by the coating residing on the capillary inner walls.

In dynamic in-tube SPME, it is assumed that an internally coated piece of fused silica capillary (in most of the cases, a piece of open tubular capillary GC column) or a fused silica capillary packed with extracting phase dispersed on an inert support (typically a piece of micro-LC capillary column) is used. In this type of configuration, the concentration profile along the axis (x) of the tubing containing the extracting phase as a function of time (t) can be described by adopting the expression for dispersion of the concentration front [22].

In static in-tube SPME, the extracting phase resides inside a protective tubing (needle), is not exposed directly to the matrix sample. The extraction occurs through the static gas phase present in the needle. In this case, the only mechanism of analyte transport is diffusion through the gaseous phase contained in the tubing. The static in-tube SPME is particularly suitable for field sampling.
1.5 Preparation of coatings on fibers

In order to prepare coatings on fused silica fibers, several approaches may be followed. In one approach, the fibers are immersed into the solution made of organic solvent and the polymeric material to be deposited for a given period of time. After removing the fibers from the solutions, the solvent is evaporated and the deposited material can be immobilized through cross linking [9]. Coatings can also be made through electrochemical deposition in which selective coatings are deposited electrochemically on the surface of metallic rods [11]. In preparing commercial coatings, the fiber is first drawn in a tower. Then, the fiber is passed through a specified diameter orifice (depends on the diameter of the fiber as well as the coating thickness) in a cup that contains the desired phase e.g., PDMS, PA that coats the fiber. As the fiber is drawn, a coating of the extracting phase is deposited on its outer surface.

1.6 Experimental parameters affecting extraction efficiency

Although the physico-chemical characteristics of the coating materials largely determine their extraction efficiency, there are several experimental parameters optimization of which generally increase extraction efficiency and improve the detection limit to a significant extent. Among such experimental factors is pH adjustment, stirring, heating, and salt addition to the matrix.
1.6.1 pH adjustment of the matrix

Since the commonly used SPME coatings are electrically neutral, they are most suitable for the extraction of neutral compounds from water (by direct or headspace extraction). Therefore, compounds which remain neutral in aqueous solution can be easily extracted by solid phase microextraction. Changing the pH of the matrix containing such neutral compounds does not yield any impact on extraction efficiency. However, compounds like organic acids and bases get dissociated into ionic species when dissolve in water. In order to extract such compounds from aqueous media, pH adjustment is required to convert the ionic species into neutral molecules. Optimum pH of the matrix, in this case, depends on the $pK_a$ or $pK_b$ values of organic acids and bases respectively. In order to make sure that 99% of the organic acid is in neutral form, the pH of the matrix should be at least two units lower than $pK_a$ values of the analytes. In case of basic compounds, similarly, pH of the matrix should be at least two units larger than their $pK_b$ values.

1.6.2 Agitation of the matrix

Although, in general, agitation of the sample matrix significantly reduces the extraction equilibrium time, the impact of agitation mostly depends on the mode of extraction. In the direct extraction mode, the coated fiber is inserted directly into the sample and the analytes are transported directly from the sample matrix to the extracting phase. In order to facilitate rapid extraction, agitation of the sample matrix is needed to
augment analyte transport from the bulk of the liquid sample to the vicinity of the fiber. Fast sample flow through the capillary (in-tube SPME), rapid fiber or vial movement, stirring or sonication are most common means employed for agitation. Agitation minimizes the effect caused by so called “depletion zone” formed close to the fiber as a result of fluid shielding and slow diffusion coefficients of analytes in liquid matrices.

In the headspace mode, agitation of the water sample to generate a continuously fresh surface would accelerate the mass transfer of less volatile analytes from the water to headspace. Once in the gaseous phase, analytes moves rapidly from the headspace to the extracting media due to their large diffusion coefficients in the gas phase.

1.6.3 Heating the matrix

Extraction temperature has a profound impact on overall extraction efficiency. However, the impact is comprised of two opposing forces: (a) diffusion of the analyte increases with the increase in temperature leading to faster mass transfer from the liquid phase to the headspace and from the headspace to the extracting sorbent. As a result, at higher temperature extraction equilibrium can be reached in relatively quickly which significantly reduces the extraction time. (b) As extraction is an exothermic process, the distribution constant decreases with increase in temperature. Therefore, extraction yield would be lower at higher temperature.

In general, if the extraction time is of major concern, the highest temperature which still provides satisfactory sensitivity should be used. When extraction sensitivity, not the extraction time, is the major concern, as in case of trace analysis, extraction
should be carried out at as low temperature as possible.

To prevent sensitivity loss at elevated extraction temperature, the extracting phase can be cooled simultaneously with heating the sample matrix. Zhang et al. [23] proposed the design of an internally cooled SMPE device which allows simultaneous cooling of the sorbent as well as heating of the sample matrix. In this assembly, two concentric fused silica capillaries are used and the sorbent is coated at the outer surface of the larger diameter capillary. The inner capillary is used to deliver liquid carbon dioxide to the coated end resulting in a significantly lower coating temperature compared to the matrix temperature. Figure 1.4 presents the design of an internally cooled SPME device [23].
Figure 1.4 Schematic of an internally cooled SPME device
(Reproduced from Ref. [23] with permission from American Chemical Society)
1.6.4 Addition of salt to the matrix

Addition of salt into the aqueous phase containing organic analytes may have profound impact on the extraction efficiency. Several research groups have studied the impact of salt addition in detail [24, 25]. Addition of salt (also called salting out) is a well known phenomenon used for improving extraction of organics from aqueous solution. Although sodium chloride salt is used predominantly in SPME practice, other salts e.g., CaCl$_2$, NH$_4$Cl, (NH$_4$)$_2$SO$_4$, MgSO$_4$, Na$_2$CO$_3$, K$_2$CO$_3$ may also be used [26]. Although addition of salt usually increases the extraction efficiency, the opposite behavior has also been reported [24, 25]. In general, the effect of salt addition increases with the increase in polarity of the compound [25].

1.6.5 Addition of organic solvents

In general, the presence of small amount of organic solvents in the analyte matrix does not have significant impact on the extraction efficiency. However, there are several reports on the subject that apparently do not agree with each other. Arthur et al. [27] reported that the presence of up to 1% methanol in aqueous solution containing aromatic hydrocarbons does not have any appreciable effect on extraction of efficiency in SPME extraction of aromatic hydrocarbons. R. Eisert [28] observed less than 10% loss in extraction yield of selected triazines when methanol content in the aqueous solution was as high as 5%. On the other hand, Urruty et al. [29] reported that for pesticide extraction, the presence of ethanol in the extracted solution is an important parameter for the
It is therefore apparent that the effect of the presence of organic solvent in the extraction matrix is compound and matrix dependent and warrants further investigation to generalize the impact of the presence of solvent in the matrix.

1.7 Derivatization

Due to their strong hydrophobicity, nonpolar analytes are easy to extract from aqueous samples using any nonpolar sorbent. But the extraction of polar analytes poses a challenge to the scientist because of their affinity for aqueous matrix. As a result such analytes are difficult to extract from environmental and biological matrices that are also polar or rich in water. To meet these challenges, derivatization techniques are frequently used. Figure 1.5 summarizes different derivatization techniques that are compatible with SPME [30]. In direct derivatization, the derivatizing agent is added to the sample vial to convert the analyte(s) into a stable nonpolar compound by chemical reaction with it followed by the extraction on the extraction fiber.
**Figure 1.5** Derivatization techniques used in solid-phase microextraction (Reproduced from Ref. [30] with permission from American Chemical Society)
When the polarity of the sorbent is sufficient for extracting polar analyte from aqueous phase, derivatization of the analytes may still be required to facilitate better separation in the GC analysis column or to increase detector response by converting the analytes into suitable form. This can be done by employing derivatization in GC injector port. Instead of using derivatization in GC injector port, another convenient way is to use in-coating derivatization following extraction.

Another interesting and potentially very useful approach for derivatization is simultaneous derivatization and extraction, performed directly on the coating. This approach yields high efficiency and can be easily used in field applications. In this case, the fiber is doped with a derivatizing reagent and subsequently is exposed to the sample. The analytes are extracted and simultaneously converted to compounds having high affinity to the coating.
1.8 References for Chapter One

Chemistry Conference and Exhibition, 1995, p. Abs. 528, University of Guelph, Guelph, ON.


Chapter Two

An Overview on Stationary Phases Used in Solid Phase Microextraction (SPME)

2.1 Introduction

Solid phase microextraction (SPME) is an environmentally benign solvent-free sample preparation technique that was developed by Pawliszyn and co-workers [1] about sixteen years ago. Because of its simplicity, cost-effectiveness, portability, solvent-free operation, environmental friendliness and ease in automation, SPME has experienced a tremendous growth since its inception in 1989. A recent literature search using SciFinder\textsuperscript{®} Scholar 2004 database on March 20, 2005 revealed that over 4,379 articles have been published on SPME in last 16 years of its existence, among which more than 750 papers have been published in 2004 alone. The search results are presented in Figure 2.1 which shows a rapid growth in the number of articles published on solid phase microextraction since its inception. Within a very short period of time SPME has already been recognized as a viable alternative to traditional sample preparation techniques (e.g., liquid-liquid extraction and solid phase extraction) that are not only hazardous due to the use of toxic organic solvents in their operation but also time consuming, labor-intensive, and cumbersome. Compared to traditional sample preparation techniques, SPME offers
many advantageous features (e.g., simplicity, speed, cost-effectiveness, ease in automation, portability, solvent-free operations, environmental friendliness, health safety and so on). Unlike liquid-liquid extraction and solid phase extraction, solid phase microextraction is an equilibrium-based non-exhaustive extraction method. In SPME the extracted amount is practically independent of the sample size.

SPME is frequently coupled to gas chromatography (GC). SPME-GC analysis consists of two major steps: (a) equilibrium extraction of the target analyte(s) from the sample matrix and (b) desorption of the extracted analyte(s) into the GC for analysis. Success in each of these steps greatly depends on the nature and property of the sorbent coatings used as the extracting phase.

Based on the substrate on which the extracting phase is coated, SPME can be classified into two general formats: (a) fiber SPME, and (b) in-tube SPME. In fiber SPME, the sorbent is coated on the outer surface of a small-diameter (typically 100 µm o.d.) solid rod called fiber. Fused silica fibers are most commonly used for this purpose. Some researchers also reported the use of metallic fibers (stainless steel, copper etc.) to facilitate rapid thermal desorption of the extracted analytes for GC analysis and to prevent frequent breakage. In in-tube SPME (capillary microextraction), the coating is created on the inner surface of a fused silica capillary. Frequently, a small piece of wall-coated capillary GC column is used for this purpose. Figure 2.2A demonstrates two different formats used in SPME. Figure 2.2B compares the two predominantly used formats in SPME showing the position of the coating.
Figure 2.1 Graphical representation of number of articles published on SPME since its inception in 1989.
Figure 2.2A Different formats of SPME (a) fiber SPME where extracting phase resides outside of a fiber, (b) in-tube SPME where extracting phase resides inside the capillary (Reproduced from Ref. [2] with permission)
(a) Coated fused silica fiber

(b) Polyimide coated fused silica capillary

(c) Enlarged cross section of a fused silica capillary

Figure 2.2B Two major formats of SPME
Although numerous inherent positive attributes have placed SPME in a superior position among currently available sample preparation techniques, the small numbers of commercially available fiber coatings are not being able to keep up with the growing demand on new sorbents needed to maintain the healthy growth of SPME and its penetration into ever increasing areas of science and technology. Lack of progress in the area of sorbent development may prove to be a serious hurdle for the future growth of SPME.

The effectiveness of solid phase microextraction depends on the analyte distribution constant (K) between the SPME coating and the sample matrix. This characteristic parameter describes the affinity of the SPME coating toward the analyte relative to similar affinity exhibited by the sample matrix. The choice of a particular coating depends on the physiochemical nature of the analytes to be extracted. The principle “like dissolves like” may serve as a rule of thumb in the selection of SPME coatings. Coating volume is another important factor that determines the method sensitivity and should be given due consideration in method development, especially for trace analysis. In general, thicker coatings provide higher sensitivity. However, thicker coatings require longer extraction times due to the slow diffusion of analyte(s) within the polymeric extraction phase. Therefore, it is important to use appropriate coating thickness depending on the required extraction sensitivity and analysis time. Depending on the format of SPME in which the extracting sorbents are used, SPME sorbents may be grouped into four major classes: (a) commercially available coatings for fiber SPME, (b) commercial GC coatings for in-tube SPME, (c) tailor-made coatings for fiber SPME, d) tailor-made coatings for in-tube SPME.
2.2 Commercially available sorbents for fiber SPME

SUPELCO Inc. has been the only commercial source of SPME fibers since 1993. Two different types of coated SPME fibers are available from SUPELCO: homogeneous polymeric sorbents and composite polymeric sorbents. Table 2.1 presents a list of commercially available SPME coatings [3].

Table 2.1 Commercially available SPME sorbents [3]*

<table>
<thead>
<tr>
<th>Sorbents</th>
<th>Polarity</th>
<th>Coating thickness (µm)</th>
<th>Maximum operating temp. (ºC)</th>
<th>Immobilization</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polydimethylsiloxane (PDMS)</td>
<td>Non-polar</td>
<td>100 30 7</td>
<td>280 280 340</td>
<td>Nonbonded Nonbonded Bonded</td>
<td>Nonpolar or moderately polar organic compounds</td>
</tr>
<tr>
<td>Polyacrylate (PA)</td>
<td>Polar</td>
<td>85</td>
<td>320</td>
<td>Partially crosslinked</td>
<td>Polar organics</td>
</tr>
<tr>
<td>Polydimethylsiloxane/Divinylbenzene</td>
<td>Bi-polar</td>
<td>65 60</td>
<td>270</td>
<td>Partially crosslinked</td>
<td>Aromatic hydrocarbons, air analysis, VOCs</td>
</tr>
<tr>
<td>(PDMS/DVB)**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carboxen/Polydimethylsiloxane (CAR/PDMS)</td>
<td>Bi-polar</td>
<td>75</td>
<td>320</td>
<td>Partially crosslinked</td>
<td>VOCs, Hydrocarbons</td>
</tr>
<tr>
<td>Carbowax/divinylbenzene (CW/DVB)</td>
<td>Polar</td>
<td>65</td>
<td>265</td>
<td>Partially crosslinked</td>
<td>Polar organics, e.g., alcohols</td>
</tr>
<tr>
<td>Carbowax templated resin (CW/TPR)</td>
<td>Polar</td>
<td>50</td>
<td>-</td>
<td>Partially crosslinked</td>
<td>Surfactants</td>
</tr>
<tr>
<td>Divinylbenzene/Carboxen/PDMS (DVB/CAR/PDMS)</td>
<td>Bi-polar</td>
<td>50/30 30/30</td>
<td>270</td>
<td>Cross-linked</td>
<td>Odors and flavors</td>
</tr>
</tbody>
</table>

* Reproduced from Ref. [3] with permission of Wiley InterScience
** In SPME literature, Divinylbenzene (DVB) actually stands for poly (divinylbenzene)
2.2.1 Homogeneous polymeric sorbents

Commercially available homogeneous polymeric sorbents include polydimethylsiloxane (PDMS) and Polyacrylate (PA). Polydimethylsiloxane (PDMS) [4] has been the most frequently used sorbent in SPME because of its inherent versatility, ruggedness, high thermal stability, and wide range of analytes that can be extracted using this phase. SUPELCO markets PDMS coated fibers in two different forms: bonded and nonbonded. The bonded PDMS coating is crosslinked on the fiber using a cross-linkable functionality present in the polymeric structure. This bonding translates into higher thermal stability (~340 °C) as well as solvent stability [3]. The only commercially available bonded PDMS fiber is the one with 7 µm coating thickness. The nonbonded PDMS coatings are immobilized either by thermal- or by UV treatment and are available in two thicknesses: 100 µm and 30 µm. Due to the absence of proper immobilization to the substrate, they possess relatively low thermal stability (280 °C) as well as low solvent resistance. As a nonpolar sorbent, PDMS extracts nonpolar analytes very well. PDMS coating can also be used to extract more polar compounds by optimizing extraction conditions such as pH, salt concentration, and temperature. One major drawback for this phase is that the fibers cannot be exposed to a matrix with a pH below 4 or above 10 [4].

Polyacrylate (PA) [5] is a highly polar sorbent, immobilized by partial cross-linking, and is available in 85 µm thickness. Being highly polar, it is frequently recommended for extracting polar analytes from different matrices [6]. Unlike other sorbents, at room temperature, polyacrylate is a rigid, low-density solid polymer. As a consequence, diffusion of analytes requires longer time resulting in longer extraction
time. Moreover, higher temperatures are required for complete desorption of the extracted analytes. Better solvent stability and longer life span have made this coating very popular for the extraction of polar analytes.

2.2.2 Polymeric composite sorbents

Sorbents of this category are made by embedding one or more porous particulate materials into a crosslinked polymer. Compared with homogeneous sorbents, these coatings possess lower mechanical stability but very high selectivity. The following commercial composite coatings are currently available from Supelco: Polydimethylsiloxane/Divinylbenzene (PDMS/DVB), Carboxen/Polydimethylsiloxane (CAR/PDMS), Carbowax/Divinylbenzene (CW/DVB), Carbowax/Templated Resin (CW/TPR), Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS).

2.2.2.1 Polydimethylsiloxane/Divinylbenzene (PDMS/DVB)

This composite sorbent was introduced in 1996 [7]. DVB is characterized by its high porosity as well as very high surface area (~750 m²/g) [7]. The pores in the polymer play a key role in retaining the analytes. DVB provides PDMS better ability to retain smaller analytes that cannot be retained by PDMS alone. Moreover, the polymer blend demonstrates better affinity for polar analytes. PDMS/DVB coating is suitable for extracting polar compounds e.g., alcohols, nitrogen containing analytes (e.g., amines), etc. [8].
2.2.2.2 Carboxen/Polydimethylsiloxane (CAR/PDMS)

This fiber was introduced into the market in 1997 [9]. Like DVB, Carboxen is also a highly porous polymeric material having high surface area (~ 715m$^2$/g) and pore volume (~ 0.78mL/g) [7]. Carboxen/PDMS provides high thermal stability (~320 °C). Volatile organic compounds (VOCs) [10] and hydrocarbons [11] are among many types of analytes that have been extracted by SPME using CAR/PDMS coating thus far.

2.2.2.3 Carbowax/Divinylbenzene (CW/DVB)

Carbowax/Divinylbenzene fiber was introduced in 1997 [12]. Blending porous divinylbenzene with relatively polar carbowax provides a composite phase that is frequently used for extracting polar organic compounds (e.g., alcohols) [13] from various matrices. Major shortcomings of this composite sorbent include the swelling tendency of Carbowax in water as well as its high sensitivity to oxygen at temperatures above 220 °C, imposing limitation in its ability to extract from aqueous media and its effectiveness for low volatile compounds. There are several approaches that may reduce the problem to some extent. Maintaining the maximum injection port temperature in the range of 180-240 °C and using catalytic purifiers to obtain oxygen-free carrier gas may improve the lifetime of the fiber [7].
2.2.2.4 Carbowax/Templated Resin (CW/TPR)

This material [14] has been designed to perform preferential extraction of polar analytes. Like other composite sorbents, CW/TPR is made by blending porous templated resin with Carbowax. A patented polymeric material SUPELCOGEL TPR-100 (CAS RN: 33972-38-2), composed of both hydrophobic and hydrophilic monomers, is used as the porous constituent in the mixture. Due to the presence of both hydrophilic and hydrophobic moieties in the polymeric network, it provides remarkable selectivity in extraction. Extraction of surfactants from aqueous media is one of the many notable applications that have been possible by the virtue of CW/TPR sorbent [15].

2.2.2.5 Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS)

This new blended extracting phase was introduced in 1999 by Supelco [16]. Divinylbenzene, a porous polymeric particulate material, contains predominantly mesopores with some micropores and macropores. As a result, it effectively extracts organic compounds with carbon atoms in the range of C$_6$-C$_{15}$. Carboxen, on the other hand, is characterized by its evenly distributed micro-, meso- and macropores. It provides highly effective extractions for C$_2$-C$_{12}$ organic compounds. Blending particles of these two highly porous polymeric materials with liquid polydimethylsiloxane yields a composite phase, which is recommended for extracting C$_3$-C$_{20}$ analytes from a wide range of polarity [17, 18].
2.3 Commercial GC coatings used in in-tube SPME

In solid phase microextraction, the fiber format has been used predominantly. The fiber is installed in a specially designed SPME syringe. Although the design of the syringe offers good protection to the fiber as well as the coating on its surface, fiber breakage, mechanical damage of the coating due to scraping, and needle bending are frequently witnessed by analytical chemists. The short length of the coated segment of the fiber provides low stationary phase loading for extraction. As a consequence, low sample capacity of the fiber imposes limitation on the sensitivity of the SPME process. Although thicker coating may increase the sample capacity to some extent, long extraction time may turn out to be the bottleneck in the whole process. Another major drawback of the fiber-SPME is the difficulty to interface with other analytical instruments e.g., HPLC, CEC, MS, FTIR etc. To overcome these format related shortcomings, in-tube SPME (or capillary microextraction, CME) has been introduced [19]. In-tube SPME is suitable for automation. Automated SPME not only shortens the total analysis time but also provides better accuracy and precision compared to manual operation.

In principle, any gas chromatographic stationary phase should be suitable for use as in-tube SPME sorbent. In fact, pieces of coated capillary GC columns (in most cases, a 60 cm segment) are commonly used for in-tube SPME [20]. In this case, the stationary phase coating on the inner surface of the capillary serves as the extracting phase. Table 2.2 lists frequently used extracting phases in in-tube SPME.
Table 2.2 Commercial GC capillary columns short pieces of which are used for in-tube SPME

<table>
<thead>
<tr>
<th>Name of the Phase</th>
<th>Vendor</th>
<th>Phase Composition</th>
<th>Chemical Structure</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP-1</td>
<td>SGE</td>
<td>100% dimethyl polysiloxane</td>
<td></td>
<td>[29]</td>
</tr>
<tr>
<td>DB-1</td>
<td>JW</td>
<td>100% dimethyl polysiloxane</td>
<td></td>
<td>[30]</td>
</tr>
<tr>
<td>SPB-1</td>
<td>SUPELCO</td>
<td>100% dimethyl polysiloxane</td>
<td></td>
<td>[26]</td>
</tr>
<tr>
<td>PTE-5</td>
<td>SUPELCO</td>
<td>Poly (5% diphenyl / 95% dimethyl siloxane)</td>
<td></td>
<td>[24]</td>
</tr>
<tr>
<td>SPB-5</td>
<td>SUPELCO</td>
<td>Poly (5% diphenyl / 95% dimethyl siloxane)</td>
<td></td>
<td>[25]</td>
</tr>
<tr>
<td>DB-Wax</td>
<td>JW</td>
<td>Poly (ethylene glycol)</td>
<td></td>
<td>[28]</td>
</tr>
<tr>
<td>Supelcowax</td>
<td>SUPELCO</td>
<td>Poly (ethylene glycol)</td>
<td></td>
<td>[24]</td>
</tr>
<tr>
<td>Omegawax 250</td>
<td>SUPELCO</td>
<td>Poly (ethylene glycol)</td>
<td></td>
<td>[21]</td>
</tr>
<tr>
<td>BP-20</td>
<td>SGE</td>
<td>Poly (ethylene glycol)</td>
<td></td>
<td>[26]</td>
</tr>
<tr>
<td>Supel-Q-PLOT</td>
<td>SUPELCO</td>
<td>Porous divinyl benzene polymer</td>
<td></td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[29]</td>
</tr>
</tbody>
</table>

References:

[24] [25] [26] [28] [29] [30] [31] [34]
Most of the sorbents used in in-tube SPME are commercially available GC stationary phases (e.g., DB-1, BP-1, SPB-1, SPB-5, PTE-5, Supelcowax, DB-5, Omegawax 250, DB-Wax, BP-20 Wax, Supel-Q-PLOT etc.).

The first report of using in-tube SPME was made by Pawliszyn and co-workers in 1997 [21]. In their experiments, 60 cm individual pieces of different GC capillary columns (Omegawax 250, SPB-1, SPB-5, uncoated fused silica capillary) were used as the extractor and each was coupled to a commercial HPLC autosampler. Six phenylureas were extracted as the test analytes using each of the above mentioned capillaries. The Omegawax 250, being the most polar phase, extracted the most and the uncoated fused silica capillary extracted the least. SPB-1 and SPB-5 coatings (nonpolar) also demonstrated poor extraction yield as was expected.

Omegawax 250 GC capillary columns use poly(ethylene glycol) as the stationary phase. It is the most frequently used sorbent in in-tube SPME for extracting polar analytes. Thus far, Omegawax 250 has been used for extracting phenylureas [21], β-blockers and metabolites in urine and serum samples [22], carbamate pesticides [23,24,25], mutagenic heterocyclic amines [26], ranitidine [19], and in drug analysis [27]. Although in both Supelcowax and Omegawax 250 coated capillaries, poly(ethylene glycol) was the common stationary phase, Omegawax 250 demonstrated higher yield in extracting carbamate from aqueous solution compared to the other [25]. In almost all of the above mentioned in-tube SPME publications SPB-1 (100% dimethyl polysiloxane), SPB-5 (poly 5% diphenyl/95% dimethyl siloxane), and uncoated fused silica capillary were used to study the importance of sorbent polarity in the extraction of polar analytes. The most polar among the used sorbents, Omegawax 250 was found to be most efficient.
Takino et al. used similar phase DB-Wax (J&W Scientific, Folsom, CA, USA) for the determination of chlorinated phenoxy acid herbicides in environmental water [28] and successfully coupled SPME-LC with EI/MS which provided enhanced selectivity and identification capability of the method.

Tan et al. [29] have successfully coupled in-tube SPME to GC/FID. One-meter segments of BP-1 (100% methylsiloxane) and BP-20 (polyethylene glycol) GC columns were used for extraction of BTEX and phenols respectively from aqueous media. Extraction was carried out by pushing the aqueous medium containing the analytes through the capillary using nitrogen pressure. Desorption of the extracted analytes was done by using a small plug of organic solvent which carried the analytes from the capillary to the GC injection port.

Although SPME has been developed to eliminate the use of toxic organic solvents in sample preconcentration step, a small amount of solvent is still being used in the desorption process, particularly when coupled to LC. To reduce the amount of toxic organic solvent used in analyte(s) desorption step after the extraction, Saito et al. [30] proposed a wire-in-tube configuration of SPME in which a 20-cm piece of DB-1 (100% polydimethylsiloxane) was used as the extractor. A stainless steel wire (diameter = 200 µm) was inserted into the capillary (d.c = 250 µm) that significantly reduced the available internal volume of the capillary (9.82 µL vs 3.53 µL) and thereby reduced the volume of solvent required for desorption. The wire-in-tube SPME was successfully used to analyze antidepressant drugs in a urine sample. Figure 2.3 demonstrates the schematic of a wire-in-tube extraction capillary.
Figure 2.3 Wire-in-tube SPME extraction capillary (Reproduced from Ref [30] with permission of Springer-Verlag)
Another GC capillary column frequently used in in-tube SPME is Supel-Q-Plot. A porous divinylbenzene polymer is used as the stationary phase in this column. Mester et al. [31] successfully coupled SPME to electrospray ionization mass spectrometer. A 60 cm piece of Supel-Q-PLOT column was used to preconcentrate and analyze trimethyl- and triethyllead species from aqueous media. This system seems to be very promising in lead speciation. The same phase has also been used for the analysis of endocrine disruptors in liquid medicines and intravenous solutions [32], daidzein and genistein in soybean foods [33], bisphenol A, alklyphenols, and phthalate esters in foods contacted with plastics [34].

2.4 Tailor-made coatings on SPME fibers

Table 2.3 presents a list of tailor-made sorbents coated on SPME fibers. For discussion purpose, they have been grouped into different classes: immobilized antibodies, metallic SPME fibers, active carbonaceous sorbents, bonded silica sorbents, PDMS film, and miscellaneous sorbents.
Table 2.3 Tailor-made sorbents on SPME fibers and their applications

<table>
<thead>
<tr>
<th>Sorbents</th>
<th>Applications</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbowax</td>
<td>2-Napthol</td>
<td>[1]</td>
</tr>
<tr>
<td>Octadecyltrichlorosilane</td>
<td>2-Napthol</td>
<td></td>
</tr>
<tr>
<td>Liquid Crystalline Poyacrylate (LCPA)</td>
<td>2-Napthol</td>
<td></td>
</tr>
<tr>
<td>Polymethylvinylchlorosilane</td>
<td>2-Napthol</td>
<td></td>
</tr>
<tr>
<td>HDEHP modified PDMS</td>
<td>Bi (III)</td>
<td>[56]</td>
</tr>
<tr>
<td>PVC Activated Charcoal Fiber Coated on Silver Wire</td>
<td>n-Alkanes</td>
<td>[42]</td>
</tr>
<tr>
<td>Graphite</td>
<td>Surfactant</td>
<td>[48]</td>
</tr>
<tr>
<td>Adjusted Active Carbon Fiber</td>
<td>BTEX</td>
<td>[47]</td>
</tr>
<tr>
<td>Graphitized Carbon Black</td>
<td>VOCs</td>
<td>[46]</td>
</tr>
<tr>
<td>Porous-Layer Activated Charcoal</td>
<td>BTEX</td>
<td>[44]</td>
</tr>
<tr>
<td>Activated Charcoal</td>
<td>PAHs</td>
<td>[45]</td>
</tr>
<tr>
<td>Activated Charcoal-PVC</td>
<td>Organophosphorus pesticides</td>
<td>[43]</td>
</tr>
<tr>
<td>Polycrystalline Graphites</td>
<td>Nonionic surfactants</td>
<td>[15]</td>
</tr>
<tr>
<td>Pencil Lead</td>
<td>Alcohols</td>
<td>[49]</td>
</tr>
<tr>
<td>PDMS</td>
<td>PAHs, PCBs</td>
<td>[4]</td>
</tr>
<tr>
<td>Anodized Aluminum Wire</td>
<td>Alcohols, BTEX, Petroleum products</td>
<td>[37]</td>
</tr>
<tr>
<td>Modified Copper Wire</td>
<td>Primary amines</td>
<td>[41]</td>
</tr>
<tr>
<td>Anodized Zinc Wire</td>
<td>VOCs</td>
<td>[40]</td>
</tr>
<tr>
<td>Copper Sulfide Wire</td>
<td>Aliphatic alcohols and amines</td>
<td>[40]</td>
</tr>
<tr>
<td>Gold Coating</td>
<td>Mercury (II) ions</td>
<td>[39]</td>
</tr>
<tr>
<td>Inorganic/Organic Mesoporous Silica</td>
<td>Aromatic hydrocarbons</td>
<td>[53]</td>
</tr>
<tr>
<td>Porous Layer Silica Bonded Phases</td>
<td>Aromatic Hydrocarbons</td>
<td>[51]</td>
</tr>
<tr>
<td>C18-bonded Silica</td>
<td>Pesticides</td>
<td>[52]</td>
</tr>
<tr>
<td>Silica Particles bonded with Phenyl, C8, C18</td>
<td>PAHs, PCBs</td>
<td>[51]</td>
</tr>
<tr>
<td>Alkyl-Diol-Silica Restricted-Access Material</td>
<td>Benzodiazepines in biological samples</td>
<td>[69]</td>
</tr>
<tr>
<td>Immobilized Theophylline Antiserum</td>
<td>Theophylline in human serum</td>
<td>[35]</td>
</tr>
<tr>
<td>Poly(pyrrole-sulfated β-cyclodextrin)</td>
<td>Cationic analytes</td>
<td>[75]</td>
</tr>
<tr>
<td>Poly(3-methylthiophene)</td>
<td>Arsenate ions</td>
<td>[74]</td>
</tr>
<tr>
<td>Salicylate PVC-based Membrane</td>
<td>Organonickel complex</td>
<td>[79]</td>
</tr>
<tr>
<td>Polyimide</td>
<td>VOCs</td>
<td>[107]</td>
</tr>
</tbody>
</table>
### 2.4.1 Immobilized antibodies

The ability to selectively recognize a target analyte at the molecular level and extract it from a given matrix is an important aspect in any chemical and biochemical analysis. Stability and commercial availability are two important factors for a molecular recognition compound being considered as a new extraction medium. Keeping these in mind, antibodies are the major candidates that have excellent potential to be used as molecular recognition-based extraction media in SPME. Antibodies possess high affinity and specific recognition ability toward their complementary antigens in biological systems. Antibodies contain several types of reactive groups that are suitable for covalent bonding with silica supports. Figure 2.4 represents a typical reaction scheme involved in antibody immobilization [35]. The credit for the first exploitation of immobilized antibody as SPME sorbent goes to Yuan et al. [35]. They reportedly immobilized theophylline antiserum on the surface of a fused silica fiber and successfully extracted theophylline from human serum. The new technique, immunoaffinity SPME, has brought new hopes for the determination of drugs in complex matrices that require very high
specificity which, in general, cannot be achieved by existing SPME sorbents.
Figure 2.4 Reactions involved in antibody immobilization (a) silanization of silica surface with APTES; (b) Surface modification with glutaraldehyde; (c) immobilization of antibody (Adapted from Ref. [35] with permission of the Royal Society of Chemistry)
N.A. Guzman [36] fabricated an improved SPME device with immobilized antibody and coupled it on-line with immunoaffinity capillary electrophoresis. In the specially designed SPME device, antigen binding fragments (Fab’) obtained from purified immunoglobulin G (IgG) antibody were immobilized by covalent bonding and were used as the SPME sorbent. Low sample volume, minimal sample preparation, high reproducibility, long lifespan, and at least 1000-fold sample preconcentration have been reported to be the major advantageous features of the new device.

2.4.2 Metallic SPME fibers

One of the major drawbacks of SPME fiber is its fragility. Almost all SPME fibers are made of fused silica. The sorbent coated end of the silica fiber which is devoid of protective polyimide coating is very fragile and needs great care in handling. Therefore, developing relatively strong SPME fibers with long life span is an important issue for the researchers working in this field.

Some researchers have proposed miniaturized metallic rods as an alternative to easily breakable silica-based SPME fibers [37,38]. In some cases, the metal itself and in other cases an oxide layer formed on the metal surface has served as the SPME sorbent.

Guo et al. [39] has successfully combined SPME with electrochemistry. An electrochemically deposited 10 µm thick gold coating on a 140 µm carbon steel electrode was used as the electrochemical extraction medium. The gold coating was used to detect inorganic mercury ions in solutions using ion-trap GC-MS. It was also able to
differentiate between free inorganic mercury and complexed mercury present in aqueous matrix. Moreover, the possibility of direct detection of metallic mercury in gas by gold coated SPME/EC has made the system very promising due to the fact that high toxicity of mercury poses a real threat to the environment.

In an attempt to develop relatively strong SPME fiber, Djozan et al.[37] developed anodized aluminum wire. They evaluated different types of aluminum-based wires: (a) polished aluminum wire (to prevent the formation of Al₂O₃ on the surface), (b) aluminum wire with oxidation product (Al₂O₃) on the surface, and (c) anodized aluminum wire as the extraction sorbent. Those researchers found that anodized aluminum wire provided the highest extraction efficiency (30 times more sensitive than the oxidized aluminum wire). The dramatic increase in sensitivity was explained by adsorptive nature of the thick (~ 20 µm) Al₂O₃ bed which was formed on the aluminum surface during the anodizing process and also the inherent porous structure of the aluminum oxide bed on the surface. Figure 2.5 illustrates the porous surface of the anodized aluminum wire [37]. Anodized aluminum wire has been used to extract aliphatic alcohols, BTEX and some petroleum products from gaseous samples. Low cost, high thermal stability (~300 °C), mechanical strength, and long life span are among the important advantages of anodized aluminum fibers.
Figure 2.5 Scanning electron micrograph of the surface of an anodized aluminum wire (Reproduced from Ref. [37] with permission of American Chemical Society)
In developing new sorbents for SPME, it is desirable to have a porous material with large surface area and high sorption capacity as well as increased selectivity, sensitivity, durability, and reproducibility. Djozan et al. [40] developed a new fiber consisting of copper wire coated with a copper sulfide coating (~ 9 µm) and efficiently extracted benzyl alcohol, benzyl amine, aliphatic amines and aliphatic alcohols directly from aqueous samples without any derivatization process. The new fiber appears to be highly selective for aliphatic amines and alcohols.

Low molecular mass aliphatic amines are important intermediates in chemical and pharmaceutical industries. They may form as biodegradation products of organic materials like proteins, amino acids or other nitrogen-containing compounds and are distributed in environmental water. Low molecular mass aliphatic amines are also important air pollutants due to their unpleasant odor and toxicity. The monitoring of alkylamines is of considerable interest as most of them are toxic, sensitizers and irritants to the skin, mucous membrane, and respiratory tract. Analysis of aliphatic amines in aqueous media is very difficult because of their high polarity. Preconcentration and derivatization before chromatographic analysis is a common practice in amine analysis. Djozan et al. [41] developed a modified copper wire (coated with a thin layer of microcrystalline CuCl) as an SPME fiber which showed high selectivity towards aliphatic amines. CuCl is very reactive because it has normally two coordinate bond structures and can accept third and fourth coordination by other ligands such as amino group to form an amino complex according to the reaction: 

\[ \text{CuCl} + n \text{ (amine)} = \text{Cu(amine)}_n\text{Cl} \]

At high temperatures (during desorption in the GC injection port), the amino complex breaks up to release free amine.
Being easy to produce, considerably adsorptive, strong enough to endure harsh operational conditions, and cheap, zinc oxide finds its application in SPME as a novel sorbent. Djozan et al. [40] prepared this sorbent by anodizing zinc wire. This fiber demonstrated good affinity toward polar analytes. Among the sulfur compounds extracted, the zinc fiber seems to have the highest sensitivity for thiophenol probably because of the presence of SH functional group in the analyte and is likely to interact selectively with ZnO coating.

Farajzadeh et al. [38] reported a metallic SMPE fiber coated with cellulose acetate and polyvinyl chloride (PVC). The fiber demonstrated high affinity toward n-alkanes (C_{10}-C_{20}). According to the inventors, this porous fiber possesses high chemical, thermal, and mechanical stability.

2.4.3 Active carbonaceous sorbents

Active carbonaceous sorbents have been proven to be excellent extraction media due to their positive attributes favorable for SPME. They are homogeneous, highly porous, stable at high temperatures, do not retain water and usually they do not manifest irreversible adsorption phenomena.

Farajzadeh et al. [42] have developed a new sorbent material by mixing activated charcoal with PVC powder in different proportions and coated on a metal wire. The resultant coating, as the developer claimed, was very firm and porous. A coating composition of 90:10 activated charcoal: PVC demonstrated high efficiency in extracting light alkanes whereas a coating with higher PVC content (25%) showed promise for a
mixture of both heavy and light alkanes. In a more recent article [43], the same group successfully extracted organophosphorus pesticides from aqueous media but this time a 70:30 activated charcoal: PVC ratio was found to be optimum.

In an attempt to develop a sorbent suitable for extracting volatile organic compounds (VOCs), Djozan *et al.* [44] reported a porous layer activated charcoal (PLAC) coated (~100 µm thickness) fiber. The new material was used to extract BTEX from aqueous media and showed significant sensitivity enhancement (at least 2 orders of magnitude) in comparison with the reported values for commercial PDMS fiber. Moreover, it provided excellent thermal stability (320 °C). In an earlier article [45], the same group utilized porous layer activated charcoal (PLAC) coated (55 µm coating thickness) fiber to extract polycyclic aromatic hydrocarbons. For this fiber, the authors had reported a significantly higher temperature (350 °C) for thermal desorption of the extracted analytes.

A fused silica fiber coated with graphitized carbon black (GCB) was reported by Mangani *et al.* [46]. The developers of this coating material found it to be suitable for extracting VOCs from different matrices.

Jia *et al.* [47] reported the use of active carbon fiber (ACF) as a sorbent in SPME. An ACF fiber was coated with a unique extraction phase possessing characteristic pore size distribution, micro pore surface area, surface chemical structure, and so on. It was claimed to be an excellent adsorbent for the removal of SO$_2$ and NO$_x$ in flue gas from coal combustion. The specific surface area and adsorption capacity was dependent on the pre-treatment and activating conditions. Two different activation processes (chemical and water vapor) were used. Water vapor activated fibers provided higher affinity to polar
compounds. On the other hand, chemically activated fibers showed higher affinity for nonpolar compounds. As the authors predicted, it is possible to fine tune the affinities to different compounds ranging from nonpolar to polar by controlling the activation process and even by combining both the chemical and water activation processes.

Kuo et al. [48] coupled SPME to direct electrospray probe (DEP) and detected trace surfactants from aqueous media. In the SPME-DEP assembly, a 2 cm long 0.3 mm o.d. graphite fiber that acts as the sorbent is inserted into a 1.5 cm long copper coil. A $10^{-9}$ M solution of Triton X-100 was used as a test sample which was successfully detected after being extracted on the graphite fiber by SPME-DEP-MS even though the same solution did not produced any response when only DEP-MS was used.

Glassy carbon, a polycrystalline graphite, has found useful applications as a stationary phase in HPLC and SFC because of its high chemical and mechanical stability as well as large surface area. Aranda et al. [15] utilized glassy carbon rods and pencil lead (both are polycrystalline graphite) as sorbents for SPME. The performances of both the sorbents were assessed by extracting a nonionic surfactant (Triton X-100). These sorbents seem promising when on-fiber derivatization is required prior to detection because the derivatizing chemicals are easily adsorbed on the surface of the sorbent. Tong et al. [49] used the same pencil lead as SPME sorbent and extracted alcohols (e.g., methanol, $t$-butyl alcohol, is amyl alcohol, $n$-propane, etc.) from water. A detection limit of 5 ng/mL was achieved for isoamyl alcohol in GC-FID.
2.4.4 Bonded silica sorbents

Slow analyte diffusion into the extraction media is considered to be a bottleneck phenomenon in SPME process. This problem becomes even more prominent in case of using thick coatings. To achieve high extraction sensitivity, it is desirable to employ thicker coating, however, use of thicker coatings leads to longer extraction time. In order to achieve higher extraction efficiency as well as faster extraction equilibrium, Lee and co-workers [50] have explored the possibility of using silica bonded phase as an SPME sorbent. Porous silica particles provided high specific surface area which led to high extraction sensitivity. A 30 µm thick coating of the bonded silica particles (C₈-, C₁₈-) was immobilized on a metal wire surface using high-temperature epoxy glue and was compared with untreated silica immobilized in the same way. The surface area of the bonded silica coating was found to be 500 times greater than that of the conventional polymer coating in SPME (0.97 x 10⁻² m² vs. 1.41 x 10⁻⁵ m²). As a result, extraction sensitivity was found to increase sharply.
Figure 2.6 Scanning electron micrographs of SPME fibers with: (A) porous layer bonded silica coating and (B) PDMS coating (Reproduced from Ref. [50] with permission of American Chemical Society)
In contrast with the diffusion controlled mass-transfer process involved in sample enrichment on conventional polymer coatings, extraction on bonded silica coatings was found to be driven by the mass transfer in the bulk solution. As a result, extraction primarily depended on the degree of sample agitation, not on the film thickness. In ideal conditions, adsorption equilibrium could be attained in few seconds. Desorption should also be faster. The 30 µm thick bonded silica coatings demonstrated higher extraction efficiency compared to a 100 µm PDMS coating. For instance, C_{8}- bonded phase extracted ~40 ng of toluene from a 0.1 ppm aqueous solution of toluene whereas PDMS coating extracted only ~ 5 ng of toluene under the same conditions. Due to the use of a metal wire as a substrate, fibers of this kind exhibited great mechanical strength. One of the major drawbacks of this fiber is that at elevated temperatures (>300 °C) the epoxy glue starts degrading and thus limiting the applications only up to 250 °C. As a continuing effort, the same group reported the application of bonded silica phase (octyl, phenyl, monomeric and polymeric octadecyl) for extracting polyaromatic hydrocarbons from aqueous solution [51]. Among all the bonded phases investigated, C_{18}- bonded silica phase showed the highest sensitivity toward PAHs.

In order to provide larger sorption capacity and higher sorption rate, Xia et al. [52] proposed a C_{18}-bonded silica-coated multifiber that provided 10 times faster sorption rate compared to commercial 100 µm PDMS fiber. In terms of analyte preconcentration ability, C_{18}-bonded silica coated fiber demonstrated 40 times more extraction sensitivity compared to PDMS coating.

Hou et al. [53] introduced a mesoporous silica material (C_{16}-MCM-41) as an SPME sorbent. The mesoporous material has been characterized by its large surface area (1028
m²g⁻¹) and a comparatively huge pore volume (0.94 cm³g⁻¹). A 100 µm thick coating was immobilized onto a stainless steel wire by epoxy resin glue and was further employed for extracting aromatic hydrocarbons. Results indicated that the new material had very high extraction efficiency and good selectivity. Compared to traditional silica-based fiber, high mechanical strength, and good chemical and thermal stability were reported to be advantageous features of this material that could potentially become a popular SPME sorbent.

2.4.5 Flat sheet membranes

In order to increase the extraction efficiency and detection limit without sacrificing analysis time, a new format of SPME named thin-film microextraction has been introduced [54]. In this new format, instead of using a polymer coated fiber, a thin sheet (1 cm x 1 cm x 0.0025 cm) of poly(dimethylsiloxane) membrane was used as the extraction phase. Having very high surface area (200 mm²) compared to 100 µm thick coated fiber (10 mm³), this membrane has extracted 20 times more PAHs compared to 100 µm thick PDMS coated fiber. The benefit of membrane SPME is not only the enhancement in sorbent surface area but also in the sorbent volume (2.55 mm³ vs 0.061 mm³, the volume of 100 µm PDMS coating). Both the factors lead to enhanced sensitivity without compromising analysis time. Figure 2.7 illustrates a typical thin-film microextraction process.
Figure 2.7 Drawing of the headspace membrane SPME system. 1. Deactivated stainless steel rod. 2. Flat sheet membrane. 3. Sample solution. 4. Teflon-coated stir bar. 5. Rolled membrane. 6. Injector nut. 7. Rolled membrane. 8. Glass liner. 9. Capillary column

(Reproduced from Ref. [54] with permission of American Chemical Society)
Merschman et al. [55] used a rectangular sorbent film (PDMS) to preconcentrate organic analytes from aqueous media. They successfully interfaced membrane SPME with infrared spectroscopy and determined the extracted analytes were analyzed by IR.

### 2.4.6 Miscellaneous sorbents

Although SPME has been introduced primarily for the preconcentration and analysis of organic compounds, it can be easily applied to the broad field of metal ion analysis by simply modifying the sorbent. Otu et al. [56] reported the solid phase microextraction of metal ions by modifying PDMS coating. A liquid ion-exchanger, di-(2-ethylhexyl)phosphoric acid (HDEHP) was used for the modification. The modified PDMS coated fiber was used to extract bismuth (III) ion from an aqueous sample.

Gorecki et al. [57] introduced Nafion® perfluorinated resin as a coating on SPME fiber for the analysis of polar compounds in liquid matrices. Results showed that compared to commercially available fibers, Nafion® performed better in extracting small alcohols. Surprisingly, Nafion® demonstrated highest affinity toward methanol, the most polar compounds among the tested materials. Moreover, Nafion® coating successfully extracted trace amount of water from organic solvents which might solve a big challenge that analytical chemists are still coping these days.

Norlin et al. [58] also used Nafion® coated fiber for nitrogen isotopic analysis of ammonium in aqueous solutions.

Jia et al. [59] designed a special SPME device that after doping with crown ether would extract metal ions from aqueous solution. He used a piece of fused silica rod,
eccentrically glued to a hydrophobic hollow-fiber membrane and then doped the membrane with dibenzo-18-crown-6 (DBC). The SPME device doped with DBC was used for extracting Hg (II) ions that were subsequently analyzed by HPLC-UV. A detection limit of 500 ppb has been estimated, although UV detector is characterized by low sensitivity.

Li et al. [60] used plasticized poly(vinylchloride) as an SPME sorbent coated on a primed steel rod housed in a syringe-like SPME device. The device was used to extract barbiturates from urine and bovine serum samples and was analyzed by CE. For urine and serum samples, the concentration ranges that could be extracted and analyzed by the method were 0.1-0.3 ppm ~1 ppm, respectively.

To detect trace levels of chemical warfare agents, Harvey et al. [61] developed a novel SPME coating BSP3 on SPME fiber consisting of hydrogen-bond acidic hexafluorobisphenol groups alternating with oligo(dimethylsiloxane) segments. The new coating demonstrated remarkable affinity toward sarin, a nerve gas. In comparison to commercial PDMS fiber, the BSP3 coated fiber showed 22-fold higher affinity towards sarin.

Fullerene has long been known as a chromatographic material for its high thermal stability (~360 °C), and good selectivity towards aromatic compounds. C. Xiao et al.[62] developed a polysilicone fullerene (PF) coating (33 µm) for SPME. They compared the efficiency of two coatings, one made of pure PF and the other made of a mixture of PDMS and PF (4:1 ratio). Coating obtained merely from PF showed better sensitivity toward the test analytes BTEX and PAHs, rather than the mixed one. A detection limit of 0.04µg.L⁻¹ was obtained for naphthalene using GC-FID.
2.5 Tailor-made coatings for in-tube SPME

Tailor-made in-tube SPME coatings can be further subdivided into two types depending on the exclusivity of their use in in-tube SPME: (1) tailor-made coatings used predominantly in in-tube SPME, and (2) tailor-made SPME coatings developed for exclusive use in in-tube SPME.

2.5.1 Tailor-made coatings used predominantly in in-tube SPME

Several tailor made SPME sorbents have found successful use in both fiber- and in-tube SPME although their use in the in-tube format predominated. Major classes in this category are: molecularly imprinted polymers (MIPs), restricted access materials (RAMs), conductive polymers and miscellaneous coatings. Table 2.4 presents a list of coatings used in the in-tube SPME format.
Table 2.4 Tailor-made sorbents used in in-tube SPME

<table>
<thead>
<tr>
<th>Sorbents</th>
<th>Application</th>
<th>Analytical Instrument</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkyldiol Silica</td>
<td>Benzodiazepines from human serum</td>
<td>HPLC-UV</td>
<td>[70]</td>
</tr>
<tr>
<td>Restricted Access</td>
<td>Propranolol</td>
<td>HPLC-UV</td>
<td>[64]</td>
</tr>
<tr>
<td>Material</td>
<td>Amides, alkanes, PAHs, chlorinated solvents</td>
<td>GC-FID</td>
<td>[98]</td>
</tr>
<tr>
<td>Molecularly Imprinted</td>
<td>BTEx</td>
<td>GC-FID</td>
<td>[101]</td>
</tr>
<tr>
<td>Polymer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDMS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polypyrrole</td>
<td>Polar pesticides</td>
<td>HPLC-ESI-MS</td>
<td>[90]</td>
</tr>
<tr>
<td></td>
<td>N-Nitrosoamines</td>
<td>HPLC-UV</td>
<td>[92]</td>
</tr>
<tr>
<td></td>
<td>Verapamil drug</td>
<td>LC-MS</td>
<td>[91]</td>
</tr>
<tr>
<td></td>
<td>In Vivo pharmacokinetic studies</td>
<td>LC-tandem MS</td>
<td>[93]</td>
</tr>
<tr>
<td></td>
<td>Aromatic compounds</td>
<td>HPLC-UV</td>
<td>[87]</td>
</tr>
<tr>
<td></td>
<td>Inorganic anions</td>
<td>IC</td>
<td>[83]</td>
</tr>
<tr>
<td></td>
<td>Organarsenic compounds</td>
<td>LC-ESI-MS</td>
<td>[82]</td>
</tr>
<tr>
<td></td>
<td>B-Blockers in urine and serum samples</td>
<td>LC-ESI-MS</td>
<td>[85]</td>
</tr>
<tr>
<td></td>
<td>Catechins, caffeine in tea</td>
<td>HPLC-ESI-MS</td>
<td>[86]</td>
</tr>
<tr>
<td></td>
<td>Stimulants in human urine and hair</td>
<td>LC-ESI-MS</td>
<td>[89]</td>
</tr>
<tr>
<td></td>
<td>Tributyltin</td>
<td>HPLC-ESI-MS</td>
<td>[85]</td>
</tr>
<tr>
<td></td>
<td>Aromatics, PAHs, aromatic amines,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>organoarsenic compounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly-N-phenylpyrrole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon deposited inside</td>
<td>VOCs</td>
<td>GC</td>
<td>[102]</td>
</tr>
<tr>
<td>capillary</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zylon fiber packed</td>
<td>Phthalate</td>
<td>HPLC-UV</td>
<td>[30]</td>
</tr>
<tr>
<td>capillary</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.5.1.1 Molecularly imprinted polymers (MIPs)

Although natural antibodies have long been used in molecular recognition but their high price, low availability, and short lifetime have led researchers to synthesize antibody mimics such as molecularly imprinted polymers (MIPs). Molecularly imprinted polymers are crosslinked macromolecules with specific binding sites for a particular analyte. In order to obtain a highly selective recognition of a certain molecule, this template molecule is incorporated in the mixture of reacting monomers during synthesis of the molecularly imprinted polymer network. After completion of the synthesis, the template molecule is extracted out leaving in the polymer a three dimensional imprint of itself. Figure 2.8 illustrates different steps involved in molecular imprinting process [63]. Mullett et al. [64] reported the application of a molecularly imprinted polymer in SPME for selective extraction of propranolol (a β-blocker compound) from biological fluids. The propranolol-imprinted polymer particles were packed in a 80-mm long PEEK tubing (1.59-mm o.d. x 0.76-mm i.d.) and both the ends were capped with a zero volume union fitted with 2-μm frit. An autosampler was used to extract target analyte and the extracted analyte were analyzed by HPLC-UV. A detection limit of 0.32 μg/mL in spiked serum sample with excellent reproducibility (RSD, <5%) was achieved in in-tube SPME using MIP-based sorbent.
**Figure 2.8** Different steps involved in MIP synthesis (Reproduced from Ref. [63] with permission of Elsevier)
The same research group [65,66] has also reported online sample preparation of verapamil and its metabolites by an MIP material coupled on-line to a restricted access material (RAM) precolumn. The RAM precolumn helped in removing bulky matrix interferences (e.g., proteins). On the other hand, MIP provided selective extraction of verapamil from the relatively clean matrix obtained after the RAM precolumn.

Verapamil-imprinted polymers were packed in a 40 mm stainless steel column (4 mm i.d.) and capped with a 2 µm frit. A detection limit of 5 ng/mL was obtained in LC-MS analysis. Another significant contribution in MIP-SPME has been reported by Koster et al. [67]. A silica fiber was coated with a 75-µm thick methacrylate polymer imprinted with clenbuterol. To compare the viability of the imprinting process, another fiber was coated with the same thickness of methacrylate but this time no imprinting was used. Experimental results indicated that the fiber with the imprinted polymer coating successfully extracted clenbuterol and its structural analogues with 75 % extraction yield, whereas nonimprinted fiber extracted only with ~5% extraction yield which clearly demonstrates the enhanced selectivity offered by an imprinted polymer in SPME. The extractability of different structural analogs of clenbuterol was also investigated. It was found that compounds having almost same spatial arrangement (e.g., bromobuterol) were extracted with higher extraction yield compared to compounds having structural differences.
2.5.1.2 Restricted access materials (RAMs)

Restricted Access Materials (RAM) were originally developed for the isolation of low molecular mass drugs from biological fluids with minimum sample treatment. Recently, it has found application in the isolation of herbicides from surface water containing high levels of humic substances [68]. Restricted access materials prevent access of macromolecules (interferences) to the specific sorbent region where analyte retention occurs.

Mullett et al. [69] introduced a biocompatible SPME fiber coated with alkyl-diol-silica (ADS) restricted access material that successfully extracted several benzodiazepines in presence of protein. The aim was to minimize the sample preparation time by eliminating protein precipitation step, and therefore, reduce the probability of sample contamination and analyte loss. The sorbent alkyl-diol-silica (ADS) possesses two different chemical surfaces (diol groups on the outer surface, and alkyl groups on the inner surface) and a pore size that prevents larger molecules (e.g., proteins) from entering into the inner surface. Hydrophilic electroneutral diol groups on the outer surface of the spherical ADS particles acts like a filter to trap bulky molecules e.g., proteins, whereas hydrophobic alkyl groups extract relatively smaller target analytes that easily penetrates the outer ADS surface. The ADS-RAM fractionated the protein present in the matrix by preventing its access to the adsorption sites, thereby allowing the low molecular mass analytes to be easily extracted and enriched into the interior of the sorbent. Calibration curve constructed for five benzodiazepines over a range 0.5-50 µg/mL demonstrated excellent linearity. Detection limits ranging from 46 to 750 ng/mL for different
benzodiazepines have been achieved in HPLC-UV.

Using the same coating, Mullett et al. [70] prepared a highly bio-compatible SPME capillary for the automated and direct in-tube extraction of several benzodiazepines from human serum. In preparing the SPME capillary, a 50 mm PEEK tubing (1.59 mm o.d. X 0.76 mm i.d.) was used and the particles were slurry packed into the capillary. After extracting several benzodiazepines from serum samples and analyzing by HPLC-UV a detection limit of 22-29 ng/mL obtained for different benzodiazepines.

Recently, the same group utilized a RAM with ion-exchange capability for monitoring drugs and metabolites in a whole blood sample [71]. A 70-mm piece of steel wire was used as the substrate on the surface of which ADS particles were glued. Figure 2.8 presents an image of the ADS particles immobilized on a SPME fiber.
Figure 2.9 SEM image of ADS particles immobilized on a fused silica fiber

(Reproduced from Ref. [71] with permission of Elsevier)
The extracted drugs and metabolites were then analyzed by LC-MS. An extensive review on biocompatible coatings (antibodies, MIPs, RAMs) for SPME by Mullett et al. [72] would provide the readers with further details for a better understanding on the topic.

2.5.1.3 Conductive polymers

A major setback SPME had experienced in its first decade was the absence of coatings suitable for extraction of ionic analytes. Because of their neutral charge, conventional commercial coatings are often unsuitable for extracting charged species. Chemical modification of the analytes by derivatization or by the addition of complexing agents (e.g., crown ethers) are the frequently used procedure to increase extraction efficiency for ionic species [73]. However, these reactions are time consuming and require expensive and toxic reagents. Besides, completeness of the derivatization reaction in dilute samples can be problematic. In an attempt to solve these problems, conducting polymers have been explored by several research groups. Conducting polymers are versatile materials that possess many positive attributes, and can be efficiently exploited for the advancement of separation science. So far, several conductive polymers have been reported as SPME sorbents among which poly-3-methylthiophene, polypyrrole and poly-N-phenylpyrrole are noteworthy. The electroactivity and reversible redox properties of these conductive polymers have made them suitable extracting phases in electrochemically controlled delivery devices and separation systems for charged species [74]. The extraction of ionic species is realized by
applying a positive/negative potential for a predetermined time. After extraction, the extracted ions are released in a small volume of 0.1 M NaCl solution by applying a negative/positive potential pulse. Table 2.5 lists coatings used in electrochemically controlled SPME.

Table 2.5 Coating used in electrochemically controlled SPME

<table>
<thead>
<tr>
<th>Sorbents</th>
<th>Application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polypyrrole</td>
<td>Aroma in wines</td>
<td>[94]</td>
</tr>
<tr>
<td></td>
<td>Chloride solution</td>
<td>[77]</td>
</tr>
<tr>
<td></td>
<td>Anions, cation</td>
<td>[78]</td>
</tr>
<tr>
<td></td>
<td>Ionic analytes</td>
<td>[79]</td>
</tr>
<tr>
<td>Poly(3-dodecylthiophene) P3DDT</td>
<td>Organometallic</td>
<td>[76]</td>
</tr>
<tr>
<td>Poly(pyrrole-sulfated β-cyclodextrin)</td>
<td>arsenobetaine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cationic analytes</td>
<td>[75]</td>
</tr>
</tbody>
</table>
T.P. Gbatu et al. [74] reported poly(3-methylthiophene) as SPME sorbent to extract ionic arsenic compounds from aqueous media without derivatization. A ∼5µm thick coating of poly(3-methylthiophene) was electrochemically deposited onto a platinum fiber. During the extraction, a positive charge was maintained on the fiber by applying an electrode potential (+ 1.2 V). At the time of desorption, the polymer was converted back to its neutral state by reversing the potential (-0.6 V) and extracted arsenate ions were expelled into a smaller volume of deionized water. Finally the ionic samples were analyzed by ICP-MS.

Temsamani et al. [75] investigated the cation uptake and release properties of a poly(pyrrrole-sulfated β-cyclodextrin) film electrode for prospective application in electrochemically aided solid phase microextraction. The experimental data revealed that by changing electrode potential, cations like K⁺, Na⁺ can be extracted and desorbed efficiently from the electrode. The same group [76] utilized poly(3-dodecylthiophene) for the preconcentration of organometallic arsenobetaine (AsB) from aqueous media. Both adsorption and desorption were done electrochemically. Extraction equilibrium was achieved within a minute. This result shows good prospect in extraction and analysis of other organometallics that still represents challenge for researchers.

Liljegren et al. [77] used microband array gold electrodes coated with polypyrrole to preconcentrate and detect chloride ions in a capillary based flow system. The results indicate that polypyrrole coated electrodes can be effectively used in miniaturized analytical flow system for analyte preconcentration. The same group [78] also described a method for the extraction, transfer, and desorption of anions and cations under controlled potential using an integrated three-electrode device which included a glassy
carbon rod, a silver tube and a stainless steel tube with polypyrrole coating. The results demonstrated that by changing the dopant ion (perchlorate for anion extraction and p-toluenesulfonic acid for cation extraction) during the electropolymerization of polypyrrole on the stainless steel, simultaneous extraction of several anions or cation can be accomplished with high extraction efficiency.

In another report, Wu et al. [79] proposed similar electrochemically controlled SPME. Exploiting conductive nature of polypyrrole film and by applying positive and negative potential, anions and cations can be extracted and thereafter the extracted cations can be desorbed just by reversing the potential.

A salicylate PVC-based membrane (as SPME sorbent) was coupled to coated graphite membrane electrodes by Ganjali et al. [80] to facilitate selective determination of trace amount of salicylate from biological sample. The new sensor could determine as low as 0.01nM salicylate ion from serum sample compared to the best reported sensor with a minimum detectable concentration limit of 0.1 nM.

Another important contribution was the introduction of polypyrrole SPME-coatings by Pawliszyn and co-workers [81]. Among various conducting polymers studied, polypyrrole and its derivatives have drawn much attention due to its commercial availability, ability to form stable polymeric films by chemical or electrochemical means on various substrate materials ranging from metals and nonmetals. Due to the inherent multifunctional properties of polypyrrole coatings (e.g., π-π interactions, acid-base interactions, hydrogen bonding, dipole-dipole interactions from polar functional groups, hydrophobic interactions between the sorbent and the analytes), they can be used for extracting a wide array of analytes. One important aspect of this polymer is that its
selectivity can easily be tuned by introducing additional functional groups into the polymer structure. Polypyrrole and its derivatives can be easily coated on the surface of metal wires by electrochemical deposition as well as the inner walls of fused silica capillaries via chemical polymerization. As a result, polypyrrole and its derivatives have been used as SPME sorbents in both fiber and in-tube format. Polypyrrole and its derivative poly-N-phenylpyrrole were introduced as SPME sorbents in 1999 by Pawliszyn and his co-workers where polypyrrole (PPY) and poly-N-phenylpyrrole (PPPY) were electrochemically coated on the surface of metal wires and utilized for extracting volatile organic compounds from aqueous solutions [81]. A detail accounts on the preparation of PPY and PPPY film on metal fibers (e.g., Pt, Au, stainless steel) as well as the coating inside the capillary was presented by Wu et al. [82]. This article also highlighted a wide range of applications of the PPY film and coating which included polar and nonpolar aromatics, polycyclic aromatic hydrocarbons, aromatic amines, organoarsenic compounds. Figure 2.9 presents SEM images of PPY and PPPY coated surface on a metal wire. Wu et al. [83] utilized polypyrrole-coated capillary for speciation of organoarsenic compounds and coupled in-tube SPME with liquid chromatography/electrospray ionization mass spectrometry. For comparison, the same extraction was carried out in four commercial capillaries (Supel-Q, Omegawax, SPB-1, SPB-5) and an uncoated fused silica capillary. As was expected, the PPY coated capillary provided the best extraction efficiency. The main driving forces for extraction for the above mentioned commercial coatings are hydrophobic interactions and the interactions from polar functional groups. On the other hand, the driving forces for extraction on PPY coating are not limited by only hydrophobic and functional groups interaction, but
they also include electrostatic interactions that occur between the charged analytes and the positively charged PPY coating.
Figure 2.10 Scanning electron micrographs of (A) Uncoated metal surface, (B) PPY-coated metal surface, (C) PPPY-coated metal surface (Reproduced from Ref. [83] with permission of Elsevier)
In a different publication [84], the same group reported the extraction of various anions (e.g., fluoride, chloride, nitrite, phosphate, sulfate, selenite, selenate, arsenate) from aqueous samples and analyzed them by ion chromatography using polypyrrole coated capillaries. Although polypyrrole seems to be a good extraction medium for ionic compounds, but a closer look at the polymeric structure reveals that every three or four polypyrrole units contain one positive charge that limits the extraction capacity of the polymer. That problem, however, can be easily minimized by incorporating cationic substituents into the polypyrrole skeleton. In a different report Wu et al. [85] employed polypyrrole coated capillary for speciation and determination of tributyltin from aqueous media. Analysis was done by HPLC-ES-MS. PPY is not only an efficient extraction medium for ionic analytes, it also efficiently extracts neutral analytes by utilizing its diverse modes of molecular level interactions. As a result, polypyrrole coating has found application in the extraction of a wide range of neutral analytes including β-blockers in urine and serum samples [86], catechin and caffeine in tea [87], aromatic compounds in aqueous samples [88], stimulants in human urine and hair samples [89], polar pesticides in water and wine samples [90], verapamil drug and its metabolites [91], and N-nitrosamines in cell cultures [92]. Recently a polypyrrole coated SPME probe has been introduced by Lord et al. [93] for in vivo pharmacokinetic studies in living animals. The same group has also combined SPME with surface enhanced laser desorption/ionization and ion mobility mass spectrometry (SPME-SELDI-IMS) using a polypyrrole coated optical fiber [94]. The tip of the fiber acts as the sorbent as well as the transmission medium for the UV laser light.

Guadarrama et al. [95] invented an SPME device consisting of a set of 12
polymeric sensors coated with polypyrrole, poly-3-methylthiophene and polyanilene. The device served as an artificial olfactory system to identify wine aroma. The device provided a noticeable difference in response toward different wines which can be achieved by an automatic headspace sampler.

2.5.2 Coatings exclusively used in in-tube SPME

In order to be able to facilitate effective sample introduction into an analytical instrument, SPME warrants that extracted analytes be desorbed as fast as possible. A delay in analysis may result in analyte loss. In an attempt to address this problem, Nardi [96] proposed an SPME sampler for long-term storage. A home-made 15-cm long 0.16 mm i.d. capillary was coated with PS255 (polydimethylsiloxane with ~1% vinyl group) with thickness of 0.5 µm. A press-fit cap was used to seal the capillary after the extraction. Even after storing for 30 days, no distinguishable loss was observed for the extracted analytes (BTEX). This extractor seems promising for samples for which immediate analysis after the extraction is not possible. Figure 2.10 shows a set of chromatograms illustrating BTEX analysis obtained after storing the extracted analytes in long-term storage assay for different period of time.
Figure 2.11 Impact of storage on extracted sample using SPME sampler for long-term storage (Reproduced from Ref. [96] with permission of Elsevier)
One of the major problems of in-tube SPME hyphenated to GC is ensuring quantitative transfer of the extracted analyte into the GC injection port. This can be done by using a small solvent plug that transfer the analyte(s) from the extraction capillary to the GC injection port [97]. L. Nardi proposed a new way to couple in-tube SPME with GC [98] that does not require a heated GC injector. He used a 9 cm long 0.25 mm i.d. capillary with 0.22 μm thick immobilized coating of PS255 stationary phase (PDMS gum phase with 1% vinyl groups) for the extraction. High-quality press-fits were made on both the ends of the extractor using a sharpened tungsten tool and an alcohol flame. Extraction was done using a capillary extraction tool comprised of a 1 mL sampling syringe, a sampling transfer-line ending with a 0.32 mm i.d. fused silica capillary and capillary extractors. One of many advantages of the proposed system is its very fast sampling step, normally a few seconds. After the extraction, the extractor was coupled to carrier gas line and the GC column using the press-fits located on both the ends of the extractor. GC elution can be performed keeping the GC oven door open or by temperature programming, if needed. Besides, the extractor can be press-fit capped with the extracted analyte(s) inside and stored for months without loosing even volatiles. The system has been proved to be effective for low volatiles (e.g., PAHs) as well as volatiles (e.g., chlorinated solvents, alkanes, amides). The newly developed in-tube SPME-HRGC is recommended especially for high-throughput analysis when detection sensitivity is not the prime objective. Similar capillary extractors were also used for negligible depletion sampling of BTEX [99], for determining partition coefficients of BTEX between cross linked polydimethylsiloxane and water [100], analysis of aromatic compounds in water [101].
McComb et al. [102] invented an INCAT (inside the needle capillary adsorption trap) device in which a colloidal graphite coating inside a steel capillary tubing is used as a sorbent. The device demonstrated some promise for VOC analysis from air as well as aqueous media.

Musshoff et al. [103] reported a fully automated headspace solid-phase dynamic extraction (HS-SPDE) followed by on-coating derivatization and gas chromatography-mass spectrometry (GC-MS). SPDE is considered to be a new generation solid-phase microextraction which uses an inside needle capillary absorption trap coated with polydimethylsiloxane. It is the first commercially available inside-needle device for headspace analysis using GC-MS. An 8-cm long stainless steel needle coated with a 50 µm film of PDMS and 10% activated carbon is used as the needle trap. The volume of the extracting phase in SPDE needle (4.40 mm³) was significantly larger than that of 100 µm PDMS SPME fiber (0.94 mm³). Apart from its advantage of higher coating volume, its robustness due to its physical construction has made SPDE very promising. Higher sample capacity and longer life-span are two major attributes of SPDE. The new device was successfully applied for the determination of cannabinoids in hair samples, pesticides in water [104], and synthetic designer drug in hair [105]. Figure 2.11 demonstrates the principle of the SPDE extraction process in extracting amphetamines and designer drugs from hair samples.
Figure 2.12 Fully automated solid-phase dynamic extraction (SPDE) process

(Reproduced from Ref. [105] with permission of Elsevier)
Koziel et al. [106] reported a needle trap device (NTD) that was used for the sampling and analysis of aerosols and airborne particulate matter from an inhaler-administered drug, spray insect repellent, and tailpipe diesel exhaust. The needle trap device was fabricated using a 40 mm long piece of stainless steel needle (0.53 mm o.d.) with 5 mm of quartz wool packing section near the tip of the needle. The quartz wool packing performs as the extracting phase. Figure 2.12 represents the schematic of a needle trap device.
Figure 2.13 Schematic representation of a needle trap device (Reproduced from Ref. [106] with permission of American Chemical Society)
2.6 References for Chapters Two


[6] Application Note 17 Supelco, Bellefonte, PA, USA.


Chapter Three

Sol-Ge Technology in Capillary Microextraction

3.1 A brief history

Sol-gel technology offers a simple but versatile approach to the synthesis of inorganic polymers and hybrid inorganic-organic materials. The history of sol-gel technology dates back to mid-1800s with Ebelmen’s [1] and Graham’s [2] studies on silica gel. Both the investigators observed that the hydrolysis of tetraethoxysilane (TEOS) under acidic conditions produced SiO$_2$ in the form of “glass-like material”. Unfortunately, extremely long drying time (one year or more) required to prevent the fracture of silica gel led to diminished the enthusiasm of the researchers and little technological development was observed in the next couple of decades.

A period between late 1800s and 1920s, however, had witnessed a surge of interest in the sol-gel process. During this time many famous scientists, including Ostwald [3] and Lord Rayleigh [4] investigated the problem of periodic precipitation phenomenon associated with the formation of Liesegang rings [5] and the growth of crystals from gels.

During the period 1950s and 1960s Roy and coworkers [6,7] succeeded in using sol-gel methods for synthesizing a large number of novel ceramic oxide compositions involving Al, Si, Ti, Zr, etc., that was impossible to obtain using ceramic powder
methods. Another important breakthrough during this period was Iler’s pioneering work on silica chemistry [8] which led to the commercial development of colloidal silica powders known as Du Pont’s colloidal Ludox spheres. Stober et al. [9] reported in 1968 that both the morphology and size of the powders could be controlled using ammonia as a catalyst for the TEOS hydrolysis reaction.

The First International Workshop on “Glasses and Glass Ceramics from Gel” was held in Padova, Italy, in 1981 and is considered to be the beginning of the present era of sol-gel science and technology [10]. Since then, there have been nine other such Workshops held biannually at different locations throughout the world. With ever increasing interest in sol-gel science and technology, last two and a half decades alone have witnessed approximately 76,000 publications. The huge surge of sol-gel articles prompted the birth of a new scientific journal “Journal of Sol-Gel Science and Technology” in 1993 which successfully brought all sol-gel scientists on a single platform.

J.D. Mackenzie [11] has classified the achievements in sol-gel chemistry during last two and a half decades (1980-2005) into two broader classes: (1) first generation of the sol-gel process and (2) second generation of the sol-gel process. The first generation sol-gel process involved in better understanding of the structure and physical chemistry of the “original-type” of liquid solution (mixture of alcohol, alkoxide, water, catalyst) which resulted in oxide gels.

Most notable advancement during this time period was the pioneering work of H. Schmidt who successfully incorporated organic material into inorganic network by sol-gel process [12] which according to J.D. Mackenzie is regarded as the beginning of
second generation. Schmidt’s invention opened up a new possibility of creating hybrid organic-inorganic materials using a very simple sol-gel methodology. The new material system was named as “Ormosils” and later renamed as “Ormocers” by the inventor himself. The credit for using organic-inorganic hybrid material as the stationary phase for electrochromatography goes to Guo and Colon [13]. Malik and co-workers introduced sol-gel column technology for capillary GC [14]. This research effort on sol-gel column technology was so well accepted among the scientific community that the article titled “Sol-gel Column Technology for High Resolution Gas Chromatography” was featured on the front cover of Analytical Chemistry (Vol. 69, No. 22, pp. 4566-4576, 1997). Since Colon and Malik’s pioneering work, a large number of research groups all over the world have been involved in developing new sol-gel hybrid organic-inorganic materials system in the area of sample preconcentration and microseparation.

Among many scientists working in the field of chromatography, Tanaka et al. made a significant contribution by developing sol-gel monolithic beds and used them as an HPLC column, [15-17]. The monolithic columns with small-sized skeletons and large through-pores can reduce the diffusion path length and flow resistance compared to a particle-packed column (most commonly used in HPLC) and thereby provide both high permeability and high column efficiency in HPLC.

3.2 Sol-gel technology for SPME

Sol-gel coating technology for the preparation of solid-phase microextraction fibers was developed by Malik and co-workers in 1997 [18]. The new coating technology
allowed the incorporation of organic polymeric material into inorganic polymeric network and chemical bonding of the created hybrid organic-inorganic sol-gel to the external surface of a fused silica fiber. Because of the chemical bonding to the fiber, such hybrid coatings are characterized by high thermal and chemical stability. Sol-gel approach offers the opportunity to create these advanced materials under mild reaction conditions (most often at room temperature). The technology is very simple, easy-to-follow, single-step and above all does not involve costly materials or reagents. One or more inorganic precursor(s), a sol-gel-active organic component, a sol-gel catalyst, water, and solvent(s) are the raw materials involved in the preparation of sol-gel hybrid organic-inorganic composite material. By changing the relative ratio of organic/inorganic materials and by adding one or more surface deactivating reagents and the polarity of the material can be controlled to some extent providing sol-gel chemists the flexibility in fine tuning the selectivity of such hybrid materials. Furthermore, the new technology permits the in situ creation of hybrid coatings on the outer surface of a fused silica fiber as well as on the inner walls of fused silica capillary. It can also be employed for the creation of a surface-bonded monolithic bed within a capillary using a properly designed sol solution. The thickness of the coating can also be conveniently controlled using this technology. Positive attributes acquired from the integration of both organic and inorganic phases as well as the intrinsic ability of these organic-inorganic materials to chemically bind to the surface of the substrate have provided the hybrid sol-gel materials with enhanced selectivity, high thermal and solvent stability, and long life span.
3.3 Sol-gel sorbents in SPME: a brief overview

An ideal sample preparation technique should be simple, inexpensive, efficient, easy to use, fast, solvent-free and compatible with a wide range of analytical separation instruments. Since solid phase microextraction (SPME) conforms to most of these criteria, it has gained enormous popularity among scientists and researchers shortly after its introduction in 1989 by Pawliszyn and co-workers.

In solid phase microextraction, sorbents/coatings play the most important role in the extraction process. Typically, a sort segment of bare fused silica fiber is coated with a polymeric material. Conventional sorbents, being merely physically bonded to the fused silica fiber surface, inherently possess low thermal and solvent stability.

In solid phase microextraction, the thickness of the sorbents is another most important factor that determines the volume of the loaded sorbent, and consequently, the amount of analyte(s) that can be extracted by it. As a result, reasonably high extraction sensitivity for an analyte can only be obtained when the sorbent has high affinity for the analyte and the sorbent coating thickness is fairly high.

Sol-gel sorbents used in SPME/ CME can be grouped into different types. Scheme 3.1 presents such a classification.
**Figure 3.1** Classification of sol-gel sorbents used in fiber-SPME/in-tube SPME (CME)
3.3.1 Polysiloxane-based sol-gel sorbents

Chemical inertness, low vapor pressure, high thermal and oxidative stability, high maximum and low minimum operating temperatures, low glass transition temperatures, low surface energies, easiness of film formation, high solubilizing power, good selectivity and tunable polarity have made polysiloxanes the most popular as well as the most commonly used sorbents in all formats of solid phase microextraction [19]. Polysiloxanes with silanol groups in their structures are sol-gel active and can participate in sol-gel reactions.

In polysiloxane-based SPME sorbents, different functional pendant groups are chemically bonded to the polysiloxane backbones. Among the most commonly used pendant groups are methyl, and phenyl groups.

3.3.1.1 Sol-gel sorbents with homogeneous polysiloxane phases

In this category of SPME sorbents, hydroxy terminated PDMS (certain types of silicone oils) have served as organic components in the prepared sol-gel extraction media. Different inorganic precursors (alkoxides of Si, Ti, and Zr) have been used for successful chemical immobilization of these organic moieties onto the surface of the substrate (outer surface of a fused silica fiber (fiber-SPME) or inner surface of a fused silica capillary (intube SPME or CME). The sol-gel reactions inherently provide molecular level homogeneity in the composition of the prepared coating and ensure its chemical bonding to the fused silica glass surface. Table 3.1 lists different homogeneous polysiloxane...
phases used as SPME sorbents.

### Table 3.1 Homogeneous polysiloxane sorbents

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Precursor</th>
<th>Organic component</th>
<th>Thermal/ Solvent stability</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sol-gel PDMS</td>
<td>MTMOS</td>
<td><img src="CH3(CH3)3Si-O-Si(CH3)3" alt="Chemical structure" /></td>
<td>320 °C</td>
<td>[18]</td>
</tr>
<tr>
<td>Sol-gel PDMS</td>
<td>MTMOS</td>
<td><img src="CH3(CH3)3Si-O-Si(CH3)3" alt="Chemical structure" /></td>
<td>300 °C</td>
<td>[21]</td>
</tr>
<tr>
<td>Sol-gel PDMS</td>
<td>MTMOS</td>
<td><img src="CH3(CH3)3Si-O-Si(CH3)3" alt="Chemical structure" /></td>
<td>350 °C</td>
<td>[22]</td>
</tr>
<tr>
<td>Sol-gel Titania PDMS</td>
<td>Titanium i-propoxide</td>
<td><img src="CH3(CH3)3Si-O-Si(CH3)3" alt="Chemical structure" /></td>
<td>Acetonitrile/water</td>
<td>[25]</td>
</tr>
<tr>
<td>Sol-gel Zirconia PDMS</td>
<td>Zirconia Butoxide</td>
<td><img src="CH3(CH3)3Si-O-Si(CH3)3" alt="Chemical structure" /></td>
<td>320 °C</td>
<td>[26]</td>
</tr>
<tr>
<td>Sol-gel poly phenylmethylvinyl siloxane</td>
<td>TEOS, VTEOS</td>
<td><img src="CH3(CH3)3Si-O-Si(CH3)3" alt="Chemical structure" /></td>
<td>350 °C</td>
<td>[50]</td>
</tr>
<tr>
<td>Sol-gel polyphenylmethyl siloxane/PDMS</td>
<td>TEOS, VTEOS</td>
<td><img src="CH3(CH3)3Si-O-Si(CH3)3" alt="Chemical structure" /></td>
<td>350 °C</td>
<td>[56]</td>
</tr>
</tbody>
</table>
Chong et al. [18] were the first to develop sol-gel coating technology [14] to prepare solid-phase microextraction fibers. Hydroxy-terminated poly(dimethylsiloxane)-the most widely used polymer as SPME sorbent was reacted with sol-gel precursor methyltrimethoxysilane (MTMOS) in presence of a sol-gel catalyst (trifluoroacetic acid containing 5% water) to produce a hybrid organic-inorganic polymeric network chemically bonded to the fused silica fiber surface. Due to the strong chemical bonding to the substrate, the hybrid material provided exceptionally high thermal and solvent stability. Moreover, the porous structure of the sol-gel coating, as is evident from the scanning electron micrograph (Figure 3.2), facilitated efficient mass transfer of analytes between the sorbent and aqueous media. This, in turn, facilitated faster extraction equilibrium compared to conventional thick PDMS coating which may take hours to reach the equilibrium. Due to the porous structure, a sol-gel hybrid organic-inorganic material provides higher surface area, as a result of which, a relatively thin coating is sufficient to achieve an analyte detection limit equals to or even lower than that achieved using conventionally coated sorbents. Another very important feature of sol-gel poly(dimethylsiloxane) coating is its ability to extract both polar and nonpolar analytes from aqueous media. This can be explained by the fact that some of the used hydroxy-terminated poly(dimethylsiloxane) molecules may retain one of their terminal hydroxyl groups (e.g., those bonded to the sol-gel network only with one end) and provide affinity towards polar analytes during extraction.
Figure: 3.2 Scanning electron microscopic image of sol-gel PDMS fiber at 3600-fold magnification (Reproduced from Ref. [18] with permission of American Chemical Society)
Since this feature is absent in conventional PDMS coating, extraction of polar compounds is impractical to achieve on conventional PDMS coated fibers. Figure 3.3 demonstrates the extraction of highly polar alcohols from aqueous solution using sol-gel PDMS coatings. The porous structure of sol-gel PDMS coating in conjunction with its high thermal stability ensures faster and complete desorption of the extracted analytes into the GC injection port and thus eliminates the analyte carryover phenomenon which is a frequent problem with the use of conventional thick PDMS coatings. Chong et al. [18] also presented a simplified way to tune the polarity of sol-gel PDMS coating by adding deactivating reagents e.g., polymethylhydroxysilane (PMHS) and/or trimethylmethoxysilane that can react with OH- present in the polymer or on the sol-gel network and thus tune the polarity of the sorbent.

Although in sol-gel chemistry, tetraalkoxysilanes [e.g., tetraethoxysilane (TEOS), tetramethoxysilane (TMOS), etc.] are the most commonly used sol-gel precursors, Chong et al. [18] utilized methyltrimethoxysilane (MTMOS) that helped prevent formation of cracks [20] particularly in thick coatings during the drying step, if tetraalkoxysilane precursors are used.
Figure 3.3 SPME-GC analysis of aliphatic alcohols (C_{10}^-, C_{12}^-, C_{14}^-, C_{16}^-, C_{18}^-)-using sol-gel PDMS fiber and GC/FID system (Reproduced from Ref. [18] with permission of American Chemical Society)
Zhou et al. [21] in fact repeated the work done by Chong et al. [18] and validated the original work by Chong et al. [18]. Moreover, new compound classes (e.g., BTEX and organochlorine compounds) have been extracted using sol-gel PDMS sorbent (40 µm thickness) and the limits of detection (LOD) were compared with the results obtained on conventional 100 µm thick coatings. As was pointed out by Chong et al. [18], being porous, sol-gel organic-inorganic hybrid material provides enhanced surface area for extraction. As a result, a sol-gel coating of moderate thickness can provide comparable or even lower detection limits which is of prime importance in ultra-trace analysis. As is indicated by the result obtained for BTEX analysis, an order of magnitude lower detection limit has been accomplished by the sol-gel PDMS coating, even though the thickness was two-fifth of the conventional PDMS coating. Another comparison was made with highly polar Polyacrylate coating for extracting organochlorine compounds. The chromatograms presented in the article clearly demonstrated the superiority of sol-gel PDMS coating over PA coating even in extracting highly polar compounds.

Bigham et al. [22] used sol-gel coatings to address several inherent format-related drawbacks of fiber SPME. In conventional SPME fibers, a small piece of fused silica fiber is used as the substrate. A thick layer of coating applied to the outer surface the fiber serves as the extracting sorbent. Due to the absence of any protective coating (e.g., polyimide), this fiber is very fragile. Moreover, as the coating stays on the external surface of the fiber, mechanical damage of the coating due to scraping and syringe needle bending is a frequent phenomenon. Due to the high fragility of fused silica fibers, only a small segment (~ 1 cm) can be used for coating resulting in low stationary phase loading for extraction. As a result, in the fiber format, SPME suffers from low sample capacity
and consequently poor method sensitivity. Additionally, this format is difficult to couple with high-performance liquid chromatography. In an attempt to eliminate these format related problems as well as to couple SPME with HPLC, in-tube SPME has been proposed [23]. It uses a coated fused silica capillary (~ 60 cm) (most commonly a piece of open tubular GC column). Although the new approach helps interfacing SPME with HPLC, other problems still remain to be solved. In the conventional coating process used to make open tubular GC columns, the stationary phase inside the fused silica capillary is physically immobilized on the surface of the substrate. Unfortunately, the lack of chemical bonds between the coating and the capillary wall results in low thermal and solvent stability to the coating. This problem is manifested by relatively low thermal stability as well as poor solvent stability of the commercial GC columns. As a result, such capillaries are not suitable for repeated SPME operations using organic or organo-aqueous HPLC solvents for the desorption of the extracted analytes. All of the underlying format related as well as coating related problems have been well addressed by Bigham et al. [22]. In the new method, a piece of sol-gel coated fused silica capillary [14] was used. Due to the strong chemical bonding of sol-gel organic-inorganic hybrid material to the inner walls, such capillaries demonstrated very high thermal stability as well as excellent stability toward conventional organic solvents. A highly nonpolar phase (PDMS) and a highly polar phase (PEG) [24] were used as new sol-gel SPME sorbents. Both phases demonstrated remarkably superior performance in direct extraction of polar and nonpolar analytes from aqueous media compared to conventional coatings.

Although sol-gel technology has been proven to be the most convenient and effective means of chemical immobilization for sorbent coatings, a narrow window of pH
stability inherent in silica based materials has imposed a serious obstruction to wide application of such materials particularly when pH adjustment to very high or very low values are inevitable for the selective extraction of target analyte(s) from a given matrix. Considering the fact that Titania possesses superior pH stability and mechanical strength compared with silica, Kim et al. [25] developed a high pH resistant, surface bonded sol-gel titania hybrid organic-inorganic coating and demonstrated its application in capillary microextraction by on-line hyphenating with high-performance liquid chromatography. The new sol-gel hybrid material generated from titanium isopropoxide (inorganic precursor) and polydimethylsiloxane (organic moiety) provided excellent performance stability even after being exposed to highly basic conditions (pH=13) for prolonged period of time (12 h). Moreover, direct chemical bonding of the TiO₂-PDMS coating to the capillary inner walls demonstrated excellent solvent stability, a prerequisite for coupling SPME with liquid-phase chromatographic techniques (e.g., HPLC, CE, CEC, etc.).

Pursuing a similar objective, Alhooshani et al. [26] reported a novel organic-inorganic hybrid zirconia coating for capillary microextraction. Although Zirconia has long been known to be better alkali resistant material than other metal oxides, such as alumina, silica, and Titania, this was the first report on sol-gel hybrid organic-inorganic zirconia sorbent used in SPME. Outstanding pH resistance, excellent chemical inertness, and high mechanical strength have made zirconia an excellent candidate for support material in the field of chromatography and membrane based separations created by using zirconium (IV) butoxide as the inorganic precursor and hydroxy-terminated polydimethylidiphenylsiloxane as the organic moiety, the new sol-gel hybrid inorganic-
organic material provided exceptionally high pH stability (pH 13, 24 h). Moreover, parts per trillion (ppt) level detection limits were achieved for both polar and non-polar analytes in CME-GC-FID experiments using sol-gel zirconia-PDMDPS as the extraction sorbent.

3.3.1.2 Mixed polysiloxane-based sol-gel sorbents

In polysiloxane based mixed sorbents, one or more organic polymeric entity is used along with PDMS to impart new selectivity to the composite material as well as some other physico-chemical advantages (e.g., higher thermal stability, additional sample capacity, greater surface area, etc.) So far, hydroxyfullerene, calix[4]arene, PVA, crown ether, DVB, PMPVS, PPhεMS have been reported in the literature [27-40].

3.3.1.2.1 Fullerene-polysiloxane mixed sol-gel sorbents

Fullerenes are closed-cage carbon molecules containing pentagonal and hexagonal rings. Because of its unique structure and properties, it has drawn wide attention immediately after its discovery in 1985[27]. The high hydrophobicity of fullerene as well as its high thermal stability, resistance to oxidation, and the capability of strong π-π and donor–acceptor interactions with analytes have made it a very promising SPME sorbent. Table 3.2 presents the important features of sol-gel hydroxyfullerene sorbent.
Table 3.2 Important features of sol-gel hydroxyfullerene sorbent

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Precursor</th>
<th>Organic Polymer</th>
<th>Thermal/Solvent Stability</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sol-gel hydroxyfullerene</td>
<td>MTMOS</td>
<td>$C_{60}(OH)_n$</td>
<td>360 °C</td>
<td>[27]</td>
</tr>
</tbody>
</table>

Yu et al. [27] exploited all these positive attributes of fullerene by developing an SPME coating using hydroxyfullerene with hydroxy terminated silicone oil (OH-TSO). A sol-gel approach was used to prepare the coating. Sol-gel solution was prepared using fixed volume (100 µL) of OH-TSO, variable amount of hydroxyfullerene (0, 10 and 20 mg), 400 µL MTMOS, 100 µL water and 200 µL TFA. Variable amount of fullerene was used to measure the contribution of hydroxyfullerene in extractions. Sol-gel hydroxyfullerene coated SPME fibers were conditioned at as high as 360 °C for 5 hours. Scanning electron micrographs (Figure 3.4) of the fullerol-coated surface revealed that such coatings possess a porous structure.
Figure 3.4 Scanning electron micrograph of sol-gel fullerol fiber at 2000-fold magnification (Reproduced from Ref. [27] with permission of Elsevier)
The high thermal and solvent stability may be attributed to the strong chemical bonding of the sorbent to the substrate, as well as the excellent thermal stability of the participating organic ingredients (fullerol and OH-TSO). Porous structure of the fullerene based sol-gel coatings facilitated faster mass transfer between the sorbent and aqueous media, and helped an extraction equilibrium to establish significantly faster than that on commercial coatings. For example, some PCBs required only 50 min to reach extraction equilibrium on a sol-gel fullerene coated fiber. Under similar conditions, a commercial 100 µL PDMS coating required even several days to reach equilibrium [28]. The highly porous structure of the coating not only helped in faster mass transfer during extraction but also aided in faster and complete desorption, minimizing the memory effect.

Sol-gel hydroxyfullerene coatings demonstrated excellent planar conformation selectivity and molecular recognition. The selectivity toward planar PCBs increased with increasing amount of fullerol added in the sol solution. It is believed [27] that strong affinity of planar PCBs toward fullerol is due to the charge-transfer between PCBs and the conjugated three-dimensional π-electronic system of fullerol. Sol-gel hydroxyfullerene coatings were also used to extract polycyclic aromatic hydrocarbons and aromatic amines. The results revealed that sol-gel fullerol coatings are not only suitable for nonpolar compounds but also very efficient in extracting polar analytes.
3.3.1.2.2 Mixed crown ether-polysiloxane sol-gel sorbents

Crown ethers are cyclic carbon compounds containing heteroatoms such as oxygen, nitrogen and sulfur. They are characterized by a cavity structure, medium polarity and strong electronegative effect of heteroatoms on the crown ether ring. They have long been used as gas chromatographic stationary phases because of their unique selectivity resulting from the cavity structure and interaction provided by highly electronegative heteroatoms on the crown ether ring. The synthesis of polymeric crown ethers is, perhaps, the easiest among all the crown ether-based phase that have so far been investigated as GC stationary phases. Poor diffusibility, low column efficiency as well as bleeding at high temperatures (due to the generation of low molecular mass crown ethers) have discouraged further research in the field. So far only one article has been published by Fine et al. in 1985 [29].

Several approaches have been suggested to avert the inherent shortcomings of low-molecular-mass crown ethers as GC stationary phases or SPME sorbents. Table 3.3 lists most common crown ether based polysiloxane sorbents used in SPME.
Table 3.3 Sol-gel crown ether sorbents used in SPME

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Precursor</th>
<th>Organic Polymer</th>
<th>Thermal/ Solvent Stability</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sol-gel Hydroxycrown ether</td>
<td>TEOS(^a)</td>
<td><img src="image" alt="Organic Polymer" /></td>
<td>350 °C</td>
<td>[30]</td>
</tr>
<tr>
<td>Sol-gel Hydroxycrown ether</td>
<td>MTMOS(^b)</td>
<td><img src="image" alt="Organic Polymer" /></td>
<td>340 °C</td>
<td>[31]</td>
</tr>
<tr>
<td>Sol-gel benzocrown ether</td>
<td>TEOS</td>
<td><img src="image" alt="Organic Polymer" /></td>
<td>350 °C</td>
<td>[34]</td>
</tr>
<tr>
<td>Sol-gel Dibenzo-18-crown-6</td>
<td>TEOS, VTOS(^c)</td>
<td><img src="image" alt="Organic Polymer" /></td>
<td>350 °C</td>
<td>[38]</td>
</tr>
<tr>
<td>Sol-gel Bisbenzocrown ether</td>
<td>TEOS</td>
<td><img src="image" alt="Organic Polymer" /></td>
<td>380 °C</td>
<td>[37]</td>
</tr>
</tbody>
</table>

\(^a\)TEOS = tetraethoxysilane; \(^b\)MTMOS= methyltrimethoxysilane; \(^c\) VTEOS= vinyltriethoxysilane.
Zeng and coworkers [30] proposed a sol-gel based methodology to coat SPME fibers with crown ethers having hydroxyl termini and successfully utilized them in phenol analysis. Sol solution was prepared by mixing 8 mg of OH-DB14C4, 90 mg OH-TSO, 10 mg PMHS, 100 µL TEOS, 50 µL 3-(2-Cycloxypropoxy)propyltrimethoxysilane (KH-560) and 100 µL methylene chloride. After thorough mixing of all the sol solution ingredients, 80 µL TFA (5% water) was added to catalyze the sol-gel reactions (hydrolysis and polycondensation) to form a three dimensional sol-gel network chemically bonded to the substrate. Solubility of OH-DB14C4 in the solution was a limiting factor that prohibited the use of higher concentrations of crown ether in the sol solution. The bare fused silica fibers were dipped into the sol solution for 30 min each time and freshly prepared sol solution was used to replace the old one after each 30 min period until the coating thickness reached 73 µm. Two other fibers having coating thickness 62 µm and 85 µm were also prepared for comparison purpose. To verify the significance of OH-DB14C4 in the sol solution, several other fibers were prepared using different amounts of OH-DB14C4 (0 mg, 4 mg, and 8 mg) keeping mass of OH-TSO (90 mg) constant. Partition coefficients (K) of different phenols between the aqueous phase and the sorbent coating containing different proportions of OH-DB14C14 and OH-TSO (0 mg/90 mg, 4 mg/90 mg, 8 mg/90 mg, 8 mg/0 mg) were determined.
Table 3.4 Comparison of partition coefficients of fibers with different compositions*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abbreviation</th>
<th>0/90 (75 µm)</th>
<th>4/90 (70 µm)</th>
<th>8/90 (73 µm)</th>
<th>8/0 (76 µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-chlorophenol</td>
<td>2CP</td>
<td>179.9</td>
<td>224.7</td>
<td>255.8</td>
<td>64.2</td>
</tr>
<tr>
<td>2-nitrophenol</td>
<td>2NP</td>
<td>150.3</td>
<td>153.8</td>
<td>202.3</td>
<td>80.9</td>
</tr>
<tr>
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<td>224.8</td>
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<td>84.8</td>
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<tr>
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<td>531.1</td>
<td>574.5</td>
<td>211.5</td>
</tr>
</tbody>
</table>

* Reproduced by permission from Anal. Chem. 73 (2001) 2429

As can be seen from the tabulated data (Table 3.4), the partition coefficient ($K$) values increased with the increase of OH-DB14C4 content. When OH-DB14C4 was used as the only sol-gel-active organic component in the sol solution, $K$ values have been reduced substantially. This was probably due to the fact that the presence of OH-TSO in the sol solution helped not only in lengthening the silica network leading to the increased surface area of the composite material but also in spreading the stationary phase uniformly on the fiber surface. Fluorescence micrograph of two fibers (one with OH-TSO, the other without OH-TSO) revealed that sol-gel OH-DB14C4/OH-TSO spreads uniformly on the substrate. On the other hand, sol-gel OH-DB14C4 (no OH-TSO)
underwent shrinkage during the gel formation leading to the formation of cracks on the surface.

Life span is one of the most important characteristics of an SPME coating. SPME coatings are damaged (frequent phenomenon for commercial fibers) due to the sudden exposure to the abruptly high temperature during analyte desorption in GC injection port and/or the presence of solvent in the matrix. Sol-gel coated OH-DB14C4/OH-TSO SPME fibers were characterized by high thermal and solvent stability. No apparent loss in extraction efficiency was observed even after being heated at 350 °C and also after 150 times repeated extractions. The presence of phenyl ring in crown ether structure, as well as the strong chemical bonding of the sol-gel network to the fused silica surface, are believed to be responsible for the high thermal and solvent stability of sol-gel OH-DB14C4/OH-TSO coating. Coatings of such high thermal stability coatings are likely to help expanding the SPME application range toward higher boiling compounds.

In SPME, affinity of an analytes for a given coating depends on their polarity following the principle “like dissolves like”. Therefore, polar CW/DVB should be able to extract polar phenols in great excess compared with the medium polar OH-DB14C4/OH-TSO. But in reality, this was not the case. The three dimensional network generated by sol-gel coating technology provided DB14C4/OH-TSO coating enhanced surface area as well as higher sample capacity that may have compensated its deficiency due to inferior polarity. Optimization of headspace SPME conditions for phenols revealed that extraction temperature of 40 °C, salt-saturate matrix of pH 1, constant stirring during extraction, extraction time of 40 min, desorption for 5 min at 260 °C yielded best result with respect to extraction.
The same group also prepared three other crown ether-based solid phase microextraction coatings by sol-gel method [31] using hydroxydibenzo-14-crown-4 (OH-DB14C4), dihydroxy-substituted saturated urushiol crown ether (DHSU14C4), and 3, 5-dibutyl-unsymmetry-dibenzo-14-crown-4 dihydroxy crown ether (DBUD14C4) and investigated their selectivity toward aromatic amines. Sol solution was prepared by thorough mixing of 8 mg OH-DB14C4, 90 mg OH-TSO, 50 µL 3-(2-Cyclooxypropoxyl)-propyltrimethoxysiloxane (KH-560), and 100 µL methylene chloride. Then 100 µL of TFA (5% water) was added and the mixture was centrifuged to remove the particulate materials. In case of other two crown ethers, 10 mg of each were used in the sol solution keeping all other ingredient unchanged. The thicknesses of sol-gel OH-DB14C4/OH-TSO, DHSU14C4-OH/TSO, and DBUD14C4/OH-TSO coatings were 65-, 70-, and 70 µm, respectively. In the course of SPME method optimization for aromatic amine extraction, optimum extraction temperature was found to be 55 °C. To increase extraction efficiency, the pH of the matrix was adjusted to 13. To compare the selectivity of different crown ethers for aromatic amine extraction, 5 different coatings were employed (OH-DB14C4/OH-TSO, DHSU14C4/OH-TSO, DBUD14C4/OH-TSO, OH-TSO, OH-DB14C4). Results obtained by those researchers suggested that OH-DB14C4/OH-TSO (65 µm) had the highest extraction efficiency. Among the three sol-gel crown ether coatings, extraction efficiency decreased with increasing number of alkyl groups (no alkyl group> n-C_{13}H_{31}> t-Bu) attached to the crown ether ring attributed to the decreased polarity of the coatings and increased steric hindrance. Among all the coatings tested, sol-gel OH-DB14C4 had the lowest extraction efficiency. In sol-gel OH-DB14C4 coating, no OH-TSO was added to lengthen the chains.
of the sol-gel network to achieve larger pore structure and increased surface area. The presence of OH-TSO in the sol-gel structure also helps the stationary phase to spread uniformly onto the surface of the substrate. Non-uniform coating, condensed network and low surface area may have been responsible for poor extraction efficiency of the prepared coating. On the other hand, all sol-gel crown ether coatings demonstrated higher extraction efficiency than sol-gel OH-TSO. This clearly demonstrated the higher selectivity of crown ethers for polar compounds. Another comparison was made between sol-gel OH-DG14C4/OH-TSO (65 µm), commercial CW-DVB (65 µm) and commercial PDMS coatings. As the extraction process is controlled by the principle “like dissolves like”, polar aromatic amines should have higher affinities for the polar coating than that for the non-polar coating. Therefore, it is expected that CW-DVB and sol-gel OH-DG14C4/OH-TSO being polar sorbents would extract higher amount of aromatic amines than a non-polar PDMS coating. The extraction results corroborated with the prediction putting PDMS coating as the least efficient for aromatic amine extraction. Among the other two polar coatings, sol-gel OH-DG14C4/OH-TSO and CW-DVB coatings, the sol-gel OH-DG14C4/OH-TSO coating demonstrated unambiguous superiority for aromatic amine extraction (Figure 3.5).
Figure 3.5 Relative extraction efficiencies of commercial PDMS fiber (100 μm), commercial CW-DVB (65 μm) and OH-DB14C4/OH-TSO (65 μm) in amine extraction (A-aniline; MT- m-toluidine; NNDEA- N,N-diethylaniline; NEMT- N-ethyl-m-toluidine; 3,4DMA- 3,4-dimehylaniline) (Reproduced from Ref. [31] with permission of Elsevier)
The inherently high surface area as well as high sample capacity of sol-gel coatings, originate from their porous structure, are believed to be responsible for the higher extraction efficiency.

All sol-gel crown ether coatings demonstrated very high thermal stability (~ 340 °C). Their extraction ability showed no significant change even after 150 extractions.

Although sol-gel DB14C4/OH-TSO has been proved to be a superior coating in many respect compared with commercial coatings, it represent a relatively low polarity coating [32,33]. Furthermore, its low solubility in sol solution limits the concentration of DB14C4 leading to low sample capacity as evidenced by the very narrow linear range of phenol [30]. In order to eliminate the inherent shortcoming of smaller crown ether, Wang et al. [34] synthesized dihydroxy-terminated benzo-15-crown-5 (DOH-B15C5). Having bigger ring size, dihydroxy-terminated benzo-15-crown-5 should provide higher polarity compared to that of smaller OH-DB14C4 because of the bigger crown ether ring and lower steric hindrance for hydroxy [34]. Moreover, higher solubility of such crown ether in sol solution should increase the sample capacity. The presence of both polar and non-polar functionality in the sol-gel composite material should facilitate extraction of both polar and nonpolar analytes, particularly aromatic compounds. The synthesis of benzo-15-crown-5 has been reported elsewhere [35]. In preparing the sol solution for sol-gel coating, 20 mg DOH-B15C5, 90 mg OH-TSO, 10 mg PMHS, 100 µL TEOS, 200 µL methylene chloride was added and thoroughly mixed. Finally 80 µL TFA (5%) was added with ultrasonic agitation and the mixture was centrifuged to remove particulate materials (if there is any). The fused silica fiber to be coated was dipped into the sol solution for 5 min. For each fiber, the coating process was repeated several times each
time for 2 min in the same sol solution until the desired thickness of the coating was obtained. Using the same sol solution repeatedly in the whole coating process (as opposed to using fresh sol solution every time after finishing the residence time [31] of the fiber in it) minimized the total coating time and reduced the material cost. The coating thickness of sol-gel DOH-B15C5/OH-TSO was determined 67 µm. Figure 3.5 shows an SEM image of sol-gel DOH-B15C5 coating illustrating its distinct porous structure.
**Figure 3.6** Scanning electron micrograph of the DOH-B15C5 coated fiber at 800-fold magnification (Reproduced from Ref. [31] with permission of Elsevier)
Using the same coating protocol, fibers having different coating thickness as well as different concentration of DOH-B15C15 were obtained. In order to assess the role of DOH-B15C15 in coating, different mass (0, 10, and 20 mg of it) was added in the sol solution that yielded the concentration of crown ether 0, 4.8, and 9.1% respectively. The reproducibility of the coating procedure was verified by coating three fibers under same conditions. The coating thicknesses were 65-, 67-, and 69 µm, respectively, which is clearly indicative of high reproducibility of the coating process. Extraction of different phenols derivatives using these fibers yielded low RSD values (in peak area) ranging from 2.20 to 4.94%. Maximum operating temperatures of different fibers were also evaluated. An SPME fiber with 100 µm thick blank sol-gel coating (no DOH-B15C5) showed cracking at 300 °C. The same cracking was observed in 30 µm blank sol-gel fiber at 320 °C. Such cracking was not apparent in 80 µm sol-gel DOH-B15C5/OH-TSO (10 mg) and 70 µm sol-gel DOH-B15C5/OH-TSO (20 mg) even after conditioning at 350 °C. Such a high operating temperature became possible due to the strong adhesion of the coating to the fused silica fiber surface through chemical bonding. Such high thermal stability also helped eliminating analyte carryover problem and expanding the application range towards high boiling compounds. It became obvious that addition of crown ether in the sol solution significantly increased the thermal stability of the composite material. In addition to high thermal stability, sol-gel DOH-B15C5/OH-TSO coating demonstrated excellent solvent stability - another indication of strong chemical bonding of the coating to the substrate. The presence of DOH-B15C5 molecules in the sol-gel network served as a selector for compounds with conjugate π electric system. Therefore, such composite sorbents should extract aromatic compounds with high efficiency. BTEX, being one of
the major environmental contaminants, were extracted using sol-gel DOH-B15C5/OH-TSO coated fiber (34 µm thickness, 10 mg crown ether). The extraction conditions were also optimized: 2 min extraction at 30 °C, 1 min desorption at 280 °C, aqueous solution saturated with NaCl and constant stirring were found to be optimum for headspace SPME of BTEX. In order to compare the selectivity of different coatings [30 µm commercial PDMS, 30 µm sol-gel PDMS, 34 µm sol-gel DOH-B15C5/OH-TSO (10 mg crown ether)], BTEX solution (10 µg/L) was employed for headspace SPME. The extraction results demonstrated that 34 µm sol-gel DOH-B15C5/OH-TSO (10 mg crown ether) fiber had the highest extraction efficiency and the 30 µm sol-gel PDMS fiber was the least efficient for BTEX extraction under the used conditions. It is believed that the presence of phenyl in DOH-B15C5 extends µ-µ interactions with BTEX compounds leading to high extraction efficiency. On the other hand, absence of such functionality in commercial PDMS coating made it inefficient for BTEX extraction.

Another class of compounds extracted by sol-gel DOH-B15C5/OH-TSO was phenols. Most phenols could be forced into the headspace by exploiting salting-out effect using NaCl. Acidifying the solution to pH 1 helps phenols maintain their neutral form. Optimum extraction conditions were: 40 min extraction at 40 °C, 2 min desorption at 300 °C, aqueous solution saturated with NaCl, pH 1 and constant stirring. Selectivity of different coatings (100 µm PDMS, 100 µm blank, 85 µm PA, and 67 µm crown ether) for phenol extraction was also studied and compared. As was expected, 67 µm DOH-B15C5 was found to be the most efficient and 100 µm PDMS the least. Although both sol-gel blank and PDMS had the same thickness, sol-gel blank demonstrated higher extraction efficiency. The excellent extraction efficiency demonstrated by sol-gel DOH-B15C5/OH-
TSO is due to enhanced surface area as well as sample capacity, hydrogen-bonding between the crown ether and phenolic compounds, polarity of the coating, complete desorption due to high thermal stability and so on. Sol-gel DOH-B15C5/OH-TSO coating also efficiently extracted a group of carcinogenic aryl amines and showed good selectivity.

Application of sol-gel DOH-B15C5/OH-TSO coating has also been extended to trace analysis of organochlorine pesticides (OCPs) in water [36]. In this case 9.1% crown ether was used in the sol solution and the coating thickness was 80 µm. As the extraction efficiency is heavily dependent upon the extraction conditions and the property of the coating, it is very important to optimize the extraction conditions as well as finding a suitable coating for extracting a particular compound class. In the extraction of OCPs using sol-gel DOH-B15C5/OH-TSO coating, extraction equilibrium was achieved in 40 min under constant agitation, solution saturated with NaCl and temperature maintained at 90°C. In a similar fashion, desorption was also very fast and completed in 60 s at 280 °C. The selectivity of sol-gel DOH-B15C5/OH-TSO coating for OCPs was compared with commercial PDMS coating. As the results indicated, extraction efficiency of the 80 µm thick crown ether coating was significantly higher than that for 100 µL PDMS coating even though the latter one has higher stationary phase loading. Thus, solid phase microextraction of OCPs using highly selective sol-gel DOH-B15C5/OH-TSO coating and thereafter detection by highly sensitive electron capture detector (ECD) has been proved to be a very powerful analytical tool for trace analysis of halogenated organic compounds.

Organophosphorus pesticides (OPs) have long been considered as a health and
environmental hazard due to its toxicity and ubiquity in nature. As a result, there is always a great demand for an analytical method that is cheap, simple, highly sensitive as well as fast that would ease the monitoring of trace levels of OPs in water, food and other matrices.

Yu et al. [37] synthesized allyloxy bisbenzo 16-crown-5 trimethoxysilane and used it as a precursor to prepare sol-gel bisbenzo crown ether/hydroxy-terminated silicone oil coating for SPME. Unlike other silicon oil based mixed sorbent systems where the loading of second organic compound is limited by its low solubility and translates into poor to moderate impact on the selectivity of the composite sorbent, allyloxy bisbenzo 16-crown-5 trimethoxysilane is highly soluble in sol solution ingredient and may be added to OH-TSO in a greater ratio. Sol solution was prepared using 75 mg of allyloxy bisbenzo 16-crown-5 trimethoxysilane, 90 µL of OH-TSO, 50 µL TEOS, 10 µL of PMHS, 300 µL of methylene chloride and 80 µL TFA (5% water). In most polysiloxane-based mixed sorbents, a second organic moiety is used in conjunction with silicone oil. But in this case, allyloxy bisbenzo 16-crown-5 trimethoxysilane was used as the sol-gel precursor. Due to high solubility of allyloxy bisbenzo 16-crown-5 trimethoxysilane in sol solution ingredients, the ratio of the mass of benzo-crown ether in this coating was about 33% which is much larger than crown ether concentration (~3%) in sol-gel SPME fiber prepared by common sol-gel method [30]. Due to the strong chemical bonding of the sorbent to the substrate, such sorbent demonstrated very high thermal stability (350 °C). Furthermore, the sol-gel crown ether coating was treated with different solvents (e.g., n-hexane, methylene chloride, acetone, and distilled water) for 1 h. No obvious change in extraction sensitivity was observed from subsequent extractions.
In developing an SPME method, finding the best sorbent is a challenging task but optimizing other variables (e.g., extraction time, ionic strength of the solution, extraction temperature are) also equally important. In any extraction process, matrix temperature has two fold influences on extraction. Higher temperature increases the diffusion coefficient of analytes in water and thus shortens the extraction time. On the other hand, elevated temperatures decrease the partition coefficient between the coating and analytes because molecular level interactions between the analytes and the coating decreases at a higher temperature. It is important to find a temperature that provides a reasonable compromise between two opposing factors. Optimum extraction temperatures were found to be 55 °C, 50 °C, 32 °C, 20 °C for honey, orange juice, water and pakchoi matrix, respectively. Extraction equilibria were achieved for most of the OPs within 120 min. Addition of NaCl helped the extraction of OPs from water, honey and orange juice matrices increasing the peak areas from 10 to 300% depending on the analytes. On the other hand, the addition of NaCl to pakchoi sample decreased the response of OPs which is because the suspended pieces of pakchoi in heterogeneous system may easily adsorb the OPs although salt can decrease the solubilities of OPs in water solution.

Sample matrix plays a very important role in extraction efficiency. When extractions are carried out from matrices other than water, dilution by water may increase extraction efficiency. As was evidenced in the extraction of OPs from different matrices, the peak area obtained by SPME-GC-FPD for homogenates (honey, orange juice, pakchoi) were less than that from aqueous solution spiked at the same concentration. Dilution with water slowly increased the peak area of the extracted analyte(s). Interestingly, when diluted to 50-100 times with water, the peak area for most OPs
increased and were higher than the SPME-GC peak areas for same concentration aqueous samples of these OPs prepared through spiking.

The same group also reported sol-gel dibenzo-18-crown-6 coating for solid phase microextraction of aliphatic amines in lake water and human urine after treating them with a new derivatizing reagent, tetrafluorobenzoic acid N-hydroxysuccinimide ester (TFBza-suc) [38]. Thermal stability and lifetime of the coating, extraction properties, and optimum extraction conditions were also investigated. Extraction sensitivity and selectivity of the new coating were also compared with two other sol-gel crown ether coatings (3’-allylbenzo-15-crown-5 and allyloxyethoxymethyl-18-crown-6) as well as commercial PDMS and PA coatings. Extraction linearity, reproducibility and method detection limits were also calculated.

Aliphatic amines are ubiquitous in nature due to their widespread use in industry as well as spontaneous generation as biodegradation products of organic compounds like proteins and amino acids or other nitrogenous compounds. Due to their toxicity and hazardous nature, low-molecular-mass amines are considered to be important air pollutants. Moreover, secondary aliphatic amines are assumed to react with nitrile to form carcinogenic nitrosamines [39]. Therefore, analysis of aliphatic amines at trace levels in biological fluids, air, and water is of great interest. GC is among many analytical approaches routinely used for aliphatic amine analysis. But because of the high polarity and hydrogen bonding properties of aliphatic amines, they often produce peak tailing and memory effects. Therefore, derivatization before extraction and/or analysis is a common practice. Derivatizing reagents, however, often interferes in separation, cause damage to the column, and in some instances, react with hydroxyl, phenol, thiol, and amine groups
leading to poor selectivity for amine detection [38]. Cai et al. [38] utilized a new derivatizing reagent TFBza-suc that specifically reacted primary and secondary aliphatic amines under mild conditions, but not with aromatic amines, hydroxyl, thiol, and phenol groups. The derivatized aliphatic amines are easy to separate by GC and detect by FID or MS. Before solid-phase microextraction, all aliphatic amines were derivatized by TFBza-suc.

To create the sol-gel coating, sol solution was prepared using one of the crown ether polymers, an alkoxyilane precursor (TEOS), a surface deactivation reagent (PMHS), a suitable solvent (methylene chloride) and a sol-gel catalyst (TFA, 5% water). Besides, VTEOS was added to impart organic character to the silica glass. The vinyl groups in VTEOS and the allyl crown ethers reacted to form cross-links in presence of free radical initiator AIBN when exposed to ultraviolet light (125 W, 366 nm). As a result, a surface bonded three dimensional network evolved. The coating thicknesses of three sol-gel coatings DB18C6/OH-TSO, B15C5/OH-TSO, and PSO18C6/OH-TSO were 80-, 84- and 82 µm, respectively. The thicknesses of two commercial coatings, PDMS and PA, were 100 and 85 µm, respectively. The extraction efficiencies of all five coatings were compared by extracting derivatized aliphatic amines under identical conditions. The results indicated that sol-gel DB18C6/OH-TSO and B15C5/OH-TSO had higher extraction efficiencies than sol-gel PSO18C6/OH-TSO. The presence of benzyl group in these crown ether is thought to be responsible for higher extraction of aliphatic amines by \( \pi-\pi \) interactions with TFBza-suc-derivatized amines. Particularly, the symmetric benzyl groups and greater number of oxygen atoms in the crown ether ring in sol-gel DB18C6/OH-TSO results in stronger \( \pi-\pi \) interactions between the coating and the
derivatized amines, and therefore, made this coating most efficient among all five coatings employed in this investigation [38]. Moreover, the extraction efficiencies of two commercial coatings were lower than the crown ether coatings, PDMS having the lowest. Enhanced surface area and sample capacity provided by sol-gel coating technology is one factor that helped achieve this enhanced extraction sensitivity. Like other sol-gel coatings, sol-gel crown ethers also demonstrated high thermal stability. The highest operating temperatures of sol-gel DB18C6/OH-TSO, B15C5/OH-TSO, and PSO18C6/OH-TSO coatings were reported to be 350, 340, and 320 °C, respectively. The presence of two benzene rings on opposite ends of the crown ether ring may have provided relatively higher thermal stability of sol-gel DB18C6/OH-TSO. Finally, the sol-gel DB18C6/OH-TSO coating was used for solid phase microextraction of derivatized aliphatic amines from lake water and urine samples. Excellent reproducibility (RSD = 3-4%), high recovery (92-109%), and low detection limits (0.05-0.005 µgL^{-1}) accomplished by the new coating have added new dimensions in amine analysis.

Considering the utmost necessity of thermally stable polar coatings in solid phase microextraction, Yun [40] proposed a α,ω-diallyltriethylene glycol/hydroxyl terminated silicone oil (DATEG/OH-TSO) -coated fiber prepared by sol-gel technology and investigated its applicability to the extraction of polar compounds (e.g., phenols) as well as nonpolar compounds (e.g., BTEX and phthalate esters from aqueous solution).

The sol solution used in preparing the coating was made using 90 mg DATEG, 90 mg OH-TSO, 100 µL TEOS, 50 µL VTEOS, 9 µL benzophenone, 120 µL methylene chloride and 120 µL TFA (5% water). As is seen from the composition, a remarkably higher amount of DATEG was added to the sol solution which was possible due to its
high solubility in the sol solution. Presence of higher mass of DATEG in sol solution would eventually translate into higher polarity of the sol-gel hybrid sorbent. As a consequence, such sorbent should be able to extract higher amounts of polar compounds from aqueous media. The fibers were dipped into the sol solution for 30 min each time. After the 30-min residence time, fresh sol solution was used to replace the previous solution. The final coating thickness was 55 µm.

Unlike commonly used sol-gel process where only one metal alkoxide (TEOS or MTMOS) is used, Yun utilized VTEOS in conjunction with TEOS which reacted with DATEG by radical cross-linking reaction under ultraviolet radiation to produce chemical bonding between crown ether and other coating ingredients.

Phenols, one of the major class of environmental pollutants, were extracted using sol-gel DATEG/OH-TSO and the results were compared with those obtained on a polar CW-DVB (65 µm) coatings. Although CW and DATEG have similar polarity, extraction results indicated higher extraction efficiency of sol-gel DATEG/OH-TSO. Enhanced surface area of the sol-gel coating is believed to be responsible for the superior performance of the sol-gel coating.

Next class of environmental pollutants extracted by the sol-gel DATEG/OH-TSO fiber was BTEX. For comparison of extraction efficiency, CW/DVB (65 µm) and PDMS (100 µm) were also employed under the same extraction conditions. As was expected, PDMS being nonpolar and the thickest coatings used, should have extracted the nonpolar BTEX with highest extraction efficiency. But the results indicated that sol-gel DATEG/OH-TSO performed best in extracting BTEX from aqueous solution (Figure 3.7). This became possible due to the fact that sol-gel DATEG/OH-TSO coating has both
polar (DATEG) and nonpolar (OH-TSO) structural features as well as enormous surface area, a characteristic feature of sol-gel coatings.
Figure 3.7 Comparison of extraction efficiencies of commercial PDMS (100 μm), commercial CW/DVB (65 μm), and DATEG/OH-TSO coatings in extracting BTEX from aqueous solution (Reproduced from Ref. [40] with permission of Elsevier)
High thermal stability of sol-gel DATEG/OH-TSO (320 °C) allowed for efficient extraction and desorption of high-boiling compounds as opposed to commercial coatings which have relatively low recommended operating temperatures (mostly <300°C). Thanks to the ability of sol-gel DATEG/OH-TSO coatings to extract and desorb high boiling compounds, SPME fibers with such coatings were used to extract several phthalate esters, a class of high-boiling contaminants. Direct-SPME of phthalate esters were done followed by desorption at 320 °C. No noticeable decline in extraction capacity even after 150 cycles of extraction-desorption clearly demonstrated superiority of this sol-gel coating compared to available commercial counterparts.

3.3.1.2.3 Mixed calix[4]arene-polysiloxane sol-gel sorbents

Calixarenes are the third generation host molecules, the first two being crown ethers and cyclodextrins. They are cyclic oligomers prepared from the reaction of phenols and aldehydes. In recent years, calixarenes have received notable attention in supramolecular chemistry. Since they possess a molecular cavity of cylindrical architecture similar to that of cyclodextrins, they can form inclusion complexes. The unique characteristics of calixarenes such as small molecular size, good film forming properties, excellent thermal stability, presence of functional groups to interact with analytes have made them promising candidates for being used in SPME sorbents. However, the poor solubility of unsubstituted calixarenes in common organic solvents as well as in chromatographic phases (e.g., polysiloxanes) has appeared to be the major obstruction to their widespread use in analytical chemistry. Table 3.5 provides a list of
sol-gel calixarene sorbents used in SPME.

**Table 3.5** Sol-gel calixarene sorbents used in SPME

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<th>Organic component</th>
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<td>[41]</td>
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<tr>
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<td>[79]</td>
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<tr>
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<td>380 ºC</td>
<td>[45]</td>
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</table>
Zeng and co-workers [41] represent the only research group involved in developing calixarene based sol-gel sorbents for solid phase microextraction. These researchers synthesized 5,11,17,23-tetra-tert-butyl-25,27-diethoxy-26,28-dihydroxycalix[4]arene and utilized it as a new SPME sorbent. A sol-gel based approach was followed to prepare the coating a fused silica fiber using calix[4]arene and hydroxyterminated silicone oil (OH-TSO) as organic components, 3-(2-Cycloxypropoxyl)propyltrimethoxysilane (KH-560) and TEOS as sol-gel precursors, poly(methylhydrosiloxane) (PMHS) as deactivating reagent, and trifluoroacetic acid (TFA) (5% water) as the sol-gel catalyst. Calix[4]arene has limited solubility in OH-TSO. To optimize the amount of calixarene that can be dissolved in OH-TSO, variable amounts of calix[4]arene (0 mg, 20 mg, 30 mg, 40 mg) were added in 90 mg OH-TSO along with other sol-gel ingredients and the sol-gel coatings were prepared. OH-TSO was added along with calix[4]arene in the sol solution in order to lengthen the silica network to achieve increased surface area of the coating. Based on SEM data, the prepared coatings had thickness estimated at 65 µm (for OH-TSO), 55 µm [for C[4] (20 mg)-OH-TSO], 60 µm [for C[4] (30 mg)-OH-TSO], 57.5 µm [for C[4] (40 mg)-OH-TSO]. As is evidenced from the thickness data, the sol solution containing 40 mg C[4] generated smaller thickness than the solution containing 30 mg C[4]. This is probably due to the fact that C[4] (40 mg) exceeded its solubility in the sol solution and eventually precipitated. All calix[4]arene coated fibers were conditioned first at 280 °C and then step by step the conditioning temperature was raised to as high as 380 °C in 20 °C increments staying for 1 h at each step. The extraction data revealed no significant loss of extraction efficiency even at 380 °C. Of course the credit goes to sol-gel coating technology that made it
possible to create coatings of such high thermal stability.

Since calix[4]arene contains phenyl termini as well as a cavity in its structure, it is expected to exhibit high selectivity toward nonpolar aromatic compounds due to π–π interaction, hydrophobic interactions and cavity-shaped cyclic molecular structure. Moreover, such coatings should efficiently extract polar aromatic amines through hydrogen bonding and dipole-dipole interactions. As was expected, calix[4]arene coatings demonstrated very high efficiency in extracting BTEX, PAHs as well as aromatic amines. To compare the extraction capability of sol-gel calix[4]arene-OH-TSO and sol-gel OH-TSO, BTEX was extracted using both the coatings under similar extraction conditions. The amount of BTEX extracted by unit volume of sol-gel calix[4]arene-OH-TSO coating was far greater than that by the sol-gel OH-TSO coating. This was clearly indicative of the positive attribute of calix[4]arene in the coating. The same trend also continued in case of extracting aromatic amines. Like other sol-gel coatings, sol-gel calix[4]arene-OH-TSO was assumed to be highly porous as manifested by its extraordinarily shorter extraction equilibrium time (20 min for fluorene in HS-SPME). On contrary, Nguyen and co-workers [42] reported 1-2 h extraction equilibrium time for PAHs on a laboratory-made PDMS coating.

Thus, sol-gel coated 5,11,17,23-tetra-tert- butyl-25,27-di-ethoxy-26,28-dihydroxycalix[4]arene coating exhibited high selectivity, sensitivity and faster extraction equilibrium for a wide range of analytes including BTEX, PAHs, and aromatic amines. Very high thermal and solvent stability, excellent coating and extraction reproducibility, long life span, and low detection limits for extracted analytes have made such a coating promising and beneficial in SPME.
In solid phase microextraction, there is a substantial lack in polar coatings. Although commercial phases like Polyacrylate (PA), Carbowax/divinylbenzene (CW/DVB), Carbowax/ Templated resin (CW/TPR) and several other custom made phases have been used as polar sorbents, all of these sorbents possess an inherent shortcoming - low thermal stability. In most of the cases, the recommended maximum operating temperatures are below 300 °C, putting a serious limitation to effective thermal desorption of polar analytes. For example, Molder et al. [43] extracted phenols from wastewater using PA fiber. After 5 min of desorption at 280 °C, a small carry-over of pentachlorophenol was observed. When desorption was done at 300 °C for 5 min, carry-over problem was gone but after 10 SPME runs the fiber demonstrated a drastic loss of extraction performance. This indicated the poor thermal stability of such phases. In an attempt to contribute to the great demand of polar sorbents, Zeng group [44] utilized previously reported sol-gel calix[4]arene-OH-TSO [41] for extracting chlorophenols. As was mentioned before, this phase demonstrated excellent thermal - (~ 380 °C) as well as solvent stability. For comparison, extraction of chlorophenols was carried out using commercial PA (85 µm), commercial PDMS/DVB (65 µm) and in-house prepared sol-gel calixarene C[4]/OH-TSO (85 µm) sorbents. As the results indicated, sol-gel C[4]/OH-TSO demonstrated highest extraction efficiency for all PCPs except 2 CP which being extremely polar showed higher affinity towards more polar PA sorbent. In case of 2,4 DCP, both C[4]/OH-TSO and PA demonstrated almost same extraction efficiency. The high extraction efficiency of C[4]/OH-TSO is believed to be due to the π-π interaction, hydrophobic interactions and cavity-shaped cyclic molecular structure.

Same C[4]/OH-TSO sorbent was also utilized for the determination of phthalate
acid esters plasticizers in polymeric materials by ultrasonic solvent extraction combined with solid phase microextraction [45]. Phthalic acid esters (PAEs) are the most commonly used additives in plastic and are believed to be the cause of serious health conditions (premature breast development in young girls, development of breast cancer in humans, and so on). Considering the serious health implications of PAEs, the US Environmental Protection Agency (EPA) and analogous regulatory agencies in several other countries have classified the commonly occurring phthalates as priority pollutants [46]. This implies a great demand of a simple, low cost and rugged method for determining PAEs in water. The sol-gel coated 5,11,17,23-tetra-tert-butyl-25,27-diethoxy-26,28-dihydroxycalix[4]arene/hydroxy-terminated silicone oil coated SPME fibers were employed to determine the contents of phthalate ester-based plasticizers in blood bags, transfusion tubing, food packaging bag, and mineral water bottle. In order to transfer PAEs from PVC products, they were cut into small pieces and placed in a vial. A small volume of methanol was added to the vial and was sonicated for 30 min. A part of the methanolic extract was then added to water and used for direct SPME. SPME experimental conditions (e.g., extraction temperature, extraction time, impact of salt addition, desorption temperature and time) were optimized to obtain the best possible and reproducible results. Experimental results demonstrated that 30 °C was the optimum temperature for PAEs extraction. Polar analytes with short alkyl chains required 60 min for equilibrium whereas nonpolar analytes with long alkyl chains required longer equilibration time due to slow diffusion. Since it is not necessary to attain equilibrium if constant extraction conditions are maintained [47], all extractions were carried out for 40 min. Different amounts of NaCl were added to verify its impact on extraction sensitivity
and a concentration of 180 g/L was found to be optimum. Desorption temperature of 300 °C was found adequate for complete release of all of the extracted PAEs in 1 min.

Relative affinity of PAEs were investigated by employing C[4]/OH-TSO and three commercially available fibers (PDMS, PA, and PDMS/DVB). The results unequivocally demonstrated the superiority of C[4]/OH-TSO among all four phases. It was assumed believed that the presence of phenyl rings and hydrophobic cavities of C[4]/OH-TSO helped extracting aromatic compounds through π-π interactions, hydrophobic interactions, and steric interactions via cavity-shaped cyclic molecular structure. Also, hydrogen bonding and dipole-dipole interactions between the analytes and the coating apparently played important roles in the extraction process.

Amines are important environmental pollutants which may occur as biodegradation products of organic matters like proteins, amino acids and other nitrogen containing organic compounds. They are also used as raw materials or intermediate products for manufacturing a wide range of industrial chemicals. Many amines possess unpleasant odor and harmful to health because of their toxicity. Due to the high polarity and solubility of low-molecular weight amines in water, it is very difficult to extract them without derivatization.

Zeng and co-workers [79] utilized previously reported calix[4]arene after slight modification in the structure that allowed headspace solid phase microextraction of underivatized aliphatic amines from aqueous solution. 25,27-dihydroxy-26,28-oxy(2′,7′-dio xo-3′,6′-diazaoctyl)oxy-p-tert-butylcalix[4]arene (amide bridged C-[4]) was synthesized in a view that the presence of polar amide bridge in the calix[4]arene structure would increase the affinity of the sorbent for amines. The fibers were prepared
following a procedure discussed before [41]. The modified calix[4]arene retained all of its positive attributes (e.g., high thermal stability (~380 °C), solvent stability, long life span, as well as highly porous surface morphology). Owing to the introduction of the polar amide bridge in calixarene molecules, the polarity of the coating increased. As a consequence, such coatings exhibited better sensitivity to most of the investigated aliphatic amines compared to commercial polar coatings PDMS/DVB and PA.

3.3.1.2.4 Mixed polyvinyl alcohol (PVA)–polysiloxane sol-gel sorbents

Lopes et al. [48] prepared a composite sol-gel sorbent using polydimethylsiloxane (PDMS) and poly (vinyl alcohol) as organic moieties. Polyvinyl alcohol was incorporated in the growing sol-gel network via polycondensation and acted as a strong cross-linking agent [49]. The thermal stability of the composite phase has been found to be superior compared to sol-gel PDMS sorbent. The improved thermal stability of sol-gel PDMS/PVA can be attributed to the additional cross-linking provided by the PVA in the reaction mixture. Another important piece of information has been obtained from the thermogravimetric analysis (TGA). Results showed that after heating at 750º C, sol-gel PDMS/PVA retains 36% of its original weight whereas sol-gel PDMS retains 30%. The retained mass represents mostly inorganic silica content of the network. It is likely that the comparatively higher inorganic content in sol-gel PDMS/PVA imparts higher thermal stability (350°C). It is worth noting that enhanced thermal stability of SPME sorbents are extremely desirable because higher thermal stability of the sorbents can extend the array
of analytes amenable to SPME toward higher-boiling compounds. Moreover, bleeding from the fiber coating during the thermal desorption is less likely to happen. The new sol-gel PDMS/PVA has shown better affinity towards polychlorinated biphenyl (PCBs) compared with sol-gel PDMS coating.

The same group also expanded the application of sol-gel PDMS/PVA sorbent by successfully employing it in trace determination of organochlorine pesticides (OCP) and organophosphorous pesticides (OPP) from herbal infusions of *Passiflora* L.

### 3.3.1.2.5 Mixed polymethylphenylvinylsiloxane (PMPVS)–polysiloxane sol-gel sorbents

Yang *et al.* [50] described sol-gel poly(methylphenylvinylsiloxane) coating which is characterized by good thermal stability, long life time, and high extraction efficiency for a wide range of compounds. Due to the inherent multifunctional properties and the features of sol-gel chemistry, sol-gel PMPVS coating was expected to demonstrate good selectivity for both polar and nonpolar analytes. The sol solution was prepared by thoroughly mixing 40 mg of PMPVS, 90 mg of OH-TSO, 100 µL of TEOS, 50 µL of VTEOS, 10 mg of PMHS and 8 mg Benzophenone, 400 µL of methylene chloride and 120 µL of TFA (5% water). Instead of using single precursor, TEOS, another precursor VTEOS was also used in conjunction with TEOS. Upon exposure to ultraviolet light, vinyl groups present in VTEOS and PMPVS reacted to form cross-links in presence of Benzophenone. Furthermore, ultrasonic drying technique was employed to overcome
shrinkage and cracking of the coating which is inevitable in conventional drying process.

Due to the presence of polarizable phenyl groups in the sol-gel PMPVS/OH-TSO coating, it is expected that such coating should demonstrate high affinity for aromatic compounds (e.g., BTX, polycyclic aromatic hydrocarbons, etc.). Two different coatings (sol-gel OH-TSO, 65 µm; and sol-gel PMPVS/OH-TSO, 55 µm) were employed for headspace SPME of BTX to compare relative extraction efficiency of both phases. As was expected, sol-gel PMPVS/OH-TSO exhibited higher affinity for BTEX due to π-π interaction between the coating and analytes. Same trend was observed in PAHs extraction from aqueous media. Sol-gel PMPVS/OH-TSO coating (35 µm) has been found to be more efficient than commercial PA (85 µm) and PDMS (100 µm) coatings. The coating showed high thermal stability (350 °C) and extended service life.

3.3.1.2.6 Mixed divinylbenzene-polysiloxane sol-gel sorbents

Nerve agents are one of the most toxic compounds. The detection and monitoring of nerve agents have received enormous importance particularly in changed post 9/11 environment in the USA because of their potential use as weapons of mass destruction. The nerve agents sarin, soman, tabun and VX are representatives of methylphosphonate nerve agents. Due to the absence of chromophore (or fluorophore) in their structure, it is difficult to use UV or fluorescence detection which are most commonly used in liquid phase separations. As a viable alternative, SPME-GC or SPME-GC/MS can be explored.

One of the major factors that inhibit the healthy growth of SPME is the availability of suitable coatings for the extraction of a particular class of compounds. In
commercial fiber manufacture process, one or more organic polymeric materials are immobilized on the surface of the silica fiber by mere physical deposition. When pure organic polymers are used as the sorbents, it is difficult to fine-tune selectivity of such sorbents. Furthermore, lack of chemical bonding between the sorbent and the substrate is considered to be responsible for low thermal and solvent stability of such phases. Sol-gel coating technology has overcome most of the above mentioned problems and provided a very simple way to synthesize hybrid organic/inorganic composite material with tunable selectivity, porous structure providing enhanced surface area, and chemical bonding to the substrate responsible for high thermal and solvent stability.

Liu et al. [51] developed a sol-gel based new sorbent using hydroxy-terminated silicone oil and divinylbenzene polymers for selective extraction of phosphates and methylphosphonates from air and water matrix. Sol solution was prepared by thoroughly mixing 180 µL of DVB, 60 mg of OH-TSO, 50 µL of VTEOS, 10 mg of PMHS, 8 mg benzophenone, 100 µL of methylene chloride and 70 µL trifluoroacetic acid (5% water). In order to prepare the coating, specially treated fused silica fibers were dipped into the sol solution and kept there for 30 min. After that, new sol solution was employed and coating continued for another 30 min. When the coating was completed, the fibers were irradiated under ultraviolet light for 30 min. Finally the fibers were thermally conditioned at 250-380 °C under nitrogen flow.

The sol solution ingredients were carefully chosen to serve specific purpose. In conjunction with commonly used precursor TEOS, a second precursor, VTEOS was used to facilitate free radical cross-linking with DVB under ultraviolet light.

In order to optimize relative proportions of OH-TSO and DVB, different ratios of
both ingredients were used and extraction efficiencies of the prepared coatings were evaluated. The following proportions of OH-TSO and DVB were used: 180 mg/0 µL; 180 mg/180 µL; and 60 mg/180 µL. The extraction results suggested that DVB played a major role in the selectivity of the composite material for extracting phosphates and methylphosphonates. The composition 60 mg OH-TSO/180 µL DVB yielded the highest extraction efficiency. Another comparison was made between sol-gel OH-TSO/DVB and the following commercial coatings: PDMS, PA, and PDMS/DVB. Sol-gel OH-TSO/DVB coating showed the best extraction efficiency among all the phases compared. Like other sol-gel coatings, sol-gel OH-TSO/DVB coating is also characterized by very high thermal stability. After heating up to 380 °C, no noticeable loss in extraction efficiency was observed. It clearly indicated the operational superiority of sol-gel OH-TSO/DVB coating. As a result of high thermal stability, it should provide higher desorption temperature for analytes with high boiling points, and also should eliminate sample carryover still considered to be a common problem for commercial coatings.

Other extraction parameters were also investigated and optimized. For headspace SPME of phosphates and methylphosphonates, 5 min extraction at 60 °C was found optimum for air samples whereas extraction 15 min extraction at 32 °C with constant stirring with 1 gm salt in a 5 mL water sample was found to be optimum for direct extraction.
3.3.1.2.7 Mixed polyphenylmethysiloxane (PPMS)–polysiloxane sol-gel sorbents

During the last couple of decades the use of pesticides has increased dramatically to enhance agricultural productivity. The high toxicity and widespread use of pesticides have been a major concern in recent years. Organochlorine pesticides (OCPs) are among the most frequently used pesticides. Their low solubility in water and resistance to metabolism has made them persistent pollutants in the environment. Some OCPs were banned in 1970 [52]. Considering their impact on public health and the environment, several approaches have been proposed to quantify OCPs in different matrices (e.g., water, plants, soils, foodstuff, etc.) [53-55].

In an effort to simplify these time-consuming methods, Cai et al. [56] developed a microwave-assisted solvent extraction (MASE) and coupled it with SPME-GC/ECD for the determination of organochlorine pesticides in Chinese teas. Polyphenylmethysiloxane (PPMS) and polymethylsiloxane (PMS) coatings were prepared using sol-gel technology and employed them as sorbents.

The sol solution was prepared by mixing 40 mg PPMS, 100 µL OH-TSO, 100 µL TEOS, 50 µL VTEOS, 10 µL PMHS, 10 mg AIBN, 800 µL methylene chloride, and 120 µL TFA (5% water). Coating was prepared by immersing a pretreated fused silica fiber into the sol solution. The coated fibers were then illuminated by ultraviolet light (125 W, 366 nm) for 60 min. followed by thermal conditioning at 100, -200, -300 and 350 °C for 1 h under nitrogen in the GC injector. The final coating thickness of the prepared sol-gel PPMS fiber was estimated at 70 µm. Following the same procedure, another fiber coated with sol-gel PMS was also prepared. The thickness of sol-gel PMS
coating was estimated at 75 µm.

Microwave-assisted solvent extraction (MASE) is a relatively new technique which has been applied to the extraction of organic compounds from different matrices [57]. In recent years, MASE followed by SPME has been proven to be a useful combination that may speed up the whole extraction and analysis process [58-61].

In order to find the most suitable sorbent for OCPs extraction, three different coatings were employed: 70 µm sol-gel PPMS, 75 µm sol-gel PMS and commercial 100 µm PDMS. Extractions of OCPs were performed in a microwave oven for 10 min. After cooling to room temperature, the extract was transferred to a vial and diluted with water to a predetermined volume for headspace SPME.

To verify the effectiveness of MAE, another extraction was carried out using ultrasonic power for an hour followed by headspace SPME.

Being nonpolar, OCPs should be extracted well on nonpolar coatings. Therefore, nonpolar sorbents like commercial PDMS as well as sol-gel PPMS and PMS should demonstrate high affinity towards OCPs. Extraction results revealed that both sol-gel PPMVS and PMVS coatings had better extraction efficiency than commercial PDMS although the latter had the highest sorbent loading. This was justified by the fact that coatings generated by sol-gel process were characterized by porous structure and enhanced surface area compared to commercial coatings. Furthermore, sol-gel PPMS coating was found to be more efficient than sol-gel PMS in extracting OCPs. The presence of phenyl groups in sol-gel PPMS is believed to be facilitating better extraction of OCPs through additional π-π interactions.

Thermal stability of an SPME sorbent is indicative of its operational superiority.
The higher the maximum operational temperature, the less is the chance of memory effect and appearance of ghost peaks in the chromatogram. Thermal stability of both sol-gel PPMS and sol-gel PMS were also investigated. No significant loss in extraction efficiency were observed after heating sol-gel PPMS coating at 350 °C and sol-gel PMS coating at 320 °C. Such high thermal stability of sol-gel coatings is due to the strong adhesion of the coating to the substrate through chemical bonding. The better thermal stability of sol-gel PPMS coating is believed to be due to the presence of phenyl group in the sorbent structure.

Finding the most suitable coating for extracting a particular group of compounds is a challenging job for separation scientists. In this regard, the optimization of SPME conditions which include extraction mode, extraction time, extraction temperature, impact of salt addition, etc. are also very important. A comparison was made between headspace SPME and direct SPME by employing 100 ng/L concentration of OCPs for equilibrium extraction. Extraction results revealed that almost all OCPs except β-HCH and aldrin have higher extraction efficiencies for headspace mode. Extraction equilibria were reached within 20 and 80 min depending on the compound. Under similar conditions, the commercial PDMS coating needed several hours to reach equilibrium [53]. Optimum extraction temperature was found to be 90 °C. Salt addition increased the extraction efficiency; 5 g of NaCl was found optimum in 15 mL aqueous solution of OCPs. Microwave assisted extraction is particularly beneficial for extracting organic compounds from solid matrices. Therefore, it has been chosen for extracting OCPs from Chinese tea samples. The optimum MASE extraction condition for OCPs from tea was 80% irradiation power. To evaluate the performances of microwave assisted solvent
extraction and ultrasonic extraction, blank green tea spiked with OCPs were employed and the extraction recovery results obtained under identical conditions were compared. The recoveries of OCPs by MASE-SPME-GC/ECD were 39.05-90.13 % for spiked 50 ng/L standard OCPs and 43.47-101.17% for spiked 100 ng/L standard OCPs. On the other hand, recoveries of OCPs by USE-SPME-GC/ECD were 1.51-27.66% for spiked 50 ng/L standard OCPs and 2.5-36.29% for spiked 50 ng/L standard OCPs. As is evident from the extraction results, MASE offered much efficient analyte recoveries with shorter extraction time. Table 3.6 provides a list of miscellaneous polysiloxane-based sol-gel sorbents used in SPME
Table 3.6 Salient features of SPME sorbents based on sol-gel PVA, polyphenylmethylsiloxane, divinylbenzene, and polymethylphenylvinylsiloxane.

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Precursor</th>
<th>Organic Component</th>
<th>Thermal/Solvent Stability</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sol-gel PVA</td>
<td>TEOS</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>350 ºC</td>
<td>[48]</td>
</tr>
<tr>
<td>Sol-gel Divinyl benzene</td>
<td>TEOS, VTEOS</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>380 ºC</td>
<td>[51]</td>
</tr>
<tr>
<td>Sol-gel polymethylphenylvinylsiloxane</td>
<td>TEOS, VTEOS</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>350 ºC</td>
<td>[50]</td>
</tr>
</tbody>
</table>
3.3.2 Non-polysiloxane sorbents

As a rule of thumb, all chromatographic stationary phases should be able to serve as SPME sorbents. Among commercially available SPME sorbents, a few are non-polysiloxane based polymers e.g., PA, CW/DVB, CW/TPR. Surprisingly, only a handful sol-gel coatings are non-polysiloxane based. A recent literature survey revealed that polyethylene glycol (superox, Carbowax 20 M), octyl-based silica, dendrimers, poly-THF, cyclodextrins, and fullerene are the only non-polysiloxane organic components used to prepare sol-gel sorbents.
Table 3.7 Non-polysiloxane sorbents used in SPME

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Precursor</th>
<th>Organic Component</th>
<th>Thermal/Solvent Stability</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Sol-gel PEG         | MTMOS     | \[
\begin{align*}
\text{O} & \text{H} \quad \text{C} \quad \text{H} \quad \text{C} \\
& \quad \text{H} \quad \text{O} \quad \text{H}
\end{align*}
\] | 300 ºC | [62] |
| Sol-gel C8          | MTMOS     | \[
\begin{align*}
\text{O} & \text{H}_2 \text{C}_2 \text{H}_3 \\
& \quad \text{O} \quad \text{H}_2 \text{C}_2 \text{H}_3 \\
& \quad \text{C}_2 \text{H}_2 \text{C}_2 \text{H}_3 \\
& \quad \text{C}_2 \text{H}_2 \text{C}_2 \text{H}_3
\end{align*}
\] | Acetonitrile/water | [66] |
| Sol-gel Hydroxyfullerene | MTMOS     | \[
\begin{align*}
\text{C}_6 & \text{H}_0 \quad \{ \text{O} \text{H} \}_a
\end{align*}
\] | 360 ºC | [27] |
| Sol-gel PEG         | -         | \[
\begin{align*}
\text{O} & \text{C}_2 \text{H}_2 \text{O} \\
& \quad \text{C}_2 \text{H}_2 \text{O} \\
& \quad \text{C}_2 \text{H}_2 \text{O} \\
& \quad \text{C}_2 \text{H}_2 \text{O}
\end{align*}
\] | 300 ºC | [22,24] |
| Sol-gel Dendrimer   | MTMOS     | ![Dendrimer Diagram]                                                                | 320 ºC | [79] |
| Sol-gel poly-THF    | MTOS      | \[
\begin{align*}
\text{O} & \text{H} \quad \{ \text{C} \text{H}_2 \}_4 \quad \{ \text{O} \text{H} \}_a
\end{align*}
\] | 350 ºC | [80] |
3.3.2.1 Polyethylene glycol-based sol-gel sorbents

The credit for the first report on utilizing Sperox-4, a PEG congener, in solid phase microextraction goes to Wang et al. [62] who exploited the widely known positive attributes of sol-gel SPME coating technology developed by Malik and co-workers [18]. Sol solution was prepared by mixing 200 mg Superox-4, 400 µL MTMOS, 200 µL acetone and 150 µL TFA (5% water). The solution was centrifuged to expel solid particulate material present in the matrix. The clear supernatant was used for sol-gel Superox-4 coating. In order to prepare the coating specially treated fused silica fibers were immersed inside the sol solution and kept there for 30 min. A 40-µm thick sol-gel coating was found to be formed during this residence time. The coated fibers were then conditioned at 300 °C for 2 h under continuous nitrogen flow.

Scanning electron microscopic investigation revealed that the coating possessed a roughened surface with a porous structure which is a highly desirable characteristic of an SPME sorbent.

In order to verify the performance of sol-gel Superox-4 coated fibers, headspace SPME of BTEX solution (10 ng/mL) was carried out. The highly porous structure of sol-gel Superox-4 coating facilitated faster mass transfer between the gas phase to the sorbent phase. Under constant stirring of the solution, extraction equilibrium was reached within 30 s for benzene and toluene, 40 s for ethylbenzene and p-xylene, 90 s for o-xylene. Under same conditions, the commercial 100 µm PDMS fiber required several minutes to reach equilibrium. The porous structure of sol-gel Superox-4 coating was also responsible for faster and complete desorption of the extracted analytes and thereby minimized the
possibility of sample carryover or memory effect are frequently encountered by commercial coatings. The complete desorption of extracted BTEX was done within 20 s at 280 °C. The porous structure of sol-gel Superox-4 coating dramatically increased its available surface area as well as sample capacity for extraction. Therefore, even a thinner sol-gel coating was likely to demonstrate a higher extraction capability compared to commercial coatings. In order to compare relative extraction efficiency of sol-gel Superox-4 and four commercial coatings (85 µm PA, 100 µm PDMS, 7 µm PDMS, and 65 µm CW/DVB), BTEX components were extracted under identical extraction conditions and analyte mass in unit volume of each coating was calculated. The extraction results (Figure 3.8) clearly demonstrated the superiority of sol-gel Superox-4 coating among all the coatings tested. Mass of BTEX absorbed per unit volume went in the following order: sol-gel Superox-4 > CW/DVB > 7 µm PDMS > 100 µm PDMS > 85 µm PA. Due to the presence of benzene ring in CW/DVB coating capable of providing π-π interactions toward the BTEX analytes, CW/DVB coating demonstrated high affinity towards aromatic compounds. Sol-gel coatings demonstrated excellent extraction efficiency, remarkably high thermal stability, and long life span. Even after 150 repeated extraction operations on sol-gel Superox-4 coating, no sign of efficiency loss was observed. Sol-gel Superox-4 coated fibers were also used to extract phenols, an environmentally important class of compounds due to their toxicity and ubiquity and widespread use in the industry. Being highly polar, PA coatings are most commonly employed for extracting phenols. In headspace-SPME using a
Figure 3.8 Comparison of analyte mass in unit volume of sorbent using five different fibers to extract BTEX from 10 ng/mL aqueous solution (1. benzene, 2. toluene, 3. ethylbenzene, 4. p-xylene, o-xylene) (Reproduced from Ref. [62] with permission from Elsevier)
sol-gel Superox-4 coating extraction equilibrium was reached within 40 and 50 min depending on the compound. Since phenols are acidic, low pH value of the matrix ensures that analytes are in neutral form and thus enhanced extraction efficiency of the coating. The pH value was adjusted to 1 by adding HCl to the matrix. Salting out is another factor that provides higher extracted amounts. To exploit this factor, 3 g of NaCl was added to 10 mL of aqueous solution. Extraction was carried out on commercial 100 μm PDMS, 85 μm PA as well as on sol-gel Superox-4 coatings. Optimum extraction temperature was found to be 30 °C. As the affinity of a particular coating is mostly determined by the principle “like dissolves like”, PDMS coating being nonpolar is not suitable for extracting polar compounds like phenols. On the other hand, PA coating being highly polar should have enhanced affinity toward polar compounds like phenols. Actually, both PA and sol-gel Superox-4 coatings demonstrated high affinity for phenols, the sol-gel Superox-4 outperforming the PA coating. It is believed that the presence of free hydroxyl group at the free end of the Superox-4 polymer chain may have enhanced the polarity of the coating to some extent and thus contributed in extraction efficiency of these polar analytes. Furthermore, highly porous nature of the sol-gel coating increased the surface area and sample capacity of the coating. As a result, a 40 μm sol-gel Superox-4 coating performed even better than a 85 μm PA coating. Unlike commercial coatings, sol-gel coatings contain both organic and inorganic components that provide unique selectivity to the coating. As a result, such coatings are suitable for both polar and nonpolar compounds. Sol-gel Superox-4 coating was also used to extract naphthalene congeners, phthalic diesters and organochlorine pesticides. Sol-gel Superox-4 coating demonstrated sufficient selectivity for all three nonpolar compound classes. It is obvious
that by varying the proportions of the sol solution ingredients or using surface
deactivation reagent (to derivatize residual silanol groups), the net composition as well as
polarity of the coating can be changed leading to a change in selectivity.

Carbowax 20 M is one of the most popular and extensively studied polar
polyethylene glycol based stationary phases used in gas chromatography. Augusto and
coworkers [63] recently reported a sol-gel method for chemical immobilization of
Carbowax 20 M onto fused silica fiber and demonstrated its performance superiority over
commercial PDMS and CW/DVB coatings in headspace SPME of BTEX. Physico-
chemical and morphological characterization of such coatings was also presented. The sol
solution was prepared by thorough mixing of 800 mg Carbowax 20M, 400 µL MTMS,
500 µL of TFA (5% water). The sol-gel coating was made by dipping fused silica fiber (1
cm) into the sol solution. The fibers were kept inside the solution for 1 h and then fresh
sol solution was used to continue the coating process. The coating process was repeated
five times so that the coating thickness became 8 ±1 µm. The fibers were then
conditioned at 230 °C under continuous helium flow for up to 60 h. As was revealed from
thermogravimetric analysis (TGA), a major loss in mass (39.7% of original coating)
ocurred at 108 °C even though the sol-gel material was dried at 110 °C overnight prior
to thermogravimetric analysis. It is believed that conventional drying in oven was not
adequate to release water and other low molecular mass reaction products entrapped
inside the sol-gel polymeric networks. Another diminutive loss in mass was observed at
around 230-250 °C. This insignificant loss in mass may have been due to loss or
degradation of unbound molecules of Carbowax 20M. Finally, a major weight loss was
observed in the temperature range 375-413 °C which accounted for 48.6 % of the initial
mass. The leftover mass (11.8%) was believed to be pure SiO₂. As the first two events of weight loss were likely to be merely due to the elimination of extraneous substances (e.g., water, low molecular mass of reaction products, unbonded molecules of Carbowax 20M etc.), the final event of weight loss was considered as characteristic thermal degradation of sol-gel Carbowax 20M and can be assigned to its maximum operating temperature.

Scanning electron micrograph of sol-gel Carbowax 20M coating [Figure 3.9] demonstrated a highly porous, sponge-like network consisting of agglomerates of microspheres with up to ~ 2 µm in diameter.
Figure 3.9 Scanning electron micrograph (600 x magnification) of a sol-gel Carbowax 20 M ormosil fiber (Reproduced from Ref. [63] with permission from Elsevier)
Several researchers pointed out that the presence of PEG in sol-gel matrix helps in controlling pore size distribution in it, assuming that PEG molecules mostly lie between micropores and mesopores (<20 nm) [64]. Perhaps the presence of mesopores and micropores in the sol-gel network are responsible for massive weight loss at 108 °C in thermogravimetric analysis even after heating at 110 °C overnight in conventional oven which demand more severe condition to expel entrapped water and other unbonded reaction products from the pores. This observation led Augusto and coworkers [63] to adopt a more stringent condition for thermal treatment, keeping the fibers at 230 °C for 50 h. As was expected, the long period of thermal conditioning of sol-gel Carbowax 20M increased the precision of analysis, bringing the RSD to very low value (2.9%).

Since Carbowax 20M not only helps controlling the pore size distribution of the sol-gel network but also gets chemically incorporated through terminal hydroxyl groups, sol-gel Carbowax 20M coated fibers demonstrated excellent selectivity toward different organic compounds. Extraction efficiency of sol-gel Carbowax 20M coated fiber was compared to that of commercial PDMS and Carbowax fiber for headspace SPME of BTEX. Extraction equilibrium was reached between 3 min and 10 min depending on the compound in sol-gel Carbowax 20M. Both commercial coatings required much longer time to reach extraction equilibrium. Similarly, complete desorption of all compounds required only 20 s. Fast mass transfer to and from the fiber coating as observed in sol-gel Carbowax 20M are typical of all sol-gel coated sorbents. This became possible due to the combination of reduced coating thickness (in this case ~8 µm, without compromising extraction sensitivity) and highly porous coating structure provided by sol-gel coating process. Experimental findings indicated that extraction efficiencies were comparable in
case of benzene and toluene. However, in case of o-xylene, the sol-gel Carbowax 20M provided significantly higher extracted amount compared to the masses extracted with 100 µm PDMS and 65 µm Carbowax-DVB fibers (230 and 540 %, respectively). Such an efficient extraction was obtained on the sol-gel carbowax fiber in spite of the fact that the sorbent volume of sol-gel Carbowax 20M (0.017 mm$^3$) was only 2.6 and 4.6% of the coating volume of 100 µm PDMS and 65 µm Carbowax-DVB coatings, respectively.

3.3.2.2 Non-polysiloxane sol-gel sorbents with alkyl ligands

$C_8$- and $C_{18}$-bonded porous silica particles have long been used as popular chromatographic stationary phases due to their chemical integrity, excellent chromatographic selectivity, and chemical inertness of the silica particles.

Tempted by their positive attributes and performance as chromatographic stationary phases as well as highly porous nature of silica particles that may have facilitated faster mass transfer of analytes during extraction, Liu et al. [65] attempted to immobilize $C_8$- and $C_{18}$-coated porous silica particles onto a stainless steel support using a high-temperature epoxy resin. Due to the high specific surface area of the immobilized porous silica particles and very thin layer of bonded phases ($C_8$- or $C_{18}$-), higher extraction sensitivity as well as faster extraction equilibrium time was observed when compared with commercial coatings. Although worked well with highly volatile compounds, its maximum operating temperature (> 250 °C) put a serious limitation on its use in the extraction of higher molecular weight compounds. Longer desorption time, sample carryover, and memory effect are among many problems encountered frequently.
when such coatings are used to extract semi-volatile or low-volatile compounds. Merely physical immobilization of coated silica particles onto the substrate using epoxy glue is to blame for poor thermal stability of the coating. Moreover, such sorbents may not provide high stability in liquid environments involving organic solvents commonly used in liquid-phase separation. In fact, almost all commercial SPME fibers have been facing thermal and solvent stability restrictions due to the lack of chemical bonding to the substrate (fiber or capillary surface).

In order to overcome the low thermal and solvent stability of C₈-coated silica particles, Gbatu et al. [66] developed a sol-gel chemistry based hydrophobic octyl coating for SPME and evaluated its applicability for extracting organometallic compounds from water followed by HPLC. Other parameters (e.g., impact of changing the ratio of sol solution ingredients, sol-gel reaction time, effect of organic solvents, effect of pH on the coating) were also investigated.

The sol solution was prepared by mixing C₈-TEOS, MTMOS, methanol, hydrochloric acid and water. As C₈-TEOS and MTMOS are the only reactants in the sol solution, their relative proportions were optimized by using different molar ratios of C₈-TEOS: MTMOS (0.5:1, 1:1, 2:1). After preparing the sol solution, one end of the previously treated fused silica fiber (1 cm) was immersed into it and held there for 20 min. During this residence time in the sol solution, a three dimensional porous network gradually evolved. In this process, a part of the sol-gel material became chemically bonded to the surface leading to the formation of a coating. The fiber was end-capped by dipping into a solution of trimethylmethoxysilane/methanol (4:1 v/v). Finally the coated fibers were thermally conditioned at 130 °C by placing them in a GC injector under
constant helium flow. Scanning electron microscopic (SEM) images were taken for different coatings that were prepared using different molar ratio of C8-TEOS/MTMOS (0.5:1; 1:1, 2:1). The SEM images revealed that the coating with C8-TEOS/MTMOS molar ratio 2:1 had some cracking. Molar ratio of 1:1 and 0.5:1 did not produce any apparent crack. All three different coatings were used for extraction of organometals in order to evaluate their extraction efficiencies. Extraction results indicated that the higher the ratio of C8-TEOS/MTMOS in sol solution, the better it extracts nonpolar analytes. But in order to prevent the likelihood of being cracked, a molar ratio of 1:1 was taken as optimum.

Optimization of hydrolysis reaction time was done using the sol solution having C8-TEOS/MTMOS in 2:1 molar ratio. Different reaction time was used for generating sol-gel coating and the resultant coatings were evaluated by extracting an aqueous solution of triphenylarsine (Ph3As), diphenylmercury (Ph2Hg) and trimethylphenyltin (TMPHt). It was found that when the hydrolysis reaction was allowed to continue for more than 4 h, extraction efficiency did not increase significantly with time. This signified the completion of hydrolysis of process and continuity of polycondensation. The estimated time of hydrolysis was in good agreement with the data obtained other researchers [13].

In HPLC, organic solvents are used to desorb the extracted analytes from the SPME fibers. However, the stability of the fiber coating towards the desorbing solvent is a limiting factor. It is worth mentioning that most of the commercial coatings, being physically immobilized on the substrate, do not survive when exposed to the common mobile phases used in HPLC [25]. The integrity of sol-gel C8- coating in organic solvents
was evaluated by exposing it to xylene and methylene chloride. In this experiment, sol-gel coatings were employed for extraction before and after exposing to organic solvents for up to 12 h. No apparent loss in extraction efficiency of the coating after such long exposure to organic solvents is indicative of excellent solvent stability of the coating. On the contrary, when commercial SPME fibers are exposed to these solvents, the coating tend swell and slip off the fiber.

Another important factor that needs to be checked is the pH stability of the coating because adjusting pH of the sample is a common practice in achieving enhanced extraction efficiency, particularly when the analytes are organic acids and bases. The sol-gel coating was found to remain intact even after being exposed to pH 0.3 and pH 13 for 16 h. High coating stability towards organic solvents and under very low and high pH conditions may be attributed to the fact that the coating is chemically bonded to the substrate.

Sol-gel coatings are characterized by porous structure which remarkably shortens the analyte extraction equilibrium time due to the faster mass transfer. Because of the same reason, sol-gel coatings also provide faster analyte desorption compared to commercial coatings. As was revealed in extraction time profile, sol-gel C₈- coated fiber required 20 to 30 min to reach extraction equilibrium depending on the compound. By comparison, in case of partially cross linked PDMS/DVB coating, extraction equilibrium was not reached even after an hour. The difference in extraction equilibrium time between the two phases may be attributed to the rate of diffusion within the coating. Sol-gel C₈- coating, being porous and thinner, favors faster diffusion resulting in shorter equilibration time.
Jinno and co-workers [67-68] interfaced solid phase microextraction with micro-LC and evaluated the performance of the hyphenated system by employing five SPME fibers: commercial polyacrylate (PA), commercial Carbowax/template (CW/TEP), sol-gel C_{11} PDMS, commercial PDMS, and methyl-octyl poly(dimethylsiloxane) for extracting benzodiazepines from human urine samples. Sol-gel C_{11} PDMS was found to offer the highest extraction efficiency for benzodiazepines in aqueous media.

3.3.3 Cyclodextrin-based non-polysiloxane sol-gel sorbents

Cyclodextrins and their derivatives have long been used as chromatographic stationary phases especially in chiral separations. Their unique properties, in particular, presence of a chiral cavity, the shape and size selectivity as well as its ability to form inclusion compounds with various analytes have made them very promising chromatographic stationary phases. Multiple retention mechanisms, including interaction of solute with the cyclodextrin cavity are said to be involved in the selectivity of these phases.

Considering the positive attributes of cyclodextrins as chromatographic stationary phases, Fan et al.[69] developed a sol-gel method for preparing sol-gel β-cyclodextrin coating for in-tube SPME coupled to HPLC for the determination of non-steroidal anti-inflammatory drugs in urine samples. In order to prepare the sol solution, 0.1 mL TEOS was added to 0.1 mL 0.01 M HCl and stirred at 60 °C until became homogeneous. Another solution was made using 0.05 g 3-glycidoxypropyltrimethoxysilane (KH-560) derivatized β-cyclodextrin dissolved in 0.3 mL acetonitrile and 0.5 mL 0.01M HCl. Both
solutions were mixed thoroughly and centrifuged. The resulting sol solution was then allowed to fill the capillary and kept inside for 20 min. The sol solution was then expelled from the capillary using slow nitrogen flow. To accomplish desired coating thickness, the coating process was repeated three times. Finally the capillary was aged for 48 hrs.

A 60 cm piece of sol-gel cyclodextrin coated capillary was used for in-tube SPME. The urine samples to be analyzed were centrifuged to remove any solid particles present in the matrix. Clean and particle-free urine samples were then diluted 10 times and spiked with analytes for extraction.

A modification in the configuration of in-tube SPME-HPLC has been proposed where the coated capillary was directly connected to the six-port injection valve minimizing the necessity of additional connecting tubes. The requirement of a costly autosampler could be replaced by using a pump and a six-port valve with a PEEK tubing as the sample loop. The sample volume could be controlled accurately and precisely by controlling the valve switch-reswitch time interval and the flow rate of the carrier solution. Moreover, as the extraction segment was independent of the analysis segment, both processes could be run simultaneously and thus minimizing the whole analysis time. The new configuration was believed to minimize the analyte accumulation problem in the mobile phase as pointed out by Raghani and Schultz [70].

Extraction profile of ketoprofen (KEP), fenbufen (FEP) and ibuprofen (IBP) on sol-gel β-cyclodextrin coating were investigated. In a carrier solution flow rate of 500 µL/min, equilibrium was reached for KEP within 69 s which corresponds to 575 µL sample volume whereas extraction equilibrium for FEP and IBP was not reached within this time period. It is known that under a given flow rate, the greater the sample volume
the higher is the extraction until the extraction equilibrium is reached. In order to make a compromise between the sample volume and method sensitivity for routine analysis, a sample volume of 250 µL was selected. The time required to obtain extraction equilibrium depends on various factors, (e.g., analyte distribution constant, volume of the coating, sample flow rate etc.). Keeping the sample volume constant, different flow rates were employed to find optimum flow rate. A flow rate of 300 µL/min was found optimum for the experiment. In general, addition of inorganic salts (e.g., NaCl) to the sample matrix increases the extraction efficiency by reducing the solubility of analyte(s) in water. An opposite trend was observed for KEP. It was explained assuming that the added salt could compete with the analytes to form inclusion compound with β-cyclodextrin and thereby affect the distribution equilibrium of the analytes. It is known that β-cyclodextrin cavity has a preferential affinity for the neutral form of acids [71]. As the analytes were weakly acidic in nature, low pH of the matrix should favor the extraction since it is likely that at low pH they remain neutral in aqueous solution. Contrary to the assumption, extraction efficiency for the drugs was increased with the increase in pH from 3 to 7. Besides inclusion complexation, there could be other factors that may play important roles in the interaction between the drugs and β-cyclodextrin. Sample matrix is such a factor. When the extraction efficiency of a standard aqueous solution and a spiked urine solution was compared, a significant loss in extraction efficiency was observed for urine sample. The co-existence of inorganic salt and protein are thought to be responsible for this loss. It is expected that sol-gel β-cyclodextrin coatings will prove to be an effective SPME sorbent not only for drugs in biological samples but also for other compounds in various matrices.
3.4 Miscellaneous sol-gel sorbents

In this category, several unique sol-gel based SPME sorbents are discussed that include low temperature glassy carbon film and sol-gel DHB film.

Teng et al. [72] proposed a method for combining solid phase microextraction with sol-gel assisted laser desorption/ionization mass spectrometry (SGALDI-MS) by using an optical fiber coated with a thin sol-gel dihydroxybenzoic acid film as the SPME fiber. A 6 cm piece of optical fiber (125 µm o.d.) was used for this experiment. A clean segment (2 cm) of this fiber was dipped into the sol solution containing 2,5-dihydroxybenzoic acid (DBH), tetraethoxysilane (TEOS), water and hydrochloric acid for one min. Due to the hydrolysis of the precursor and subsequent condensation of its hydrolysis products as well as DBH, a thin film was formed on the outer surface of the optical fiber. Extraction of the target analyte(s) was carried out by immersing the coated end of the fiber into the solution containing the analyte(s) for 1 min. After the extraction, the fiber was placed on a sample target. Finally the sample target was directly inserted into the mass spectrometer for SGALDI-MS analysis. Benzo[a]pyrene has been extracted successfully from an aqueous solution using sol-gel DBH coated fiber. It is possible to extend the application of SPME-SGALDI by carefully selecting suitable sol-gel material.

Glassy carbon is one of the most extensively studied solid sorbent used in solid-phase extraction (SPE) [73], high performance liquid chromatography (HPLC) [74], supercritical fluid chromatography (SFC) [75], and gas chromatography (GC) [76]. Glassy carbon has unique chemical and structural features in compared to other forms of carbon. It consists of long sheets of hexagonally oriented sp$^2$- hybridized carbon atoms.
The flat surface of the glassy carbon leads to unique retention and selectivity characteristics, producing greater shape selectivity than is obtained on silica bonded phases [77].

Considering the unique shape selective property of glassy carbon, Giardina et al. [78] proposed a method of utilizing glassy carbon as an SPME sorbent. In preparing the coating, porous silica was coated with LTGC by dissolving it into heptane/methylene chloride mixture and then slowly drying the solvent system that leaves a thin coating of LTGC onto the silica particles. The coated silica particles were then thermally treated at different temperatures ranging from 300 °C to as high as 1000 °C for at least 10 hrs. The coating was made on 1.5 cm segments of stainless steel fiber (~127 µm o.d.). To immobilize the LTGC coated silica particles onto the stainless steel fibers, a sol-gel solution was prepared by mixing formamide with Kasil No. 1 (SiO₂/K₂O). The fibers were first soaked with sol-gel solution immediately followed by dipping into the LTGC coated silica particles. The fibers were then gently tapped to remove any excess LTGC-coated silica particles. The process continued for 10 times to form a coating of desired thickness.

Selectivity of immobilized LTGC-coated silica has been thoroughly investigated by using a wide range of aromatic hydrocarbons and some taste and odor contaminants (e.g., geosmin, 2-methylisoborneol, and 2,4,6- trichloroanisole) commonly found in water supplies. Experimental data revealed that LTGC coating had the highest affinity for molecules possessing the greatest cross-sectional surface area and polarizability. Another important finding was that the selectivity of LTGC increased as a function of LTGC processing temperature.
3.5 References for Chapter Three


Chapter Four

Capillary Microextraction on Sol-Gel Benzyl Terminated Dendrimer Coating

4.1 Introduction

Solid-phase microextraction (SPME) [1] is now considered to be a fairly mature sample preparation technique with a wide variety of applications ranging from environmental to biomedical to agricultural, and a host of other samples of scientific and industrial importance. SPME successfully overcomes the inherent shortcomings of conventional sample preparation methods by completely eliminating the use of organic solvents and by integrating a number of sample handling operations such as extraction, preconcentration, and sample introduction for instrumental analysis. In addition, SPME is a simple, inexpensive, easy-to-automate, portable, and fast sample preparation technique. Due to these positive attributes, SPME has experienced an explosive growth since its inception over a decade ago.

SPME is based on the distribution of analytes between the sample matrix and the extracting phase coated either on the outer surface of a solid fiber (fiber SPME) or on the inner surface of a fused silica capillary (in-tube SPME or capillary microextraction, CME) [2]. Various SPME coatings have been successfully used to accomplish solvent-free extraction of analytes from different matrices. Among the used SPME coatings are polydimethylsiloxane (PDMS)[1], Polyacrylate [3], Carbopack [4], polyimide [5],
polypyrrole [6], molecularly imprinted materials [7,8], Carbowax/divinylbenzene (CW/DVB) [9], polydimethylsiloxane/divinylbenzene (PDMS/DVB) [10], polydimethylsiloxane/Carboxane (PDMS/Carboxane) [11], Carbowax/templated resin (CW/TPR) [11], sol-gel PDMS [12,13], sol-gel PEG [2,14], and sol-gel crown ether [15,16] that have been successfully applied for the extraction of analytes from different matrices. The extraction affinity is determined by various types of intermolecular and steric interactions between the analyte species and the coating of the extracting phase. Thus, selective extraction of analytes can be achieved based on their polarity, hydrophobicity, chemical composition, shape/size, etc. Selective extraction by SPME has often been performed based on solute polarity. However, such an approach is not very effective for samples where both polar and nonpolar contaminants are present, and both types need to be analyzed. For such samples, it is very important to have a sorbent that can extract both polar and nonpolar compounds simultaneously with high extraction sensitivity.

Most of the SPME coatings that have been used so far are based on linear organic polymers. Linear polymers have some inherent shortcomings for being used as SPME coatings. They possess a wide range of molecular weight distribution responsible for considerable variations in their physical properties [17]. The large dispersity of linear polymers makes it difficult to achieve batch-to-batch reproducibility. Moreover, linear polymers are highly viscous and poorly soluble in common organic solvents, putting limitations in their effective use as SPME coatings.

Dendrimers [18, 19] are highly branched macromolecules that can easily overcome many of the inherent shortcomings of linear polymers. Dendrimers are created
in a step-wise fashion from simple branched monomeric units, the nature and functionality of which can be easily controlled and varied. The supramolecular properties of dendrimers can be effectively tailored by creating desired functional groups on either the core [20], the peripheral surface [21], the branching unit [22], or at multiple sites within the dendrimer [23]. Dendritic macromolecules possess physical properties that, in many cases, greatly differ from those of their linear analogs. For example, their monodisperse structure is built in generations: layer by layer around a core moiety [24]. In organic solvents, they exhibit high solubility and low viscosity compared with their linear analogs [25]. These discrepancies in physical properties are reflections of the fundamental differences in the molecular architectures of these two types of macromolecules providing drastically different numbers of terminal functional groups [26].

Dendrimers possess open and vacuous structures characterized by channels and pockets, especially in higher generations [27]. Unlike 1st and 2nd generations, the higher generation dendrimers have greater internal surface area compared with the external surface area [28]. Therefore, 3rd and higher generation dendrimers should be well suited for applications where high surface area (both internal and external) is a prerequisite. Because of their tree-like branched architecture, functionalized dendrons are potential candidates for novel sorbents in analytical sample enrichment and separations. This opens new possibilities in achieving enhanced selectivity, sensitivity, and performance in chromatographic separations and sample preparations.

To date, in the area of analytical separations, dendrimers have been used as: (a) pseudo-stationary phases in electrokinetic chromatography [29-31], (b) bonded stationary
phases in capillary electrochromatography [32], (c) chiral stationary phases in HPLC [33], and (d) GC stationary phases [34].

Effective immobilization of the polymeric coating on fused silica fiber or capillary inner surface is a prerequisite for the maximum utilization of its analytical potential. However, it is often difficult to achieve acceptable degree of immobilization of thick SPME stationary phase coatings through conventional approaches [35]. As has been pointed out by Chong et al. [12], the absence of chemical bonds between the polymeric coating and the fused silica fiber surface is responsible for low thermal and solvent stability of conventionally coated SPME fibers. Low thermal stability of thick coatings essentially leads to incomplete sample desorption and sample carryover problems. On the other hand, low solvent stability of coatings presents a significant obstacle to the hyphenation of in-tube SPME (capillary microextraction) with liquid-phase separation techniques since organic or organo-aqueous liquids are employed for the desorption of analytes from the SPME coating used for extraction [36,37].

Most of the difficulties associated with the creation and immobilization of thick stationary phase coatings on the fused silica surface can be effectively addressed by using sol-gel coating technology [11,14,38,39] In the context of SPME, major advantageous features of sol-gel technology are as follows: (1) it combines the surface treatment, deactivation, coating, and stationary phase immobilization into a single-step procedure making the whole SPME fiber/capillary manufacturing process fast, efficient, and cost-effective; (2) it creates chemical bonds between the fused silica surface and the sol-gel coating; (3) it provides SPME coatings with high thermal and solvent stabilities, and thereby opens the possibility to expand the SPME application range toward higher
boiling compounds on one hand, and thermally labile compounds on the other; (4) it provides an effective pathway to combine organic and inorganic material properties to achieve enhanced selectivity; (5) it provides SPME coatings with a porous structure responsible for increased surface area allowing the use of thinner coatings to achieve acceptable extraction phase loading and sample capacity.

A recent publication from our group [61] describes sol-gel dendrimer coatings in analytical microextraction. In this dissertation, we provide a detail account on sol-gel approach to in situ creation of dendritic sorbent on the inner walls of fused silica capillaries to achieve solventless extraction of both polar and nonpolar trace analytes from aqueous samples.

4.2 Experimental section

4.2.1 Equipment

Sol-gel dendrimer CME-GC experiments were carried out on a Shimadzu model 17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector (FID) and a programmed temperature vaporizer (PTV) for sample introduction. An in-house built gravity-fed sample dispenser was used to deliver the aqueous samples through the sol-gel dendrimer coated capillary during CME experiments. A Fisher Model G-560 Ginie 2 vortex (Fisher Scientific, Pittsburgh, PA) was employed for thorough mixing of different solutions. A Microcentaur model APO 5760 microcentrifuge (Accurate Chemical and Scientific Corporation, Westbury, NY)

177
was employed (@ 13000 RPM, 15682 g) to separate particulates from the sol solutions used for coating the extraction capillaries as well as the GC columns. A homemade, gas pressure-operated filling/purging device [Figure 4.1] was used to fill the fused silica capillary with the sol solution or purge it with helium gas at various stages of coating and extraction procedures. A Barnsted Model 04741 Nanopure deionized water system (Barnsted/Thermodyne, Dubuque, IA) was used to obtain 17.2 MΩ ultra pure water. A JEOL model JSM-35 scanning electron microscope was used to obtain SEM images of the sol-gel dendrimer coatings. On-line data collection and processing were done using ChromPerfect software (Version 3.5) for Windows (Justice Laboratory Software, Denville, NJ).

### 4.2.2 Chemicals and materials

Fused-silica capillary (250 µm i.d.) with an external protective polyimide coating was purchased from Polymicro Technologies Inc. (Phoenix, AZ). A two-way fused silica press-fit connector (Polymicro Technologies Inc., Phoenix, AZ) was used to interface the extraction capillary with the front end of the GC capillary column inside the chromatographic oven. Benzyl-terminated dendron with triethoxysilyl-derivatized stem was synthesized in one of our laboratories following a procedure described elsewhere [34]. Hydroxy-terminated PDMS was purchased from United Chemical Technologies, Inc. (Bristol, PA). Two types of trimethoxysilyl-derivatized poly(ethylene glycol) (M-SIL-5000 and SIL-3400) were obtained from Shearwater Polymers (Huntsville, AL). Acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, nonyl aldehyde, \( m- \)
tolualdehyde, undecylic aldehyde, butyrophenone, valerophenone, hexanophenone, heptanophenone, benzophenone, 2-chlorophenol, 3,4-dichlorophenol, 3,5-dimethylphenol, and 2,4,6-trichlorophenol were purchased from Aldrich Chemical Co. (Milwaukee, WI) and n-decyl aldehyde was purchased from Sigma Chemical Co. (St. Louis, MO). Methanol (HPLC grade) and all borosilicate glass vials were purchased from Fisher Scientific (Pittsburgh, PA).

4.2.3 Preparation of sol-gel dendrimer coated extraction capillaries

Preparation of sol-gel coated extraction capillaries involves five distinct and sequential operations: (1) cleaning and pretreatment of the fused silica capillary, (2) preparation of the sol solution, (3) coating the fused silica capillary with the sol solution, (4) thermal conditioning of the coated fused silica capillary, (5) rinsing of the coating with organic solvents to remove unbonded materials (if there is any).

4.2.3.1 Cleaning and hydrothermal treatment of the fused silica capillary

In order to clean the inner surface of the fused silica capillary from organic contaminants, it was first sequentially rinsed with methylene chloride and methanol. One milliliter of each solvent was passed through the capillary sequentially using the filling/purging device (Figure 4.1). The rinsed fused silica capillary was then purged with
helium for 30 min followed by hydrothermal treatment. Hydrothermal treatment is a very important step that helps generating adequate surface silanol groups required for the formation of strong chemical bonds between the substrate and the growing sol-gel network. To perform hydrothermal treatment, the cleaned fused silica capillary was filled with Nanopure deionized water using the filling/purging device and then the water was flushed out of the capillary with the aid of helium gas pressure. The capillary was then purged with helium gas for 30 min so that only a thin layer of water remained on the surface of the capillary. At this point, both the ends of the fused silica capillary were sealed with oxy-acetylene flame. The sealed capillary was then heated at 250 °C in a GC oven for 2 hours. Both the ends were then cut open with a ceramic wafer. One end of the fused silica capillary was then connected to the GC injection port with the help of a graphite ferrule and the capillary was heated again in the GC oven at 250 °C for 2 hours using a continuous helium flow through the capillary. After this, the capillary was ready for coating.
Figure 4.1 Schematic of a homemade capillary filling/purging device for preparation of capillary microextraction capillaries and open-tubular sol-gel GC columns.
4.2.3.2 Preparation of the sol solution

The used sol solution consisted of an alkoxide precursor, a sol-gel-active (either hydroxy or alkoxy silane terminated) organic polymer, one or more surface deactivating reagents, appropriate organic solvent(s), and a sol-gel catalyst. Table 4.1 presents the names, functions, and chemical structures of different ingredients of the sol solution used in preparing sol-gel dendrimer coated microextraction capillaries. The sol solution was prepared by dissolving methyltrimethoxysilane (sol-gel precursor, 5 µL), phenyl-terminated 3rd generation dendrimer with a triethoxysilyl containing root (sol-gel active organic ligands, 50 mg), hexamethyldisilazane (surface deactivation reagent, 10 µL), polymethylhydrosiloxane (surface deactivation reagent, 25 µL), and trifluoroacetic acid (containing 5% water) (sol-gel catalyst, 50 µL) in methylene chloride (solvent, 900 µL). After adding all the ingredients, the resultant solution was vortexed (5 min) for thorough mixing of the constituents followed by centrifugation (5 min) to remove any precipitates from the solution before using it for coating.
Table 4.1 Name, function and chemical structure of sol-gel dendrimer coating solution ingredients

<table>
<thead>
<tr>
<th>Name</th>
<th>Function</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyltrimethoxysilane</td>
<td>Sol-gel precursor</td>
<td><img src="image" alt="CH3O-Si-OCH3" /></td>
</tr>
<tr>
<td>Phenyl-terminated dendrimer with a triethoxysilyl root</td>
<td>Sol-gel precursor containing a dendritic ligand</td>
<td><img src="image" alt="CF3COOH" /></td>
</tr>
<tr>
<td>Trifluoroacetic acid/Water 95:5 (v/v)</td>
<td>Catalyst</td>
<td>CF3COOH</td>
</tr>
<tr>
<td>Methylene Chloride</td>
<td>Solvent</td>
<td><img src="image" alt="CH2Cl2" /></td>
</tr>
<tr>
<td>Hexamethylenedisilazane (HMDS)</td>
<td>Deactivating reagent</td>
<td><img src="image" alt="H3C-Si=NH-Si=CH3" /></td>
</tr>
<tr>
<td>Polymethylhydrosiloxane (PMHS)</td>
<td>Deactivating reagent</td>
<td><img src="image" alt="H3C-Si-O-((Si-O)mSi-CH3" /></td>
</tr>
</tbody>
</table>

4.2.3.3 Coating of the fused silica capillary with sol solution

A hydrothermally treated fused silica capillary (3 m x 250 μm i.d.) was installed in the gas pressure operated filling/purging device (Figure 1) to fill the capillary with the specially designed sol solution. After filling, the sol solution was kept inside the capillary for 30 min to facilitate the formation of a surface-bonded sol-gel dendrimer coating. During this in-capillary residence time of the sol solution, a sol-gel hybrid organic-inorganic network was evolving within the sol solution. A part of this sol-gel material
growing in the vicinity of the fused silica capillary walls ultimately became bonded to the fused silica capillary walls via condensation reaction with silanol groups on the surface. The free unbonded portion of the sol solution was then expelled from the capillary under helium pressure (50 psi) and the coated capillary was purged with helium for an hour. The continuous flow of helium facilitated the evaporation of volatile solvents.

4.2.3.4 Thermal conditioning of the coated capillary

The sol-gel coated capillary was thermally conditioned in a GC oven using temperature-programmed heating from 40 °C to 300 °C @ 1 °C min⁻¹, and holding the capillary at the final temperature for 5 h under helium purge. The purpose of the conditioning was to stabilize the coated sorbent and remove the non-bonded volatile components of the sol solution left in the coating.

4.2.3.5 Rinsing the capillary with organic solvents to remove unbonded materials

Before using in extraction, the coated capillary was rinsed sequentially with 2 mL each of methylene chloride and methanol followed by drying in a stream of helium under the same temperature-programmed conditions as in 4.2.3.4, except that the capillary was held at the final temperature for 30 min. The capillary was further cooled down to
ambient temperature and cut into 13-cm long pieces that were further used to perform microextraction.

**4.2.4 Preparation of sol-gel PDMS coated capillary columns for GC**

A 10 m long piece of uncoated fused silica capillary (250 µm i.d.) was accurately wrapped on a GC column basket in coils. The capillary was sequentially rinsed with 1 mL each of methylene chloride and methanol followed by purging with helium for 30 min. The column was then hydrothermally treated following the procedure described in Section 4.2.3.1. The hydrothermally treated column was installed on the filling/purging device (Figure 4.1) and the helium flow was adjusted to 40 psi.

The coating solution for the sol-gel PDMS GC column was prepared as follows: 50 mg of PDMS was dissolved in 900 µL of methylene chloride. A 5 µL volume of methyltrimethoxysilane (MTMOS), 25 µL of polymethylhydrosiloxane (PMHS), and 50 µL of trifluoroacetic acid (TFA) (5% water v/v) were sequentially added into the vial containing the polymer solution. In order to ensure proper mixing of the ingredients, the sol solution was vortexed after adding each ingredient. As the hydrolysis reaction begins as soon as the sol-gel catalyst is added, a 10-min period was allowed for hydrolysis reaction to proceed. At the end of this 10-min period, 20 µL NH₄F solution (20 mg/mL in methanol) was added to the sol solution to facilitate faster condensation. The resulting sol solution was centrifuged and the clear supernatant was transferred to a clean vial using a micro pipet for further use in column coating.
The sol solution was then introduced into the hydrothermally treated fused silica capillary using helium gas pressure in the filling/purging device (Figure 4.1). Once the capillary was filled with sol-gel coating solution, the filling/purging device was carefully depressurized and the free exit end of the capillary was sealed with a rubber septum while keeping the other end of the capillary inside filling/purging device. The sol solution was kept inside the capillary for 10 min. During this in-capillary residence time of the sol solution, a surface bonded hybrid organic-inorganic film gradually evolved. After this, the sol solution was expelled from the fused silica capillary under helium pressure. The column was then purged with helium for 60 min. The next step was the thermal conditioning of the coated column. For this, one end of the column was connected to the GC injection port using a graphite ferrule and a continuous flow of helium was maintained through the column. The other end of the capillary was kept free inside the GC oven. The column was heated from 40 °C to 150 °C at a rate of 1°C/min, maintained this temperature for 5 h, then heated to 330 °C at a rate of 5 °C/min and holding at this final temperature for 1 h.

After the thermal conditioning, the column was rinsed with 2 mL of methylene chloride/methanol mixture (75:25 v/v), purged with helium for 1 h and then conditioned again from 40 °C to 330 °C at a rate of 5 °C/min, holding it at final temperature for 30 min.
4.2.5 Preparation of sol-gel PEG coated capillary column for GC

A 10-m long piece of fused silica capillary was first cleaned and hydrothermally treated as described in section 4.2.4. It was then installed on the filling/purging device (Figure 4.1) to carry out the coating process.

A sol-gel coating solution was prepared as follows: 35 mg of methoxypoly(ethylene glycol)-silane (PEG 1) and 15 mg of poly (ethylene glycol)-bis silane (PEG2) (sol-gel active organic ligands) were dissolved in 600 µL of methylene chloride (solvent) contained in a polypropylene microcentrifuge vial. A Scientific Products model S8223 Vortex shaker aided the dissolution process (5 min). Then 5 µL of MTMOS (precursor), 10 µL of bis(trimethoxysilylethyl)benzene (precursor), and 5 µL of HMDS (deactivating reagent) were sequentially added to the microcentrifuge vial and thoroughly mixed for 5 min to obtain a homogeneous solution. After this, 50 µL of 95% TFA (acid catalyst containing 5% water) was added to the solution and thoroughly mixed. After 10 min, a 20-µL volume of NH₄F solution (20 mg/mL in methanol) was introduced into the vial. The volume of the solution was made up to 1000 µL by adding the required amount of methylene chloride, and the mixture was thoroughly vortexed. The resulting solution was centrifuged at 13 000 rpm (15,682g) for 5 min. The precipitate at the bottom of the vial, if any, was discarded, and the top clear sol solution was used to fill the hydrothermally treated fused-silica capillary using a helium pressure of 50 psi. After a set period of in-capillary residence time (10-20 min), the solution was expelled from the capillary under the same helium pressure and the capillary was subsequently
purged with helium at 50 psi for an additional 60 min. This was followed by temperature-programmed heating in a GC oven from 40 to 150 °C at 2 °C min⁻¹ with a hold time of 300 min at 150 °C and then from 150 to 280 °C at 6 °C min⁻¹, holding it at 280 °C for 120 min. Keeping the temperature programming rate at 6 °C min⁻¹, the column was further conditioned in small steps, holding the column for 120 min at each of the following final temperatures: 300, 320, and 340 °C. The column was then rinsed with 2 mL of methylene chloride and conditioned again from 40 to 320 °C at 6 °C min⁻¹. While conditioning, the column was purged with helium at 1 mL min⁻¹.

4.2.6 Gravity-fed sample dispenser for capillary microextraction

A gravity-fed sample dispenser was used for capillary microextraction (Figure 4.2). It was constructed by modifying a Chromaflex AQ column (Kontes Glass Co., Vineland, NJ) that consisted of a thick-walled Pyrex glass cylinder concentrically placed in an acrylic jacket. Deactivation of the inner surface of the glass cylinder was accomplished by treating with HMDS solution (5% v/v solution in methylene chloride) followed by heating at 250 °C for 1 hour in an inert gas environment. The cylinder was then cooled to ambient temperature, thoroughly rinsed with methanol and deionized water, and dried in a helium gas flow. The system was then reassembled and was ready for use as a sample delivery device in capillary microextraction.
**Figure 4.2** Schematic of a gravity-fed sample dispensing unit used in sol-gel dendrimer capillary microextraction.
4.2.7 Deactivation of glassware

All glassware used in this study was first cleaned using Sparkleen detergent, thoroughly rinsed with deionized water followed by drying at 150 °C for 2 hours. The inner surface of the dried glassware was then treated with a 5% v/v solution of HMDS in methylene chloride followed by heating in an oven at 250 °C for 8 hours under helium flow. The glassware was then rinsed sequentially with methylene chloride and methanol and dried in the oven at 100 °C for 1 hour. Before use, all glassware was thoroughly rinsed with deionized water and dried at room temperature in a continuous flow of helium.

4.2.8 Preparation of standard sample solutions for sol-gel dendrimer CME

All stock solutions were prepared by dissolving 50 mg of each analyte in 5 mL of methanol in a 10 mL deactivated amber glass vial to obtain a concentration of 10 mg/mL. The solution was further diluted to 0.1 mg/mL in methanol. The final solution was prepared by further diluting this solution in water to achieve µg/mL to ng/mL level concentrations depending on the compound class.

4.2.9 Extraction of analytes on sol-gel dendrimer coated capillaries

A 13-cm long piece of the sol-gel dendrimer-coated capillary (250 µm i.d.) was conditioned in a GC oven using a temperature program (from 40 °C to 300 °C @ 10 °C min⁻¹, held at the final temperature for 30 min) and simultaneously purging the capillary
with helium. The conditioned capillary was vertically connected to the lower end of the gravity-fed sample dispenser (Figure 4.2) using a plastic nut and a ferrule. A 50-mL volume of the aqueous sample containing trace concentrations of the target analytes was added to the inner glass cylinder of the sample dispenser through the inlet located at the top. A small helium gas pressure (5 psi) was maintained in the system to assist the sample flow. The solution was allowed to pass through the capillary for 30 min. During this time, the analyte molecules were extracted by the sol-gel dendrimer coating as the sample passed through the capillary, and the system moved towards an extraction equilibrium. The capillary was further purged with helium gas for 1 min to remove residual water from the capillary walls.

4.2.10 Transferring the extracted analytes to the GC Column and gas chromatographic analysis of the extracted analytes

The extracted analytes were transferred from the microextraction capillary to the GC column via thermal desorption. For this, the CME capillary was first installed on the GC injection port and securedly interfaced with the GC capillary column. To facilitate the installation, both the GC injection port and the oven were cooled to 30 °C, and the quartz wool was removed from the injection port glass liner. The CME capillary with the extracted analytes in the coating was then introduced into the GC injection port from the bottom end of the port so that ~8 cm of the capillary remained inside the injection port. A graphite ferrule was used to make an air-tight connection between the capillary and the injection port. The lower end of the capillary (residing inside the GC oven) was
connected to the inlet end of GC capillary column using a deactivated press-fit quartz connector. Figure 4.3 demonstrates the connection of the extraction capillary with the GC analysis column using a press-fit quartz connector. The temperature of the PTV injection port was then rapidly raised to 300 °C @ 100 °C min⁻¹ to desorb the analytes from the extraction capillary into the carrier gas flow, keeping the GC oven temperature at 30 °C during the whole desorption process (5 min). Under these conditions, the desorbed analytes were efficiently carried over by the flow of helium to the lower end of the extraction capillary and/or front end of the GC column held at 30 °C. As soon as the desorbed analytes reached the cooler CME capillary-GC column coupling zone residing inside the GC oven (30 °C), the analytes were focused into a narrow band. To facilitate transport of the focused zone through the GC column and its separation into individual components, the GC oven temperature was then programmed as follows: from 30 °C to 300 °C @ 15 °C min⁻¹ with a 10 min hold at the final temperature.
Figure 4.3 Schematic representation of the connection of the extraction capillary with the analysis column inside the GC oven using a press-fit quartz connector
4.2.11 Calculation of the limit of detection (LOD) for individual analyte

The limit of detection (LOD) for an analyte is the smallest concentration that can be detected reliably. The LOD is related to both the signal and the noise of the system and usually is defined as the concentration corresponding to a peak whose signal-to-noise (S/N) ratio is 3:1.

In order to calculate the limit of detection (LOD), each compound was extracted individually under same extraction conditions and the peak height of the analyte was calculated in µV. The noise was calculated in µV from the baseline of the chromatogram using the ChromPerfect for Windows (Version 3.5) computer software (Justice Laboratory Software, Mountain Views, CA). The limit of detection of the compound was calculated through intrapolation using the analyte peak height and the corresponding value of the noise.

4.3 Results and discussion

The branched architecture of dendrimers makes them promising candidates for use as extracting materials with distinct advantages over linear polymers used for the same purpose. The main objective of the present work was to investigate the possibility of using benzyl terminated dendrimers as a novel extraction medium for solid phase microextraction. This was accomplished by creating immobilized dendrimer coatings on the fused silica capillary inner surface using principles of sol-gel chemistry.
4.3.1 Sol-gel dendrimer coating and chemical aspects of its preparation

Sol-gel column technology [38] provides an elegant single-step procedure for creating organic-inorganic hybrid stationary phase coatings (both thick and thin) inside the capillary that can be further used to perform capillary microextraction [2] or high-resolution gas chromatographic separations. Sol-gel technology also allows the creation of hybrid coatings on the outer surface of a solid fiber [12] that can be used in conventional fiber-based SPME analysis. In both instances, the coating is chemically bonded to the substrate, and provides high thermal stability required for SPME-GC analysis. Thanks to chemical bonding to the substrate, sol-gel coatings also possess high solvent stability required for hyphenating SPME with liquid-phase separation techniques (e.g., HPLC, MEKC, CEC, etc.) that use organo-aqueous mobile phases.

If an organic polymer or ligand is to undergo sol-gel reaction, it has to be sol-gel-active. The dendrimer used in this study contained ethoxysilyl groups in its root making the dendrimer molecules sol-gel active. Details of the synthesis of sol-gel active dendrimers can be found elsewhere [34].

The ingredients used in the sol-gel dendrimer coating are presented in Table 4.1. As can be seen in Table 4.1, methyltrimethoxysilane (MTMOS) is the second sol-gel precursor used in the coating solution. Under the experimental conditions used, both MTMOS and the triethoxysilyl moieties in the benzyl-terminated dendron can get hydrolyzed in the presence of the sol-gel catalyst, trifluoroacetic acid (TFA), as presented in the reaction Scheme 4.1.
The hydrolyzed precursors can then undergo polycondensation reactions in a variety of ways to create a sol-gel network. Scheme 4.2 represents a simplified model for the polycondensation reaction of hydrolyzed MTMOS.

The growing chain of the sol-gel polymer can also undergo polycondensation reaction with hydrolyzed triethoxysilyl root of the dendron to form an organic-inorganic hybrid network with the chemically incorporated dendrimers as an organic constituent (Scheme 4.3).

The condensation reaction can also take place with the participation of silanol groups on the inner surface of the fused silica capillary. The sol-gel dendritic network developed in the vicinity of the fused silica capillary inner surface can get chemically anchored to the column walls (Scheme 4.4) forming a surface-bonded extracting phase film, and remain as such when the sol-gel solution is expelled after 30 min of residence inside the capillary.

Both polymethylhydrosiloxane (PMHS) and hexamethyldisilazane (HMDS) used in the sol solution as surface deactivation reagents lack sol-gel active sites. Therefore, it can be assumed that they rather get physically incorporated in the sol-gel network, and subsequently react with the silanol groups (Scheme 4.5) during the post-coating thermal conditioning process. This results in a three-dimensional deactivation process taking place within the entire thickness of the sol-gel coating [38] as opposed to traditional two-dimensional deactivation process which is confined only to the capillary surface. Thus the sol-gel technology used for the coating process elegantly combines column deactivation, coating, and extracting phase film immobilization in a simple and effective manner.
Scheme 4.1 Hydrolysis reaction of (a) methyltrimethoxysilane; (b) phenyl-terminated dendrimer with triethoxysilane derivative.
Scheme 4.2 Polycondensation reaction of hydrolyzed methyltrimethoxysilane.
Scheme 4.3 Condensation of phenyl-terminated sol-gel active dendron with the growing sol-gel network formed from MTMOS.
Scheme 4.4 Chemical anchoring of sol-gel dendrimer stationary phase.
Scheme 4.5(b) Deactivation of fused silica capillary inner surface.
4.3.2 Characterization of surface morphology and determination of coating thickness using scanning electron microscopy

Figures 4.4 and 4.5 represent two scanning electron micrographs (SEM) of the inner surface of the sol-gel dendrimer coated capillary. Remarkable uniformity in coating thickness is evident from the SEM image presented in Figure 4.4. The coating thickness was estimated at 0.5 \( \mu \text{m} \). (Figure 4.4). Moreover, sol-gel dendrimer coating possesses a roughened, porous texture (Figure 4.5) with enhanced surface area which is favorable for extraction.
Figure 4.4 Scanning electron microscopic image of a 250 µm i.d. sol-gel dendrimer coated microextraction capillary illustrating the coating thickness. Magnification: 10,000x
Figure 4.5 Scanning electron microscopic image of a 250 µm i.d. sol-gel dendrimer coated microextraction capillary illustrating the typical roughened surface obtained by sol-gel coating process. Magnification: 10,000x
4.3.3 Determination of extraction kinetics for both polar and nonpolar analytes

Figure 4.6 illustrates the CME kinetic profile of a nonpolar analyte (phenanthrene) and a polar analyte (2,4,6-trichlorophenol) extracted on a sol-gel benzyl-terminated dendrimer coated capillary. Extractions were carried out using aqueous samples containing 100 ppb concentration of each analyte. Both for the polar and nonpolar analytes, extraction equilibria were attained within 30 min (Figure 4.6).

Based on these kinetic data, a 30-min extraction time was further used for all samples to ensure that the extraction equilibrium was reached.
Figure 4.6 Illustration of the extraction kinetics of a nonpolar compound (phenanthrene) and a polar compound (2,4,6-trichlorophenol) obtained on a 13 cm x 250 µm i.d. sol-gel dendrimer coated microextraction capillary using 100 ppb aqueous solutions. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 10-50 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 ºC, final 300 ºC; GC oven temperature programmed from 30 ºC (hold for 5 min) to 300 ºC at a rate of 15 ºC min⁻¹; helium carrier gas; FID temperature 350 ºC.
4.3.4 Applications of sol-gel dendrimer coated microextraction capillaries

Sol-gel dendrimer coated capillaries were used to extract a variety of analytes from a wide polarity range (nonpolar to highly polar) and of environmental, biomedical and ecological importance. Test analytes included polycyclic aromatic hydrocarbons (PAHs), aldehydes, ketones, alcohols, phenols and butylated hydroxy toluene (BHT) – a widely used preservative. The extracted solutes were further analyzed by GC-FID.

4.3.4.1 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are among the most common environmental pollutants found in air, water, and soil in the USA and other industrialized countries where petroleum products are heavily used. Toxicity, mutagenicity, and carcinogenicity of these compounds in animals [41] has prompted US Environmental Protection Agency (EPA) to place 16 unsubstituted PAHs in its list of 129 priority pollutants [42]. Among the 16 EPA promulgated unsubstituted PAH, 5 were extracted and analyzed using sol-gel dendrimer coated microextraction capillaries. Table 4.2 provides a list of 5 selected unsubstituted PAHs, their chemical structures as well as pertinent physico-chemical properties. Capillary microextraction results have been presented in Table 4.3 (run-to-run reproducibility data) and Table 4.4 (capillary-to-capillary reproducibility data). Calculated limit of detection data for the selected PAHs have been presented in Table 4.5. Figure 4.7 represents a gas chromatogram of these unsubstituted polycyclic aromatic hydrocarbons from EPA priority pollutants list. They
were extracted from an aqueous sample (each PAH at 10 ppb) using a sol-gel dendrimer coated microextraction capillary. As can be seen from the data presented in Table 4.2, the detection limits obtained for PAHs in CME-GC-FID range between 2.1 ppt and 3.6 ppt. These values are comparable to or better than the detection limits reported in the literature for conventionally coated SPME fibers. For instance, Doong et al.[42] reported a detection limit of 0.25 ng/mL (250 ppt) for fluoranthene obtained by SPME-GC-FID on a commercial 100-µm PDMS coated fiber, which is more than two order of magnitude higher than the value 0.002 ng/mL (2ppt) obtained on sol-gel dendrimer CME-GC-FID.
Table 4.2 Chemical structures and pertinent physical properties of polyaromatic hydrocarbons (PAHs) extracted using sol-gel dendrimer coating

<table>
<thead>
<tr>
<th>Name of the analyte</th>
<th>Chemical structure</th>
<th>Molecular weight (g/mol)</th>
<th>Melting point (°C)</th>
<th>Boiling point (°C)</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthene</td>
<td><img src="image" alt="Acenaphthene" /></td>
<td>152.20</td>
<td>92.5</td>
<td>150.0</td>
<td>0.8987</td>
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<tr>
<td>Fluorene</td>
<td><img src="image" alt="Fluorene" /></td>
<td>166.22</td>
<td>114.8</td>
<td>295.0</td>
<td>1.203</td>
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<tr>
<td>Phenanthrene</td>
<td><img src="image" alt="Phenanthrene" /></td>
<td>178.23</td>
<td>99.2</td>
<td>340</td>
<td>0.9820</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td><img src="image" alt="Fluoranthene" /></td>
<td>202.26</td>
<td>107.8</td>
<td>384</td>
<td>1.252</td>
</tr>
<tr>
<td>Pyrene</td>
<td><img src="image" alt="Pyrene" /></td>
<td>202.26</td>
<td>156</td>
<td>404</td>
<td>1.271</td>
</tr>
</tbody>
</table>
Figure 4.7 CME-GC analysis of PAHs at 10 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min$^{-1}$, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C, programmed at a rate of 15 °C min$^{-1}$; helium carrier gas; FID temperature 350 °C. Peak identification: (1) acenaphthene, (2) fluorene, (3) phenanthrene, (4) fluoranthene, and (5) pyrene
Table 4.3 Run-to-run peak area reproducibility for PAHs in capillary microextraction using sol-gel dendrimer coatings*

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>A x 10^-4</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Run 4</th>
<th>Run 5</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthene</td>
<td>3.10</td>
<td>3.45</td>
<td>3.42</td>
<td>3.04</td>
<td>3.37</td>
<td>3.28</td>
<td>5.27</td>
<td></td>
</tr>
<tr>
<td>Fluorene</td>
<td>4.79</td>
<td>5.25</td>
<td>5.19</td>
<td>4.73</td>
<td>5.13</td>
<td>5.01</td>
<td>4.27</td>
<td></td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>5.65</td>
<td>6.07</td>
<td>5.96</td>
<td>5.95</td>
<td>5.89</td>
<td>5.90</td>
<td>2.33</td>
<td></td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>6.27</td>
<td>6.46</td>
<td>5.95</td>
<td>5.95</td>
<td>5.89</td>
<td>6.38</td>
<td>1.46</td>
<td></td>
</tr>
<tr>
<td>Pyrene</td>
<td>6.24</td>
<td>6.55</td>
<td>6.65</td>
<td>6.63</td>
<td>6.63</td>
<td>6.48</td>
<td>2.56</td>
<td></td>
</tr>
</tbody>
</table>

*CME- GC analysis of PAHs at 10 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min⁻¹, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C, programmed at a rate of 15 °C min⁻¹; helium carrier gas; FID temperature 350 °C. Peak identification: (1) acenaphthene, (2) fluorene, (3) phenanthrene, (4) fluoranthene, and (5) pyrene.

A= Actual computer output for peak area in arbitrary unit.
Table 4.4 Capillary-to-capillary peak area reproducibility for PAHs in capillary microextraction using sol-gel dendrimer coatings*

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>Capillary 1</th>
<th>Capillary 2</th>
<th>Capillary 3</th>
<th>Capillary 4</th>
<th>Capillary 5</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthene</td>
<td>1.94</td>
<td>2.04</td>
<td>1.98</td>
<td>2.04</td>
<td>2.00</td>
<td>2.00</td>
<td>2.08</td>
</tr>
<tr>
<td>Fluorene</td>
<td>4.24</td>
<td>4.21</td>
<td>4.42</td>
<td>4.12</td>
<td>4.40</td>
<td>4.28</td>
<td>2.58</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>4.85</td>
<td>4.93</td>
<td>4.81</td>
<td>4.66</td>
<td>4.81</td>
<td>4.81</td>
<td>2.04</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>6.45</td>
<td>6.46</td>
<td>6.92</td>
<td>6.32</td>
<td>6.54</td>
<td>6.54</td>
<td>3.46</td>
</tr>
<tr>
<td>Pyrene</td>
<td>7.88</td>
<td>8.27</td>
<td>9.04</td>
<td>7.88</td>
<td>8.27</td>
<td>8.27</td>
<td>5.72</td>
</tr>
</tbody>
</table>

*CME- GC analysis of PAHs at 10 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min⁻¹, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C, programmed at a rate of 15 °C min⁻¹; helium carrier gas; FID temperature 350 °C. Peak identification: (1) acenaphthene, (2) fluorene, (3) phenanthrene, (4) fluoranthene, and (5) pyrene.

A= Actual computer output for peak area in arbitrary unit.
Table 4.5: Limits of detection (LOD) data for PAHs in CME-GC-FID using sol-gel dendrimer microextraction capillaries.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (ppb)</th>
<th>Measured Peak Height (µV) (H x 10^{-3})</th>
<th>Limit of Detection (S/N 3), ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthene</td>
<td>10</td>
<td>5.79</td>
<td>3.6</td>
</tr>
<tr>
<td>Fluorene</td>
<td>10</td>
<td>12.95</td>
<td>2.3</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>10</td>
<td>14.19</td>
<td>2.1</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>10</td>
<td>13.25</td>
<td>22</td>
</tr>
<tr>
<td>Pyrene</td>
<td>10</td>
<td>12.72</td>
<td>2.3</td>
</tr>
</tbody>
</table>

*CME- GC analysis of PAHs at 10 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min^{-1}, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C, programmed at a rate of 15 °C min^{-1}; helium carrier gas; FID temperature 350 °C. Peak identification: (1) acenaphthene, (2) fluorene, (3) phenanthrene, (4) fluoranthene, and (5) pyrene.

H= Actual computer output for peak height in µV.
4.3.4.2 Aldehydes and ketones

The sol-gel dendrimer-coated CME capillaries were further used to extract trace levels of aldehydes and ketones (carbonyl compounds) in aqueous samples. Carbonyl compounds play an important role in aquatic oxidation processes. In natural waters, these compounds can be produced by the photodegradation of dissolved natural organic matter [43] as well as a product of microbiological processes [44]. In recent years, carbonyl compounds have been receiving much attention as disinfection and oxidation by-products formed during drinking water treatment processes. Many of these by-products have been shown to carcinogens or carcinogen suspects [45-47]. The polar nature and enhanced reactivity of carbonyl compounds in water matrices often impose the need for their derivatization prior to their extraction and/or detection by chromatographic techniques [48-49]. However, derivatization of these analytes, especially when present in trace concentrations, may complicate the analytical process, leading to poor accuracy and reproducibility. Figure 4.8 is a gas chromatogram representing a mixture of 4 underivatized aldehydes. Table 6 provided a list of these underivatized aldehydes. Microextraction results have been presented in Table 4.7, Table 4.8, and Table 4.9 represents the calculated limit of detection (LOD) values for these underivatized aldehydes.
Table 4.6 Chemical structures and pertinent physical properties of aldehydes extracted using sol-gel dendrimer coating

<table>
<thead>
<tr>
<th>Name of the analyte</th>
<th>Chemical structure</th>
<th>Molecular Weight (g/mol)</th>
<th>Melting Point (ºC)</th>
<th>Boiling Point (ºC)</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonyl aldehyde</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>142.2406</td>
<td>63</td>
<td>93 at 23 mm Hg</td>
<td>0.823</td>
</tr>
<tr>
<td>m-Tolualdehyde</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>120.1506</td>
<td>-</td>
<td>199</td>
<td>1.019</td>
</tr>
<tr>
<td>n-Decylaldehyde</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>156.2674</td>
<td>7</td>
<td>207.209</td>
<td>0.825</td>
</tr>
<tr>
<td>n-Undecylaldehyde</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>170.2942</td>
<td>-</td>
<td>109-115 at 5 mm</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.8 Capillary microextraction-GC analysis of aldehydes at 100 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min\(^{-1}\), final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min\(^{-1}\); helium carrier gas; FID temperature 350 °C. Peak identification: (1) nonylaldehyde, (2) \(m\)-tolualdehyde, (3) \(n\)-decylaldehyde, and (4) \(n\)-undecylaldehyde
Table 4.7 Run-to-run peak area reproducibility for aldehydes in capillary microextraction using sol-gel dendrimer coatings*

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Run 4</th>
<th>Run 5</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonylaldehyde</td>
<td>3.14</td>
<td>3.46</td>
<td>3.58</td>
<td>2.99</td>
<td>3.02</td>
<td>3.24</td>
<td>7.36</td>
</tr>
<tr>
<td>n-Decylaldehyde</td>
<td>17.43</td>
<td>16.20</td>
<td>16.65</td>
<td>18.15</td>
<td>16.63</td>
<td>17.01</td>
<td>4.09</td>
</tr>
</tbody>
</table>

*Capillary Microextraction- GC analysis of aldehydes at 100 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min⁻¹, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min⁻¹; helium carrier gas; FID temperature 350 °C. Peak identification: (1) nonylaldehyde, (2) m-tolualdehyde, (3) n-decylaldehyde, and (4) n-undecylic aldehyde

A= Actual computer output for peak area in arbitrary unit.
Table 4.8 Capillary-to-capillary peak area reproducibility for aldehydes in capillary microextraction using sol-gel dendrimer coatings*

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>A x 10⁻⁴</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Run 1</td>
<td>Run 2</td>
<td>Run 3</td>
<td>Run 4</td>
<td>Run 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonylaldehyde</td>
<td>2.99</td>
<td>3.60</td>
<td>3.61</td>
<td>2.91</td>
<td>3.10</td>
<td>3.25</td>
<td>9.20</td>
</tr>
<tr>
<td>m-Tolualdehyde</td>
<td>8.91</td>
<td>9.27</td>
<td>9.41</td>
<td>10.84</td>
<td>8.76</td>
<td>9.63</td>
<td>6.79</td>
</tr>
<tr>
<td>n-Decyl aldehyde</td>
<td>16.37</td>
<td>15.43</td>
<td>17.88</td>
<td>19.94</td>
<td>17.35</td>
<td>17.41</td>
<td>8.97</td>
</tr>
<tr>
<td>n-Undecyl aldehyde</td>
<td>21.18</td>
<td>17.09</td>
<td>19.75</td>
<td>19.41</td>
<td>19.97</td>
<td>19.73</td>
<td>7.60</td>
</tr>
</tbody>
</table>

*Capillary Microextraction-GC analysis of aldehydes at 100 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min⁻¹, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min⁻¹; helium carrier gas; FID temperature 350 °C. Peak identification: (1) nonylaldehyde, (2) m-tolualdehyde, (3) n-decylaldehyde, and (4) n-undecylic aldehyde

A= Actual Computer output for peak area in arbitrary units.
Table 4.9 Limits of detection (LOD) for aldehydes in CME–GC-FID using sol-gel dendrimer microextraction capillaries*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (ppb)</th>
<th>Measured peak height (µV) (H x 10^{-3})</th>
<th>Limit of detection (S/N 3), ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonylaldehyde</td>
<td>100</td>
<td>10.53</td>
<td>19.4</td>
</tr>
<tr>
<td>m-Tolualdehyde</td>
<td>100</td>
<td>36.86</td>
<td>5.6</td>
</tr>
<tr>
<td>n-Decyl aldehyde</td>
<td>100</td>
<td>63.07</td>
<td>3.3</td>
</tr>
<tr>
<td>n-Undecyl aldehyde</td>
<td>100</td>
<td>58.49</td>
<td>3.5</td>
</tr>
</tbody>
</table>

*Capillary Microextraction-GC analysis of aldehydes at 100 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min^{-1}, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min^{-1}; Helium carrier gas; FID temperature 350 °C. Peak identification: (1) nonylaldehyde, (2) m-tolualdehyde, (3) n-decylaldehyde, and (4) n-undecylaldehyde.

H= Actual computer output for peak height in µV.
Ketones were extracted from an aqueous solution containing 100 ppb of each analyte. The data presented in Table 4.9 indicates that the detection limits obtained for underivatized aldehydes in CME-GC-FID using a sol-gel dendrimer coated microextraction capillary range between 3.5 ppt and 19.4 ppt. These values are fairly comparable to the values reported in the literature, which were achieved through derivatization process using commercial SPME fibers. For instance, Chancho et al. [48] reported a detection limit of 0.02 ng/mL (200 ppt) for decanal obtained by SPME-GC-ECD on a commercial SPME fiber having 65 µm thick DVB-PDMS coating, which is significantly higher than the value 0.003 ng/mL (3 ppt) obtained on sol-gel dendrimer CME-GC-FID. The same trend was also observed for other aldehydes. It is worth mentioning that ECD often provides higher sensitivity than FID for oxygenated compounds like aldehydes.

Table 4.10 provides a list of 5 ketones which were extracted and analyzed using sol-gel dendrimer coated capillaries. Capillary microextraction results are presented in Table 4.11, Table 4.12 and Table 4.13. Figure 4.9 represents a gas chromatogram of the mixture of 5 underivatized ketones extracted from an aqueous solution containing 100 ppb of each analyte. Excellent reproducibility for the capillary microextraction of ketones (RSD values for run-to-run reproducibility ranges from 1.37 % to 3.39%, while RSD values for capillary-to-capillary reproducibility ranges from 2.08 % to 6.45 %) is a good indicator of the superior performance of the sol-gel dendrimer coated microextraction capillaries as well as the method developed in these experiments.
Table 4.10 Chemical structures and pertinent physical properties of ketones extracted using sol-gel dendrimer coating

<table>
<thead>
<tr>
<th>Name of the analyte</th>
<th>Chemical structure</th>
<th>Molecular weight (g/mol)</th>
<th>Melting point (°C)</th>
<th>Boiling point (°C)</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrophenone</td>
<td><img src="image1" alt="Chemical structure" /></td>
<td>148.2042</td>
<td>11-13</td>
<td>220-222</td>
<td>0.988</td>
</tr>
<tr>
<td>Valerophenone</td>
<td><img src="image2" alt="Chemical structure" /></td>
<td>162.231</td>
<td>-9</td>
<td>105-107 at 5 mm Hg</td>
<td>0.988</td>
</tr>
<tr>
<td>Hexanophenone</td>
<td><img src="image3" alt="Chemical structure" /></td>
<td>176.2578</td>
<td>25-26</td>
<td>265.1</td>
<td>0.958</td>
</tr>
<tr>
<td>Heptanophenone</td>
<td><img src="image4" alt="Chemical structure" /></td>
<td>190.2846</td>
<td>17</td>
<td>155 at 15 mm Hg</td>
<td>0.946</td>
</tr>
<tr>
<td>Benzophenone</td>
<td><img src="image5" alt="Chemical structure" /></td>
<td>18.2214</td>
<td>48.5</td>
<td>305.4</td>
<td>1.5893</td>
</tr>
</tbody>
</table>
Table 4.11 Run-to-run peak area reproducibility for ketones in capillary microextraction using sol-gel dendrimer coatings*

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>A x 10^-4</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Run 1</td>
<td>Run 2</td>
<td>Run 3</td>
<td>Run 4</td>
<td>Run 5</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>Butyrophenone</td>
<td>3.65</td>
<td>3.76</td>
<td>3.64</td>
<td>3.73</td>
<td>3.64</td>
<td>3.68</td>
<td>1.37</td>
</tr>
<tr>
<td>Valerophenone</td>
<td>6.38</td>
<td>6.58</td>
<td>6.17</td>
<td>6.30</td>
<td>6.14</td>
<td>6.31</td>
<td>2.52</td>
</tr>
<tr>
<td>Hexanophenone</td>
<td>7.75</td>
<td>8.25</td>
<td>8.09</td>
<td>8.13</td>
<td>8.28</td>
<td>8.10</td>
<td>2.33</td>
</tr>
</tbody>
</table>

*Capillary Microextraction-GC analysis of ketones at 100 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min⁻¹, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min⁻¹; helium carrier gas; FID temperature 350 °C. Peak identification: (1) butyrophenone, (2) valerophenone, (3) hexanophenone, (4) heptanophenone, and (5) benzophenone.

A= Actual computer output for peak area in arbitrary unit.
Figure 4.9 Capillary Microextraction-GC analysis of ketones at 100 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min⁻¹, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min⁻¹; helium carrier gas; FID temperature 350 °C. Peak identification: (1) butyrophenone, (2) valerophenone, (3) hexanophenone, (4) heptanophenone, and (5) benzophenone.
Table 4.12 Capillary-to-capillary peak area reproducibility for ketones in capillary microextraction using sol-gel dendrimer coatings*

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Run 4</th>
<th>Run 5</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrophenone</td>
<td>3.30</td>
<td>30.98</td>
<td>3.18</td>
<td>2.96</td>
<td>3.21</td>
<td>3.15</td>
<td>3.70</td>
</tr>
<tr>
<td>Valerophenone</td>
<td>6.28</td>
<td>5.87</td>
<td>6.12</td>
<td>5.88</td>
<td>6.32</td>
<td>60.91</td>
<td>3.12</td>
</tr>
<tr>
<td>Heptanophenone</td>
<td>8.29</td>
<td>9.02</td>
<td>9.71</td>
<td>9.20</td>
<td>10.02</td>
<td>9.25</td>
<td>6.45</td>
</tr>
<tr>
<td>Benzophenone</td>
<td>6.99</td>
<td>6.60</td>
<td>6.72</td>
<td>6.83</td>
<td>6.93</td>
<td>6.81</td>
<td>2.08</td>
</tr>
</tbody>
</table>

*Capillary Microextraction- GC analysis of ketones at 100 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min⁻¹, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min⁻¹; helium carrier gas; FID temperature 350 °C. Peak identification: (1) butyrophenone, (2) valerophenone, (3) hexanophenone, (4) heptanophenone, and (5) benzophenone

A= Actual computer output for peak area in arbitrary unit.
**Table 4.13** Limits of detection (LOD) for ketones in CME-GC-FID with sol-gel dendrimer microextraction capillaries

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (ppb)</th>
<th>Measured Peak Height (µV) (H x 10^{-3})</th>
<th>Limit of Detection (S/N 3), ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrophenone</td>
<td>200</td>
<td>12.60</td>
<td>44.3</td>
</tr>
<tr>
<td>Valerophenone</td>
<td>100</td>
<td>23.11</td>
<td>11.7</td>
</tr>
<tr>
<td>Hexanophenone</td>
<td>50</td>
<td>29.95</td>
<td>3.7</td>
</tr>
<tr>
<td>Heptanophenone</td>
<td>25</td>
<td>33.10</td>
<td>1.9</td>
</tr>
<tr>
<td>Benzophenone</td>
<td>100</td>
<td>16.86</td>
<td>15.2</td>
</tr>
</tbody>
</table>

*Capillary Microextraction-GC analysis of ketones at 100 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min^{-1}, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min^{-1}; helium carrier gas; FID temperature 350 °C. Peak identification: (1) butyrophenone, (2) valerophenone, (3) hexanophenone, (4) heptanophenone, and (5) benzophenone

H= Actual computer output for peak height in µV.
4.3.4.3. Phenols

The next class of compounds that were extracted using sol-gel dendrimer coated capillaries was phenols. The presence of phenolic compounds in the environment is of great concern because of their role in drinking water pollution [50], their toxicity [51], and widespread use in the industry [52,53]. Due to their toxicity and persistence in the environment, 11 phenolic compounds have been included in EPA Priority Pollutant list [54]. Since phenolic compounds are highly polar, it is quite difficult to extract them directly from aqueous media. Derivatization, pH adjustment and/or salting-out are often used to facilitate the extraction [2, 55]. To avoid the analytical complexity due to derivatization, HPLC is a frequent choice for the analysis of phenolic compounds [56, 57].

In the present study, no solute derivatization, pH adjustment or salting out of the aqueous sample was used to extract phenolic compounds from the aqueous medium. Still, sol-gel dendrimer coated microextraction capillaries provided lower detection limits compared to other reports in the literature. For example, we achieved a detection limit of 0.22 ppb for 4-chloro, 3-methyl phenol which is lower than the value (1.4 ppb) obtained by Buchholz et al.[2] on a SPME fiber with 95-µm thick polyacrylate coating. Same trend was also observed for other phenolic compounds. A list of phenolic compounds used in this experiment is given in Table 4.14. Run-to-run and capillary-to-capillary microextraction reproducibility results are provided in Table 4.15 and Table 4.16, respectively. A gas chromatogram obtained from these experiments is shown in Figure 4.10. Like other analytes extracted and analyzed using sol-gel dendrimer coated microextraction capillaries and GC-FID, phenolic compounds were also characterized by
high run-to-run and capillary-to-capillary microextraction reproducibility as evidenced by low RSD values in both instances. This has become possible due to the excellent material property of the dendrimer-based sorbent acquired by sol-gel coating technology developed by Malik and co-workers.

Table 4.14 Chemical structures and pertinent physical properties of phenols analyzed using sol-gel dendrimer coating

<table>
<thead>
<tr>
<th>Name of the analyte</th>
<th>Chemical structure</th>
<th>Molecular weight (g/mol)</th>
<th>Melting point (ºC)</th>
<th>Boiling point (ºC)</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Chlorophenol</td>
<td><img src="image1" alt="Chemical structure" /></td>
<td>152.20</td>
<td>92.5</td>
<td>150.0</td>
<td>0.8987</td>
</tr>
<tr>
<td>2,5-Dimethylphenol</td>
<td><img src="image2" alt="Chemical structure" /></td>
<td>166.22</td>
<td>114.8</td>
<td>295.0</td>
<td>1.203</td>
</tr>
<tr>
<td>3,4-Dichlorophenol</td>
<td><img src="image3" alt="Chemical structure" /></td>
<td>178.23</td>
<td>99.2</td>
<td>340</td>
<td>0.9820</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td><img src="image4" alt="Chemical structure" /></td>
<td>202.26</td>
<td>107.8</td>
<td>384</td>
<td>1.252</td>
</tr>
<tr>
<td>4-Cloro-3-methylphenol</td>
<td><img src="image5" alt="Chemical structure" /></td>
<td>202.26</td>
<td>156</td>
<td>404</td>
<td>1.271</td>
</tr>
</tbody>
</table>
Table 4.15: Run-to-run peak area reproducibility for phenols in capillary microextraction using sol-gel dendrimer coatings

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>A x 10^-4</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Run 4</th>
<th>Run 5</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Chlorophenol</td>
<td>1.58</td>
<td>1.75</td>
<td>1.59</td>
<td>1.64</td>
<td>1.51</td>
<td>1.62</td>
<td>5.29</td>
<td></td>
</tr>
<tr>
<td>2,5-Dimethylphenol</td>
<td>2.90</td>
<td>2.82</td>
<td>2.88</td>
<td>2.84</td>
<td>2.90</td>
<td>2.87</td>
<td>1.26</td>
<td></td>
</tr>
<tr>
<td>3,4-Dichlorophenol</td>
<td>1.85</td>
<td>1.92</td>
<td>1.84</td>
<td>2.04</td>
<td>2.05</td>
<td>1.94</td>
<td>5.06</td>
<td></td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>15.46</td>
<td>16.31</td>
<td>15.16</td>
<td>15.43</td>
<td>15.75</td>
<td>15.57</td>
<td>2.15</td>
<td></td>
</tr>
<tr>
<td>4-Chloro-3-methylphenol</td>
<td>6.40</td>
<td>6.35</td>
<td>6.35</td>
<td>6.42</td>
<td>6.39</td>
<td>6.38</td>
<td>0.49</td>
<td></td>
</tr>
</tbody>
</table>

*Capillary Microextraction- GC analysis of phenols at 10 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C/min, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min⁻¹; helium carrier gas; FID temperature 350 °C. Peak identification: (1) 2-chlorophenol, (2) 2,5-dimethylphenol, (3) 3,4-dichlorophenol, (4) 2,4,6-trichlorophenol, and (5) 4-chloro-3-methylphenol.

A= Actual computer output for peak area in arbitrary unit.
Table 4.16: Capillary-to-capillary peak area reproducibility for phenols in capillary microextraction using sol-gel dendrimer coatings*

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>A x 10^{-4}</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Run 4</th>
<th>Run 5</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Chlorophenol 2,5-Dimethylphenol 3,4-Dichlorophenol 2,4,6-Trichlorophenol 4-Chloro-3-methylphenol</td>
<td>1.37</td>
<td>1.27</td>
<td>1.31</td>
<td>1.13</td>
<td>1.36</td>
<td>1.29</td>
<td>7.59</td>
<td></td>
</tr>
<tr>
<td>2,5-Dimethylphenol 3,4-Dichlorophenol 2,4,6-Trichlorophenol 4-Chloro-3-methylphenol</td>
<td>2.69</td>
<td>3.04</td>
<td>2.72</td>
<td>2.69</td>
<td>2.69</td>
<td>2.76</td>
<td>5.58</td>
<td></td>
</tr>
<tr>
<td>3,4-Dichlorophenol 2,4,6-Trichlorophenol 4-Chloro-3-methylphenol</td>
<td>1.84</td>
<td>1.92</td>
<td>1.95</td>
<td>1.84</td>
<td>1.89</td>
<td>1.89</td>
<td>2.53</td>
<td></td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol 4-Chloro-3-methylphenol</td>
<td>14.61</td>
<td>15.49</td>
<td>14.53</td>
<td>14.05</td>
<td>14.17</td>
<td>14.60</td>
<td>3.87</td>
<td></td>
</tr>
<tr>
<td>4-Chloro-3-methylphenol</td>
<td>6.99</td>
<td>6.41</td>
<td>6.06</td>
<td>5.99</td>
<td>6.94</td>
<td>6.48</td>
<td>7.37</td>
<td></td>
</tr>
</tbody>
</table>

*Capillary Microextraction-GC analysis of phenols at 10 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C/min, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min⁻¹; helium carrier gas; FID temperature 350 °C. Peak identification: (1) 2-chlorophenol, (2) 2,5-dimethylphenol, (3) 3,4-dichlorophenol, (4) 2,4,6-trichlorophenol, and (5) 4-chloro-3-methylphenol.

A= Actual computer output for peak area in arbitrary unit.
Table 4.17 Limits of detection (LOD) for phenols in CME-GC-FID using sol-gel dendrimer microextraction capillaries*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (ppb)</th>
<th>Measured peak height (µV) ((H \times 10^{-3}))</th>
<th>Limit of detection ((S/N 3)), ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Chlorophenol</td>
<td>100</td>
<td>3.78</td>
<td>840</td>
</tr>
<tr>
<td>2,5-Dimethylphenol</td>
<td>100</td>
<td>8.36</td>
<td>320</td>
</tr>
<tr>
<td>3,4-Dichlorophenol</td>
<td>100</td>
<td>6.49</td>
<td>160</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>100</td>
<td>18.26</td>
<td>220</td>
</tr>
<tr>
<td>4-Chloro-3-methylphenol</td>
<td>100</td>
<td>11.44</td>
<td>260</td>
</tr>
</tbody>
</table>

*Capillary Microextraction- GC analysis of phenols at 10 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C/min, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min⁻¹; helium carrier gas; FID temperature 350 °C. Peak identification: (1) 2-chlorophenol, (2) 2,5-dimethylphenol, (3) 3,4-dichlorophenol, (4) 2,4,6-trichlorophenol, and (5) 4-chloro-3-methylphenol.

H= Actual computer output for peak height in µV.
Figure 4.10 Capillary Microextraction-GC analysis of phenols at 10 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C/min, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min⁻¹; helium carrier gas; FID temperature 350 °C. Peak identification: (1) 2-chlorophenol, (2) 2,5-dimethylphenol, (3) 3,4-dichlorophenol, (4) 2,4,6-trichlorophenol, and (5) 4-chloro-3-methylphenol.
4.3.4.4 CME of butylated hydroxytoluene (BHT)

Butylated hydroxytoluene (BHT) was another compound that was extracted on sol-gel dendrimer coated capillaries. Figure 4.11 represents a gas chromatogram of butylated hydroxytoluene (BHT) obtained in a CME-GC-FID experiment using a sol-gel dendrimer extraction capillary. Table 4.18 lists the chemical structure and other pertinent properties of BHT. Data obtained from capillary microextraction of BHT using sol-gel Dendrimer coated capillaries are presented in Table 4.19 and Table 4.20. Calculated limit of detection data is presented in Table 4.21.

BHT is one of the most commonly used additives (preservative) used in rubber, petroleum products, organic solvents, plastics, foods, edible fats and oils, cosmetics [58]. Although controversial, BHT is suspected to possess carcinogenic properties [59]. Therefore, the Joint Expert Committee on Food Additives (JECFA) of the Word Health Organization (WHO) has set the acceptable daily intake (ADI) of BHT at 0-0.3 mg/kg body weight. Recently Tombeshi and Freije [60] proposed an SPME-GC/MS technique for the determination of BHT. In SPME-GC-FID, they achieved a detection limit of 4.2 ng/mL using a 100-μm thick PDMS coating on the SPME fiber. By comparison, a detection limit of 3.0 pg/mL was achieved in the present study using CME-GC-FID with a sol-gel dendrimer-coated microextraction capillary. This corresponds to three orders of magnitude improvement in the detection limit and extraction sensitivity.
Table 4.18 Chemical structure and pertinent physical properties of butylated hydroxy toluene (BHT) extracted using sol-gel dendrimer coating

<table>
<thead>
<tr>
<th>Name of the analyte</th>
<th>Chemical structure</th>
<th>Molecular weight (g/mol)</th>
<th>Melting point (ºC)</th>
<th>Boiling point (ºC)</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butylated hydroxytoluene (BHT)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>220.354</td>
<td>71</td>
<td>265</td>
<td>1.048</td>
</tr>
</tbody>
</table>

Table 4.19 Run-to-run peak area reproducibility for BHT in capillary microextraction using sol-gel dendrimer coatings*

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>A x 10⁻⁴</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Run 4</th>
<th>Run 5</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butylated hydroxytoluene</td>
<td>7.31</td>
<td>7.75</td>
<td>7.27</td>
<td>7.97</td>
<td>8.14</td>
<td>7.69</td>
<td>5.06</td>
<td></td>
</tr>
</tbody>
</table>

*Capillary Microextraction-GC analysis of BHT at 10 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 ºC, program rate 100 ºC min⁻¹, final 300 ºC; GC oven temperature programmed from 30 ºC (hold for 5 min) to 300 ºC at a rate of 15 ºC min⁻¹; helium carrier gas; FID temperature 350 ºC.

A= Actual computer output for peak area in arbitrary unit.
Table 4.20: Capillary-to-capillary peak area reproducibility for BHT in capillary microextraction using sol-gel dendrimer coatings*.

<table>
<thead>
<tr>
<th>Compounds name</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Run 4</th>
<th>Run 5</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butylated Hydroxytoluene</td>
<td>7.21</td>
<td>6.99</td>
<td>8.00</td>
<td>7.74</td>
<td>7.22</td>
<td>7.43</td>
<td>5.67</td>
</tr>
</tbody>
</table>

*Capillary Microextraction-GC analysis of BHT at 10 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min⁻¹, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min⁻¹; helium carrier gas; FID temperature 350 °C.

A= Actual computer output for peak area in arbitrary unit.
Table 4.21: Limit of detection (LOD) data for BHT in CME-GC-FID using sol-gel dendrimer microextraction capillaries*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (ppb)</th>
<th>Measured peak height (µV) (H x 10^{-3})</th>
<th>Limit of detection (S/N 3), ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butylated hydroxytoluene</td>
<td>10</td>
<td>5.85</td>
<td>3.0</td>
</tr>
</tbody>
</table>

*Capillary Microextraction- GC analysis of BHT at 10 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min^{-1}, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min^{-1}; helium carrier gas; FID temperature 350 °C.

H= Actual computer output for peak height in µV.
Figure 11 Capillary Microextraction-GC analysis of BHT at 10 ppb concentration using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min⁻¹, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min⁻¹; helium carrier gas; FID temperature 350 °C.
4.3.4.5. Alcohols

Figure 4.12 represents a gas chromatogram of a mixture of alcohols (10 ppb concentration of each). Extraction of these polar compounds was conducted from aqueous samples without any derivatization, pH adjustment or salting-out effects. The presented data shows excellent affinity of the sol-gel dendrimer coating for these highly polar analytes that are often difficult to extract from aqueous media in the underivatized form using commercial coatings. Excellent symmetrical peak shapes and high detection sensitivity (Table 4.25) are indicative of outstanding performance of the used sol-gel dendrimer coating in CME and excellent deactivation characteristics of sol-gel PEG column used for the GC analysis of the extracted alcohols.

As is revealed from the data presented in the text, run-to-run and capillary-to-capillary repeatability data for peak area obtained in CME-GC-FID experiments are quite satisfactory. For most solutes, these RSD values were under 5%. For the polar analytes, the RSD values were higher than those for nonpolar analytes. Retention time repeatability data for PAHs, aldehydes, ketones, phenols, and alcohols were characterized by RSD values of less than 0.14 %.

Unique molecular architecture of dendrimers and the ability of sol-gel dendrimer coatings to provide efficient and reproducible extraction of both polar and nonpolar compounds with high detection sensitivity makes dendrimer-based materials very promising in analytical extraction technology.
Table 4.22 Chemical structures and pertinent physical properties of alcohols extracted using sol-gel dendrimer coating

<table>
<thead>
<tr>
<th>Name of the analyte</th>
<th>Chemical structure</th>
<th>Molecular weight (g/mol)</th>
<th>Melting point (°C)</th>
<th>Boiling point (°C)</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Octanol</td>
<td>−O−</td>
<td>130.2296</td>
<td>-15</td>
<td>195</td>
<td>0.826</td>
</tr>
<tr>
<td>1-Nonanol</td>
<td>−O−</td>
<td>144.2564</td>
<td>-8 to -6</td>
<td>125</td>
<td>0.828</td>
</tr>
<tr>
<td>1-Decanol</td>
<td>−O−</td>
<td>158.2832</td>
<td>6</td>
<td>230</td>
<td>0.829</td>
</tr>
<tr>
<td>1-Undecanol</td>
<td>−O−</td>
<td>172.32</td>
<td>11</td>
<td>146 at 30 mm Hg</td>
<td>0.832</td>
</tr>
<tr>
<td>1-Dodecanol</td>
<td>−O−</td>
<td>186.3368</td>
<td>22-26</td>
<td>260.262</td>
<td>0.833</td>
</tr>
</tbody>
</table>
Table 4.23 Run-to-run peak area reproducibility for alcohols in capillary microextraction using sol-gel dendrimer coatings*

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>A $\times 10^{-4}$</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Run 1</td>
<td>Run 2</td>
</tr>
<tr>
<td>1-Octanol</td>
<td>12.60</td>
<td>13.15</td>
</tr>
<tr>
<td>1-Decanol</td>
<td>10.91</td>
<td>11.29</td>
</tr>
<tr>
<td>1-Undecanol</td>
<td>9.35</td>
<td>9.61</td>
</tr>
<tr>
<td>1-Dodecanol</td>
<td>13.82</td>
<td>15.44</td>
</tr>
</tbody>
</table>

*Capillary Microextraction-GC analysis of alcohols at ppb level concentrations using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min$^{-1}$, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min$^{-1}$; helium carrier gas; FID temperature 350 °C. Peak identification: (1) 1-octanol (500 ppb), (2) 1-nonanol (100 ppb), (3) 1-decanol (30 ppb), (4) 1-undecanol (20 ppb), and (5) 1-dodecanol (50 ppb). A= Actual computer output for peak area in arbitrary unit.
Table 4.24 Capillary-to-capillary peak area reproducibility for alcohols in capillary microextraction using sol-gel dendrimer coatings*

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>Capillary 1</th>
<th>Capillary 2</th>
<th>Capillary 3</th>
<th>Capillary 4</th>
<th>Capillary 5</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Octanol</td>
<td>12.35</td>
<td>12.77</td>
<td>13.52</td>
<td>14.21</td>
<td>14.41</td>
<td>13.46</td>
<td>6.64</td>
</tr>
<tr>
<td>1-Nonanol</td>
<td>12.73</td>
<td>12.94</td>
<td>13.61</td>
<td>14.42</td>
<td>13.91</td>
<td>13.46</td>
<td>2.50</td>
</tr>
<tr>
<td>1-Decanol</td>
<td>10.97</td>
<td>11.37</td>
<td>11.25</td>
<td>11.47</td>
<td>11.77</td>
<td>11.01</td>
<td>4.27</td>
</tr>
</tbody>
</table>

*Capillary Microextraction- GC analysis of alcohols at ppb level concentrations using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min⁻¹, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min⁻¹; helium carrier gas; FID temperature 350 °C. Peak identification: (1) 1-octanol (500ppb), (2) 1-nonanol (100 ppb), (3) 1-decanol (30 ppb), (4) 1-undecanol (20 ppb), and (5) 1-dodecanol (50 ppb).

A= Actual computer output for peak area in arbitrary unit.
**Table 4.25** Limits of detection (LOD) for alcohols in CME-GC-FID using sol-gel dendrimer microextraction capillaries*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (ppb)</th>
<th>Measured peak height (µV) ($H \times 10^{-4}$)</th>
<th>Limit of detection (S/N 3), ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Octanol</td>
<td>500</td>
<td>3.93</td>
<td>11.2</td>
</tr>
<tr>
<td>1-Nonanol</td>
<td>100</td>
<td>5.29</td>
<td>2.3</td>
</tr>
<tr>
<td>1-Decanol</td>
<td>30</td>
<td>4.39</td>
<td>1.0</td>
</tr>
<tr>
<td>1-Undecanol</td>
<td>20</td>
<td>3.16</td>
<td>1.0</td>
</tr>
<tr>
<td>1-Dodecanol</td>
<td>50</td>
<td>2.83</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*Capillary Microextraction-GC analysis of alcohols at ppb level concentrations using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min$^{-1}$, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min$^{-1}$; helium carrier gas; FID temperature 350 °C. Peak identification: (1) 1-octanol (500 ppb), (2) 1-nonanol (100 ppb), (3) 1-decanol (30 ppb), (4) 1-undecanol (20 ppb), and (5) 1-dodecanol (50 ppb). H= Actual computer output for peak height in µV.
Figure 4.12 Capillary Microextraction-GC analysis of alcohols at ppb level concentrations using sol-gel dendrimer coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, program rate 100 °C min⁻¹, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C min⁻¹; helium carrier gas; FID temperature 350 °C. Peak identification: (1) 1-octanol (500 ppb), (2) 1-nonanol (100 ppb), (3) 1-decanol (30 ppb), (4) 1-undecanol (20 ppb), and (5) 1-
dodecanol (50 ppb).

### 4.4 Conclusion

For the first time, sol-gel dendrimer coated capillaries were used for solvent-free microextraction and preconcentration in chemical analysis. Both polar and nonpolar analytes were efficiently extracted from aqueous samples on the same sol-gel dendrimer capillary and provided excellent detection sensitivity. Parts per trillion level detection limits were achieved in CME-GC-FID using sol-gel dendrimer-coated extraction capillaries. It should be possible to further enhance the extraction sensitivity by using capillaries with (1) larger-diameters (e.g., 320 µm, 520 µm), (2) greater lengths (3) thicker CME coatings, and (4) sol-gel monolithic extraction beds, or any combination of these factors. Since sol-gel dendrimer extraction phase shows excellent thermal and solvent stability, sol-gel dendrimer CME capillaries are suitable for coupling with both GC and HPLC.
4.5 References for Chapter Four


246
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Chapter Five

Capillary Microextraction on Sol-Gel Poly-Tetrahydrofuran Coating

5.1 Introduction

Solid-phase microextraction (SPME) [1] is an excellent solventless alternative to the traditional sample preparation techniques like liquid-liquid extraction (LLE), Soxhlet extraction, solid-phase extraction (SPE), etc. It is a simple, sensitive, time-efficient, cost-effective, reliable, easy-to-automate, and portable sample preparation technique. In SPME, analyte enrichment is accomplished by using a sorbent coating in two different formats: (a) conventional fiber format [1] and (b) the more recently developed “in-tube” format [2]. In its conventional format, SPME uses a sorbent coating on the external surface of a fused silica fiber (typically 100-200 µm in diameter) covering a short segment at one of the ends. In the in-tube format, the sorbent coating is applied to the inner surface of a capillary. SPME completely eliminates the use of organic solvents in sample preparation, and effectively integrates a number of important analytical steps such as sampling, extraction, preconcentration, and sample introduction for instrumental analysis. Thanks to these positive attributes, SPME has experienced a rapid growth over the last decade.

Despite rapid advancements in SPME applications, a number of important
problems in the area of sorbent coatings still remain to be solved. First, existing SPME coatings are designed to extract either polar or nonpolar analytes from a given matrix. For example, being a nonpolar sorbent, polydimethylsiloxane (PDMS) shows excellent selectivity toward nonpolar analytes. The polar polyacrylate coating, on the other hand, demonstrates excellent selectivity toward polar compounds. Such an approach is not very convenient for samples where both polar and nonpolar contaminants are present and both need to be analyzed. For such applications, it is important to have a sorbent that can extract both polar and nonpolar compounds with high extraction sensitivity needed for trace analysis. Second, in conventional SPME only a short length of the fiber is coated with sorbent. The short length of the coated segment on the SPME fiber translates into low sorbent loading which, in turn, leads to low sample capacity. This imposes a significant limitation on the sensitivity of the classical fiber SPME. Improving sensitivity is still a major challenge in SPME research. This is particularly important for analyzing ultra-trace contaminants that are present in the environment. One possible way of improving extraction sensitivity in SPME is through an increase in the coating thickness [3, 4]. However, equilibration time rapidly increases with the increase in coating thickness because of the dynamic diffusion-controlled nature of the extraction process [3]. As a consequence, both extraction and subsequent desorption processes become slower, resulting in longer total analysis time. Moreover, immobilization of thicker coating on fused silica surface is difficult to achieve by conventional approaches [5] indicating to the necessity of an alternative approach to effective immobilization of thick coatings. Third, low thermal and solvent stability of SPME coatings represents a major drawback of conventional SPME technology, and is a direct consequence of the poor
quality of sorbent immobilization. With a very few exceptions, SPME fibers have been traditionally coated by mere physical deposition of the sorbent in the form of a surface coating. The absence of chemical bonds between the sorbent coating and the fused silica surface is considered to be the main reason for low thermal and solvent stability of SPME fibers [6]. Low thermal stability of thick coatings forces one to use low desorption temperatures to preserve coating integrity, which in turn, leads to incomplete sample desorption and sample carryover problems. Besides, low solvent stability of the coating poses a significant obstacle to reliable hyphenation of in-tube SPME with liquid-phase separation techniques (e.g., HPLC) that employ organic or organo-aqueous mobile phases [3]. It is evident that future advancements in SPME would greatly depend on new developments in the areas of sorbent chemistry and coating technology that will allow preparation of chemically immobilized coatings from advanced material systems providing desired selectivity and performance in SPME.

One possible approach to address most of the problems described above is to use sol-gel technology to create sorbent coatings [6-9]. Sol-gel chemistry provides a simple and convenient pathway leading to the synthesis of advanced material systems that can be used to prepare surface coatings [10,11]. In the context of fused silica fiber/capillary-based SPME, major advantages offered by sol-gel technology are as follows: (1) it combines surface treatment, deactivation, coating, and stationary phase immobilization into a single-step procedure making the whole SPME fiber/capillary manufacturing process very efficient and cost-effective; (2) it creates chemical bonds between the fused silica surface and the created sorbent coating; (3) it provides surface-coatings with high operational stability ensuring reproducible performance of the sorbent coating under
operation conditions involving high temperature and/or organic solvents, and thereby it expands the SPME application range toward both higher-boiling- as well as thermally labile analytes; (4) it provides the possibility to combine organic and inorganic material properties in extracting sorbents providing tunable selectivity; (5) it offers the opportunity to create sorbent coatings with a porous structure which significantly increases the surface area of the extracting phase and provides acceptable stationary phase loading and sample capacity using thinner coatings.

A number of shortcomings inherent in conventional fiber SPME originate from the design and physical construction of the fiber and the syringe-like SPME device. These include susceptibility of the fiber to breakage during coating or operation, mechanical damage of the coating due to scraping, and operational uncertainties due to needle bending. In-tube SPME [2], also termed capillary microextraction (CME) [7], is practically free from these inherent format-related shortcomings of conventional fiber SPME. It uses a fused silica capillary (generally a small piece of GC column) with a stationary phase coating on the inner surface to perform extraction. The protective polyimide coating outside the capillary remains intact and provides reliable protection against breakage. Moreover, this format provides a simple, easy, and convenient way to couple SPME to high-performance liquid chromatography.

Despite numerous advantageous features, in-tube SPME still has drawbacks that originate mainly from the deficiency of the coating technique used to prepare the extraction capillary. Conventional static coating technique, commonly employed to prepare GC capillary columns (short segments of which are used for in-tube SPME), is not suitable for generating thick coatings necessary for enhanced extraction sensitivity in
SPME. Besides, in general, a conventionally prepared coating is not chemically bonded to the fused silica capillary surface. As a consequence, such coatings exhibit low thermal and solvent stability. Recently, sol-gel capillary microextraction (CME) has been proposed [7] to address the above-mentioned problems through *in situ* creation of surface-bonded coatings via sol-gel technology capable of providing both thick and thin coatings on the capillary inner walls.

In both conventional fiber SPME and CME, the sorbent coating plays a critically important role in the extraction process. To date, several types of sorbent coatings have been developed and used for extraction. These coatings can be broadly divided into two major types: (1) single-phase- and (2) composite coatings. Single-phase SPME coatings include polydimethylsiloxane (PDMS) [12], Polyacrylate [3], Carbopack [13], polyimide [14], polypyrrole [15], and molecularly imprinted materials [16,17]. Among the composite coatings are Carbowax/divinylbenzene (CW/DVB) [18], polydimethylsiloxane/divinylbenzene (PDMS/DVB) [19], polydimethylsiloxane/Carboxane (PDMS/Carboxane) [20], and Carbowax/templated resin (CW/TPR) [20].

In recent years, sol-gel SPME coatings [6,7,21-27] have drawn wide attention due to their inherent advantageous features and performance superiority over traditional coatings (both non-bonded and cross-linked types). Sol-gel PDMS [6,7,22,28] coatings possess significantly higher thermal stability (> 360 °C) than their conventional counterparts for which the upper temperature limit generally remains within 200-270 °C [29]. High thermal and solvent stability have been demonstrated for other sol-gel coatings: sol-gel PEG [23] (320 °C), sol-gel crown ethers [25] (340 °C), sol-gel
hydroxyfullerene [27] (360 °C), sol-gel polymethylphenylvinylsiloxane [26](350 °C), and sol-gel phenyl-functionalized coating [69] (350 °C).

Sol-gel PEG coating [23] has been recommended for polar analytes. Sol-gel crown ether coating [24-25] demonstrated higher extraction efficiencies for aromatic amines compared to CW-DVB fiber. Gbatu et al. [21] described the preparation of sol-gel octyl coatings for SPME-HPLC analysis of organometallic compounds from aqueous solutions. Compared with the commercial SPME coatings, a hydroxyfullerene-based sol-gel coating [27] showed higher sensitivity, faster mass transfer rate for aromatic compounds and possessed molecular planarity recognition capability for polychlorinated biphenyls (PCBs). Yang et al. [26] prepared sol-gel poly (methylphenylvinylsiloxane) (PMPVS) coating using sol-gel technology that provided very high extraction efficiency for aromatic compounds.

Poly-THF (also called polytetramethylene oxide, PTMO) is a hydroxy-terminated polar material that has been used as an organic component to synthesize organic-inorganic hybrid materials [30-35]. Sol-gel poly-THF has been used as a bioactive bone repairing material [36], and as a proton conducting solid polymer electrolyte that might allow the operation of high temperature fuel cells [37]. Little work has been devoted to explore the potential of the sol-gel poly-THF material for use as an extraction medium in analytical chemistry. In this chapter, we describe a sol-gel chemistry-based approach to in situ creation of poly-THF based hybrid organic-inorganic coatings on the inner walls of fused silica capillaries and demonstrate the possibility of using such coatings to achieve parts per trillion (ppt) and parts per quadrillion level detection limits for both polar and nonpolar analytes after extraction from aqueous sample matrices.
5.2 Experimental

5.2.1 Equipments

Capillary microextraction-gas chromatography (CME-GC) experiments with sol-gel poly-THF coating as the extracting phase were carried out on a Shimadzu model 17A GC system (Shimadzu Corporation, Kyoto, Japan) equipped with a programmed temperature vaporizer (PTV injector) and a flame ionization detector (FID). An in-house designed liquid sample dispenser (Figure 5.1) was used to perform CME via gravity-fed flow of the aqueous samples through the sol-gel poly-THF coated capillary. A Fisher Model G-560 Genie 2 Vortex (Fisher Scientific, Pittsburgh, PA) was used for thorough mixing of sol solution ingredients. A Microcentaur model APO 5760 microcentrifuge (Accurate Chemical and Scientific Corporation, Westbury, NY) was used for centrifugation (at 13000 rpm, 15682g) of sol solutions made for coating the microextraction capillaries. An Avatar model 320 FTIR System (Nicolet Analytical Instruments, Madison, WI) was used to obtain the IR spectra of poly-THF, sol-gel solution, and sol-gel poly-THF sorbent. A JEOL model JSM-35 scanning electron microscope was used for the investigation of the coated capillary surface. A homebuilt, gas pressure-operated filling/purging device [38] was used to perform a number of operations: (a) filling the extraction capillary with the sol solution, (b) expelling the solution from the capillary after predetermined period of in-capillary residence, and (c) purging the microextraction capillary with helium. Ultra pure (17.2 MΩ) water was obtained from a Barnsted Model 04741 Nanopure deionized water system (Barnsted/
Thermodyne, Dubuque, IA). ChromPerfect (Version 3.5 for Windows) computer software (Justice Laboratory Software, Denville, NJ) was used for on-line collection, integration, and processing of the experimental data.
Figure 5.1 Schematic of a gravity-fed sample dispensing unit for capillary microextraction with a sol-gel poly-THF coated capillary
5.2.2 Chemicals and materials

Fused silica capillary (320 µm i.d.) with a protective polyimide coating on the external surface was purchased from Polymicro Technologies Inc. (Phoenix, AZ). Poly-THF 250 was a gift from BASF Corporation (Parsippany, NJ). Acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, \textit{n}-nonanal, \textit{n}-undecanal, \textit{n}-dodecanal, \textit{n}-tridecanal, valerophenone, hexanophenone, heptanophenone, decanophenone, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 4-chloro-3-methyl phenol, and pentachlorophenol were purchased from Aldrich Chemical Co. (Milwaukee, WI); \textit{n}-decyl aldehyde, 1-nonanol, 1-decanol, 1-undecanol, and 1-tridecanol were purchased from Acros Organics (Pittsburgh, PA). Lauryl alcohol was purchased from Sigma Chemical Co. (St. Louis, MO). HPLC-grade methanol and methylene chloride and all borosilicate glass vials were purchased from Fisher Scientific (Pittsburgh, PA).

5.2.3 Preparation of sol-gel poly-THF coated microextraction capillaries

Preparation of sol-gel Poly-THF coated microextraction capillaries involves five distinct and sequential operations: (1) cleaning and pretreatment of the fused silica capillary, (2) preparation of the sol solution, (3) coating the fused silica capillary with the sol solution, (4) thermal conditioning of the coated fused silica capillary, and (5) rinsing of the coating with organic solvents to remove unbonded materials (if there is any).
5.2.3.1 Cleaning and hydrothermal treatment of the fused silica capillary

The fused silica capillary inner surface must be cleaned before coating with the sol solution. In order to clean the surface from organic contaminants, it was rinsed with methylene chloride and methanol. One mL of each solvent starting with methylene chloride was passed through the capillary sequentially using the filling/purging device (Figure 5.2). The rinsed fused silica capillary was then purged with helium for 30 min followed by hydrothermal treatment. Hydrothermal treatment generated adequate surface silanol groups required for strong chemical bonding between the substrate and the growing sol-gel network. To perform hydrothermal treatment, the cleaned fused silica capillary was filled with Nanopure deionized water using the filling/purging device and then the water was flushed out of the capillary with the aid of helium gas pressure. The capillary was then purged with helium gas for 30 min so that only a thin layer of water remained on the surface of the capillary. At this point, both ends of the fused silica capillary were sealed with oxyacetylene flame. The sealed capillary was then heated at 250 °C in a GC oven for 2 hours. Both ends were then cut open with a ceramic wafer. One end of the fused silica capillary was further connected to the GC injection port with the help of a graphite ferrule and was heated again in the GC oven at 250 °C for another 2 more hours with a continuous helium flow through the capillary. After this, the capillary was ready for coating.
Figure 5.2 Schematic of a homemade capillary filling/purging device for the preparation of capillary microextraction capillaries and open-tubular sol-gel GC columns
5.2.3.2 Preparation of the sol solution

A sol solution designed to prepare a hybrid sol-gel organic-inorganic coating consisted of an alkoxide precursor, a sol-gel-active (either hydroxy or alkoxy terminated) organic polymer, one or more surface deactivating reagent, appropriate organic solvent(s), and a sol-gel catalyst. Table 5.1 presents the name, function, and chemical structure of different ingredients used to prepare the coating solution to create sol-gel poly-THF coatings for CME. The sol solution was prepared by dissolving methyltrimethoxysilane (sol-gel precursor, 250 µL), hydroxy-terminated poly(tetrahydrofuran), (250 mg), hexamethyldisilazane (surface deactivation reagent, 20 µL), and trifluoroacetic acid (containing 5% water) (sol-gel catalyst, 100 µL) in methylene chloride (solvent, 400 µL). After adding all the ingredients, the resultant solution was vortexed for 3 min for thorough mixing of the constituents followed by centrifugation for 5 min to remove any precipitates from the sol solution before it was used for coating.
Table 5.1 Names, functions and chemical structures of sol-gel poly-THF coating solution ingredients

<table>
<thead>
<tr>
<th>Name</th>
<th>Function</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyltrimethoxysilane (MTMOS)</td>
<td>Sol-gel precursor</td>
<td><img src="CH3O-Si-OCH3" alt="Structure" /></td>
</tr>
<tr>
<td>Polytetrahydrofuran</td>
<td>Organic ligand</td>
<td><img src="OCH3" alt="Structure" /></td>
</tr>
<tr>
<td>Trifluoroacetic acid / water 95:5 (v/v)</td>
<td>Catalyst</td>
<td><img src="H-O-(CH2)4-O" alt="Structure" />n H)</td>
</tr>
<tr>
<td>Methylene chloride</td>
<td>Solvent</td>
<td><img src="CF3COOH" alt="Structure" /></td>
</tr>
<tr>
<td>Hexamethyldisilazane</td>
<td>Deactivating reagent</td>
<td><img src="CH2Cl2" alt="Structure" /></td>
</tr>
</tbody>
</table>

![Structure](CH3) ![Structure](OCH3) ![Structure](CF3COOH) ![Structure](CH2Cl2)
5.2.3.3 Coating fused silica capillary with sol solution

In order to coat a fused silica capillary with the previously prepared sol solution, a 3-m piece of hydrothermally treated fused silica capillary (320 µm i.d.) was installed on the gas pressure-operated filling/purging device (Figure 5.2) that was used to fill the capillary with specially designed sol solution. After filling, the sol solution was kept inside the capillary for 60 min to facilitate the formation of a surface-bonded sol-gel poly-THF coating. During this residence period of the sol solution inside the capillary, a sol-gel hybrid organic-inorganic polymeric network evolved within the sol solution, and part of it ultimately became bonded to the fused silica capillary inner surface via condensation reaction with surface silanol groups. The bonded part of the sol-gel material served as the CME coating. The free unbonded portion of the sol solution was then expelled from the capillary under helium pressure (50 psi) and the coated capillary was purged with helium for an hour. The continuous flow of helium facilitated the evaporation of volatile solvents.

5.2.3.4 Thermal conditioning of the coated capillary

Thermal conditioning of the sol-gel coating was a very important step in post-coating processing because it (1) helped the sol-gel reaction to go to completion, (2) eased removal of entrapped solvents, and unreacted sol-gel ingredients, (3) ensured strong immobilization of the coating on the surface, and (4) facilitated the deactivation
reactions on the fused silica surface and/or the sol-gel coating matrix and thus helped
tuning the net polarity as well as the selectivity of the resultant sorbent. The sol-gel
coated capillary was thermally conditioned in a GC oven using temperature-programmed
heating from 40 °C to 300 °C @ 1 °C min⁻¹, and holding the capillary at the final
temperature for 5 h under helium purge.

**5.2.3.5 Rinsing the capillary with organic solvents to remove unbonded materials**

Before using in extraction, the coated capillary was rinsed sequentially with 2 mL
each of methylene chloride and methanol followed by drying in a stream of helium under
the same temperature-programmed conditions, as in section 4.2.3.4 except that the
capillary was held at the final temperature for 30 min. The capillary was further cooled
down to ambient temperature and cut into 13-cm long pieces that were further used to
perform microextraction.

**5.2.4 Preparation of sol-gel PDMS coated capillary columns for GC**

A 10-m long piece of uncoated fused silica capillary (250 µm i.d.) was accurately
wrapped on a GC column basket in coils. The capillary was sequentially rinsed with 1
mL each of methylene chloride and methanol followed by purging with helium for 30
min. The column was then hydrothermally treated following the procedure described in
Section 5.3.2.1. The hydrothermally treated column was installed on the filling/purging device (Figure 5.2) and the helium flow was adjusted to 40 psi.

The coating solution for the sol-gel PDMS column was prepared as follows: 25 mg PDMS was dissolved in 900 µL of methylene chloride. A 5 µL volume of methyltrimethoxysilane (MTMOS), 25 µL of polymethylhydrosiloxane (PMHS), and 50 µL trifluoroacetic acid (TFA) (5% water v/v) were sequentially added to the vial containing the polymer solution. In order to ensure proper mixing of the ingredients, the sol solution was vortexed after adding each ingredient. The hydrolysis reaction began as soon as the sol-gel catalyst is added, and a 10 min period was allowed for hydrolysis reaction to proceed. At the end of this 10 min period, 20 µL NH₄F solution (20 mg/mL in methanol) was added to the sol solution to facilitate faster condensation. The resulting sol solution was centrifuged and the clear supernatant from the top was transferred to a clean vial using a micro pipet for further use in column coating.

The sol solution was then introduced into the hydrothermally treated fused silica capillary using gas pressure (40 psi) in the filling/purging device (Figure 5.2). Once the capillary was filled with sol-gel solution, the filling/purging device was carefully depressurized and the free exit end of the capillary was sealed with a rubber septum while keeping the other end of the capillary inside filling/purging device. The sol solution was kept inside the capillary for 10 min. During this in-capillary residence time of the sol solution, a surface bonded hybrid organic-inorganic film gradually evolved. After this, the sol solution was expelled from the fused silica capillary under helium pressure. The column was then purged with helium for 60 min. The next step was the thermal conditioning of the coated column. For this, one end of the column was connected to the
GC injection port using a graphite ferrule and a continuous flow of helium (1 mL/min) was maintained through the column. The other end of the capillary was kept free inside the GC oven. The column was heated from 40 °C to 150 °C at a rate of 1°C/min, maintained at 150 °C for 5 h, then heated to 330 °C at a rate of 5 °C/min and maintained at this final temperature for 1 h.

After the thermal conditioning, the column was rinsed with 2 mL of methylene chloride/methanol mixture (75:25 v/v), purge with helium for 1 h and then conditioned again from 40 °C to 330 °C at a rate 5 °C/min, holding it at final temperature for 30 min.

5.2.5 Preparation of sol-gel PEG coated capillary column for GC

A 10 m long (250 µm i.d.) piece of fused silica capillary was first cleaned and hydrothermally treated as described in section 5.3.2.1. It was then installed on the filling/purging device to carry out the coating process.

A sol-gel coating solution was prepared as follows: 35 mg of methoxypoly(ethylene glycol)-silane (PEG 1) and 15 mg of poly (ethylene glycol)-bis silane (PEG2) (sol-gel active organic ligands) were dissolved in 600 µL of methylene chloride (solvent) contained in a polypropylene microcentrifuge vial. A Scientific Products model S8223 Vortex shaker aided the dissolution process (5 min). Then 5 µL of MTMOS (precursor), 10 µL of bis(trimethoxysilylethyl)benzene (precursor), and 5 µL of HMDS (deactivating reagent) were sequentially added to the microcentrifuge vial and thoroughly mixed for 5 min to obtain a homogeneous solution. After this, 50 µL of 95%
TFA (acid catalyst containing 5% water) was added to the solution and thoroughly mixed. After 10 min, a 20-µL volume of NH₄F solution (20 mg/mL in methanol) was introduced into the vial. The volume of the solution was made up to 1000 µL by adding the required amount of methylene chloride, and the mixture was thoroughly vortexed. The resulting solution was centrifuged at 13 000 rpm (15,682 g) for 5 min. The precipitate at the bottom of the vial, if any, was discarded, and the top clear sol solution was used to fill the hydrothermally treated fused-silica capillary using a helium pressure of 50 psi. After a set period of in-capillary residence time (10-20 min), the solution was expelled from the capillary under the same helium pressure and the capillary was subsequently purged with helium at 50 psi for an additional 60 min. This was followed by temperature-programmed heating in a GC oven from 40 to 150 °C at 2 °C min⁻¹ with a hold time of 300 min at 150 °C and then from 150 to 280 °C at 6 °C min⁻¹, holding it at 280 °C for 120 min. Keeping the temperature programming rate at 6 °C min⁻¹, the column was further conditioned in small steps, holding the column for 120 min at each of the following final temperatures: 300, 320, and 340 °C. The column was then rinsed with 2 mL of methylene chloride and conditioned again from 40 to 320 °C at 6 °C min⁻¹. While conditioning, the column was purged with helium at 1 mL min⁻¹.

5.2.6 Cleaning and deactivation of glassware

To avoid any contamination of the standard solutions from the glassware, all glassware used in the current study was thoroughly cleaned with Sparkleen detergent.
followed by rinsing with copious amount of deionized water and drying at 150 °C for 2 hours. To silanize the inner surface of the dried glassware, they were treated with a 5% v/v solution of HMDS in methylene chloride followed by heating in an oven at 250 °C for 8 hours under helium purge. The silanized glassware was then rinsed sequentially with methylene chloride and methanol and dried in an oven at 100 °C for 1 hour. Prior to use, all glassware were rinsed with generous amounts of deionized water and dried at room temperature in a flow of helium.

5.2.7 Preparation of standard solutions for CME on sol-gel poly-THF coated capillaries

All stock solutions, except those for polycyclic aromatic hydrocarbons, were prepared by dissolving 50 mg of each analyte in 5 mL of methanol in a deactivated amber glass vial (10 mL) to obtain a solution of 10 mg/mL. The polycyclic aromatic hydrocarbon solutions were prepared by dissolving 50 mg of each analyte in 5 mL of tetrahydrofuran. The solution was further diluted to 0.1 mg/mL in methanol. The final aqueous solution was prepared by further diluting this solution with water to achieve µg/mL to ng/mL level concentrations depending on the compound class. Freshly prepared aqueous solutions were used for extraction.
5.2.8 Gravity-fed sample dispenser for capillary microextraction

A gravity-fed sample dispenser was used for capillary microextraction (Figure 5.1). It was built by modifying a Chromaflex AQ column (Kontes Glass Co., Vineland, NJ), which consists of a thick-walled Pyrex glass cylinder concentrically placed in an acrylic jacket. Since glass surfaces tend to adsorb polar analytes, the inner surface of the glass cylinder was deactivated by treating with HMDS solution as described before. The cylinder was then cooled down to ambient temperature, thoroughly rinsed with methanol and deionized water, and dried in a helium gas flow. The system was then reassembled.

5.2.9 Extraction of analytes on sol-gel poly-THF coated capillaries

A 12.5 cm long segment of the sol-gel poly-THF coated capillary (250 µm i.d.) was conditioned under helium purge in a GC oven using a temperature program (from 40 °C to 320°C @ 10 °C/min, held at the final temperature for 30 min). The conditioned capillary was then vertically connected to the lower end of the gravity-fed sample dispenser (Figure 1) using a plastic connector. A 50 mL volume of the aqueous sample containing trace concentrations of the target analytes was added to the inner glass cylinder through the sample inlet located at the top of the dispenser. The solution was passed through the capillary for 30 min to facilitate the extraction equilibrium to be established. The capillary was then detached from the dispenser and purged with helium.
for 1 min to remove residual water from the capillary walls.

### 5.2.10 Transferring the extracted analytes to the GC column and gas chromatographic analysis of the extracted analytes

The extracted analytes were transferred from the microextraction capillary to the GC column via thermal desorption. For this, the CME capillary was first installed on the GC injection port and securely interfaced with the GC capillary column. To facilitate the installation, both the GC injection port and the oven were cooled to 30 °C, and the quartz wool was removed from the injection port glass liner. The CME capillary with the extracted analytes in the coating was then introduced into the GC injection port from the bottom end of the port so that ~8 cm of the capillary remained inside the injection port. A graphite ferrule was used to make an air-tight connection between the capillary and the injection port. The lower end of the capillary (residing inside the GC oven) was connected to the inlet end of GC capillary column using a deactivated press-fit quartz connector. Figure 5.3 illustrates the connection of the extraction capillary with the GC analysis column using a press-fit quartz connector.
Figure 5.3 Schematic representation of the interface between the extraction capillary and the analysis column inside the GC oven using a press-fit quartz connector.
Installation and interfacing of the extraction capillary with the GC column were followed by thermal desorption of extracted analytes from the installed sol-gel poly-THF coated microextraction capillary. For this, the temperature of the PTV injection port was rapidly raised from 30 °C to 300 °C @ 100 °C /min while keeping the GC oven temperature at 30 °C (5 min). Under these temperature program conditions, the extracted analytes were effectively desorbed from the sol-gel poly-THF coating and were transported b the cooler coupling zone consisting of the lower end segment of the microextraction capillary and/or to the front end of the GC column - both located inside the GC oven and maintained at 30 °C. As the desorbed analytes reached the cooler interface zone (30 °C), they were focused into a narrow band. On completion of the 5-min desorption and focusing period, the analytes in this narrow band were analyzed by GC using temperature-programmed operation as follows: from 30°C to 300°C @ 20 °C /min with a 10 min hold time at the final temperature.

5.3 Results and discussion

5.3.1 Sol-gel chemistry of the coating process

Sol-gel chemistry is an elegant synthetic pathway to advanced materials [8, 39-41] that can be effectively utilized to create surface-bonded organic-inorganic hybrid coatings on the outer surface of conventional SPME fibers [6] as well as on the inner walls of a capillary for use in CME [7] (in-tube SPME). Additionally, sol-gel technology
can be used for creating both thin [21] and thick [24] coatings employing a wide variety of sol-gel-active organic ligands [21, 23-27].

Polytetrahydrofuran (poly-THF) [42] is a medium polarity polymer with terminal hydroxyl groups that can be utilized to bind this polymer to a sol-gel network via polycondensation reaction. It consists of tetramethylene oxide repeating units, and is synthesized through cationic ring opening polymerization of tetrahydrofuran (Scheme 5.1) using various initiators [43].
Scheme 5.1 Cationic ring opening polymerization of tetrahydrofuran
Table 5.1 lists the chemical ingredients used in this work to prepare the sol solution for the fabrication of a sol-gel poly-THF coated capillaries.

In order to create the sol-gel poly-THF coating in situ, the sol solution was kept inside the capillary for 60 min. During this in-capillary residence time, an organic-inorganic hybrid sol-gel network was evolving in the sol solution and a thin layer of this network located in close vicinity of the fused silica capillary walls had the opportunity to become chemically bonded to the capillary inner surface as a result of condensation reaction with the silanol groups on the capillary walls. The in situ creation of a highly stable, deactivated sol-gel coating involved the following processes: (1) catalytic hydrolysis of the alkoxide precursors, (2) polycondensation of the hydrolyzed precursor with other sol-gel-active components of the sol solution, (3) chemical bonding of poly-THF to the evolving sol-gel network, (4) chemical anchoring of the evolving hybrid organic-inorganic polymer to the inner walls of the capillary, and (5) derivatization of residual silanol groups on the coating by using HMDS. Reactions leading to the creation of a surface-bonded sol-gel poly-THF coating are presented in schemes 5.2-5.6. Hydrolysis of the sol-gel precursor, methyltrimethoxysilane (MTMOS), in presence of the sol-gel catalyst (TFA) is presented in scheme 5.2.

The hydrolysis products of the precursor can then undergo polycondensation reactions in a variety of ways to create a three-dimensional sol-gel network. One possible route for this process is presented in scheme 5.3.
Scheme 5.4 represents a simplified model for the polycondensation reaction of the growing sol-gel network with poly-THF to chemically incorporate the polymer in the resultant organic-inorganic hybrid network structure.

Scheme 5.5 represents the chemical anchoring process of the evolving network to the inner surface of the capillary.

Hexamethyldisilazane, used in the coating solution, is capable of deactivating the residual silanol groups on the stationary phase coating during thermal conditioning of the coated capillary as presented in scheme 5.6.
Methyltrimethoxysilane

**Scheme 5.2** Hydrolysis of the sol-gel precursor, MTMOS
Scheme 5.3 Polycondensation of hydrolyzed MTMOS
Scheme 5.4 Chemical incorporation of poly-THF into the sol-gel network
Scheme 5.5 Chemical anchoring of the sol-gel poly-THF hybrid organic-inorganic polymer to the silanol groups on the fused silica capillary inner walls
5.6 Deactivation of residual silanol groups by derivatization with hexamethyldisilazane
5.3.2. Characterization of sol-gel poly-THF sorbent

5.3.2.1 Fourier-transform infrared spectroscopic characterization of the sol-gel poly-THF coating

Fourier-transform infrared spectroscopic (FT-IR) measurements were performed in the range of 600-4000 cm\(^{-1}\) using a Avatar Model 320 FTIR system. Instrumental resolution was set at 4 cm\(^{-1}\).

Figure 5.4 represents three IR spectra representing pure poly-THF (left), sol solution having all ingredients except poly-THF (middle), sol-gel poly-THF coating (right). In the bottom spectrum, the IR data contain the feature (Si-O-C) identified by 1045 cm\(^{-1}\) that confirms the successful bonding of poly(tetrahydrofuran) to the hydrolyzed product of the alkoxide precursor [35]. The broad band at 3357 cm\(^{-1}\) clearly demonstrates the presence of hydroxyl chain ends in pure poly-THF. In the FT-IR spectrum for sol-gel poly-THF, similar band for OH- groups is seen to be present but in a lesser magnitude. This indicates that some of the OH- groups present in poly-THF have been used up in the process of condensation reaction that occurred to incorporate poly-THF moieties into the silica network.
Figure 5.4 IR spectra of pure polytetrahydrofuran (left), sol solution having all ingredients except polytetrahydrofuran (middle), sol-gel polytetrahydrofuran coating (right).
5.3.2.2 Scanning electron microscopy (SEM)

Surface morphology and the coating thickness of sol-gel poly-THF coated microextraction capillaries were investigated using scanning electron microscopy.

Figure 5.5 (a, b) represents a scanning electron micrographs (SEM) of a sol-gel poly-THF coated capillary.

From Figure 5.5a the coating thickness was estimated at 0.5 μm. As can be seen from the image, sol-gel poly-THF coating is remarkably uniform in thickness.
Figure 5.5a Scanning electron microscopic image of a 320 µm i.d. sol-gel poly-THF coated fused silica capillary used in CME.
**Figure 5.5b** Scanning electron microscopic image illustrating porous structure of sol-gel poly-THF coating in a 320 µm i.d. sol-gel poly-THF coated fused silica capillary used in CME
Figure 5.5b illustrates porous structure of the sol-gel poly-THF coating. It represents the surface view of the coating obtained at a magnification of 10,000x. It reveals the underlying porous structure of the sol-gel poly-THF coating. Due to the porous nature, the sol-gel poly-THF extraction medium possesses enhanced surface area, an advantageous feature to achieve enhanced sample capacity. The porous structure also facilitates efficient mass transfer through the coating, which in turn, translates into reduced equilibrium time during extraction.

5.3.2.3 Thermogravimetric analysis

Thermogravimetric analysis is a valuable tool for determining the thermal stability and composition of a material. Thermal stability is a measure of the capability of a material to preserve its properties upon heating. It is an important characteristic, which determines the maximum operating temperature of a given sorbent. The most common method of determining thermal stability of a sorbent is the non-isothermal TGA, in which the change in weight is determined while the temperature is increased in a linear manner. The data obtained via this process is called a thermogram, which is a graph of mass change versus temperature. Figure 5.6a represents a thermogram of pure poly-THF. Degradation begins at ca. 320 °C. Figure 5.6b represents a thermogram of sol-gel poly-THF sorbent. As can be seen, the hybrid material demonstrated at least 100 °C higher thermal stability compared to pure poly-THF. This was possible due to the inherent positive attribute of sol-gel
processing technology.

Figure 5.6a TGA curve of pure poly-THF for programmed heating (10 °C/min) under N₂ (Reproduced from Ref. [67] with permission of Elsevier)
Figure 5.6b TGA curve of sol-gel poly-THF for programmed heating (10 °C/min) under N$_2$ [TGA 2950, TA Instruments, Inc. DE, USA]
5.3.3 Determination of the extraction kinetics

SPME is a non-exhaustive extraction technique. Quantitation by SPME is based on solute extraction equilibrium established between the sample solution and the coating. Therefore, the time required to reach the equilibrium is particularly important. The time required for establishing equilibrium is dependent on the partition coefficient of the analyte and on the agitation of the sample matrix. For volatile analytes, the extraction equilibrium time is generally shorter in extraction from headspace. In order to extract the maximum amount of analyte, the extraction equilibrium time has to be reached. But this may be too long and impractical for many compounds. For instance, Doong et al. [63] reported that fluoranthene and pyrene required 540 min to reach extraction equilibrium on a commercial PDMS (30 µm) fiber in direct extraction. The long equilibrium time of high molecular-weight PAHs is due to their low water solubilities and diffusion coefficients. The diffusivities of high molecular-weight PAHs in the aqueous solution range from $6.3 \times 10^{-6}$ to $7.4 \times 10^{-6}$ cm$^2$/s which can be even smaller ($10^{-8}$ to $10^{-9}$ cm$^2$/s) in polymeric materials [64, 65].

Figure 5.7 represents the extraction kinetic profiles of different PAHs in direct-SPME using commercial PDMS (30 µm) fiber [63].
Figure 5.7 Extraction kinetic profiles of PAHs for direct-SPME using a commercial PDMS (30 µm) fiber (Reproduced from Ref. [63] by permission from J. Chromatogr. Sci.)
On the contrary, like other sol-gel sorbents, sol-gel poly-THF coating possesses a porous structure which has been revealed by the SEM images (Figure 5.4 b). Such porous structure should significantly increase the available surface area of the extracting phase. Due to the inherent porous structure, sol-gel poly-THF provides enhanced extracting phase loading and consequently high sample capacity. Lee and coworkers [68] reported that unlike SPME with polymer coated fiber, which involves a diffusion-limited process, the kinetics of SPME with a porous layer are mainly controlled by mass transfer in the bulk solution. Therefore, the extraction rate depends greatly on the degree of agitation rather than on film thickness. In capillary microextraction, the aqueous solution containing the analytes is under continuous flow due to gravity, and the extracting phase reaches the extraction equilibria within a short period of time. In the present study, sol-gel poly-THF coated microextraction capillary reached extraction equilibrium in a significantly shorter time even for high molecular-weight polycyclic aromatic hydrocarbons. For example, extraction equilibria were reached for two polycyclic aromatic hydrocarbons fluoranthene and pyrene within 30 min.

Figure 5.8 illustrates the CME kinetic profiles of two nonpolar analytes (fluoranthene and pyrene), two moderately polar analytes (heptanophenone and dodecanal) and a highly polar analyte (pentachlorophenol) extracted on a sol-gel poly-THF coated capillary. Extractions were carried out using aqueous solutions of fluoranthene (10 ppb), pyrene (10 ppb), dodecanal (20 ppb), heptanophenone (20 ppb), and pentachlorophenol (50 ppb). As can be seen, both nonpolar, moderately polar, and highly polar compounds reached respective equilibria within 30 min. This is indicative of
the fast mass transfer in the sol-gel poly-THF coating. Based on these experimental results, further experiments in this work were carried out using a 30-min extraction time.
Figure 5.8 Illustration of the extraction kinetics of nonpolar (fluoranthene and phenanthrene) and moderately polar (heptanophenone and dodecanal) compounds extracted on a 12.5 cm x 320 µm i.d. sol-gel poly-THF coated capillary using 10 ppb aqueous solution of each analyte in a mixture. Extraction kinetics of the highly polar compound, pentachlorophenol, was obtained separately on a 12.5 cm x 320 µm i.d. sol-gel poly-THF coated capillary using 50 ppb aqueous solution.

Extraction conditions: Extraction time, 10-50 min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C.
5.3.4 Determination of the analyte enrichment factors of sol-gel poly-THF sorbent

One of the major attributes of solid phase microextraction is its ability to preconcentrate the analyte(s) in the extracting phase, and therefore, it is important to measure the analyte(s) enrichment factor for a particular extracting phase in conjunction with a particular class of compounds. In order to compare relative extraction efficiency of different extracting phases, a commercial 100 µm PDMS coated fiber, commercial 85 µm PA coated fiber, and a sol-gel Poly-THF coated microextraction capillaries were employed. One µL of 1 mg/mL solution of n-undecanol (containing 1 ng of the analytes) was injected directly into the GC injection port under splitless mode and the corresponding peak area was recorded. A 20 ppb aqueous solution of fluorene and 100 ppb aqueous solution of n-undecanol were used for direct SPME using SPME fibers and the sol-gel poly-THF microextraction capillary. The volume of the sol-gel Poly-THF coating was calculated using the equation 1.

\[ V_f = 2\pi r d_f L \]  
Equation 5.1

Where,

- \( V_f \) = Volume of the sol-gel poly-THF extracting phase
- \( r \) = Radius of the extraction capillary
- \( L \) = Length of the extraction capillary
- \( d_f \) = Thickness of the coating

The volume of the sol-gel Poly-THF extraction phase (coating thickness ~ 0.5...
µm) in a 12.5 cm capillary (320 µm i.d.) has been calculated as 0.063 µL. The sorbent volume in commercial 100 µm PDMS (0.660 µL) and 85 µm PA (0.500 µL) has been found in the literature [66].

Direct extraction was carried out using the commercial fibers and sol-gel Poly-THF coated microextraction capillary for 30 min. The area obtained from each extraction (arbitrary unit) corresponded to the amount of analyte(s) present into the extracting phase. The concentration of analyte in the extracting phase was calculated using equation 2.

\[
C = \frac{\text{Mass of the analyte extracted into the sorbent (mg)}}{\text{Volume of the sorbent (mL)}} \quad \text{Eq. 5.2}
\]

Finally, the analyte enrichment factor was calculated using equation 3.

\[
\text{Enrichment Factor} = \frac{\text{Concentration of analyte in the extracting phase}}{\text{Original concentration of analyte in the sample matrix}} \quad \text{Eq. 5.3}
\]

The results obtained in these experiments have been presented in Figure 5.9 and Table 5.2. As can be seen from the table, sol-gel Poly-THF coated microextraction capillaries demonstrated excellent analyte enrichment capability.
**Figure 5.9** Comparison of extraction efficiencies of commercial PDMS (100 µm), PA (85 µm) and sol-gel poly-THF (0.5 µm) coatings [Y-axis represents computer output for peak area in arbitrary unit]
Table 5.2 Analyte enrichment factors of commercial PDMS (100 µm), commercial PA (85 µm) and sol-gel poly-THF sorbents for \( n \)-undecanol*

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Phase volume (µL)</th>
<th>Concentration of ( n )-undecanol in the phase after preconcentration (mg/mL)</th>
<th>Analyte enhancement factor for ( n )-undecanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDMS (commercial), 100 µm</td>
<td>0.660</td>
<td>0.0353</td>
<td>353</td>
</tr>
<tr>
<td>PA (commercial), 85 µm</td>
<td>0.500</td>
<td>0.0916</td>
<td>915</td>
</tr>
<tr>
<td>Sol-gel poly-THF, 0.5 µm</td>
<td>0.063</td>
<td>1.593</td>
<td>16,000</td>
</tr>
</tbody>
</table>

* Fiber SPME (using 100 µm PDMS and 85 µm PA fibers)/ capillary microextraction (using sol-gel poly-THF coated capillary) - GC analysis of fluorene (20 ppb) and \( n \)-undecanol (100 ppb). Extraction time, 30 min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 350 °C (280 °C for fiber SPME), at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C.
5.3.5 Determination of thermal stability of the sol-gel poly-THF coating

Thermal stability of an SPME sorbent characterizes resistance to degradation when heated. High thermal stability of a SPME sorbent is a highly desired attribute, particularly when it is used in combination with GC. As sol-gel process allows to incorporation organic moieties into inorganic polymeric network through chemical bonding, the resulting hybrid materials generally show higher thermal stability compared to pure organic polymers. This is no exception for sol-gel poly-THF.

In order to determine the thermal stability of sol-gel poly-THF coating, microextraction capillaries were conditioned at 300 °C, 320 °C, 340 °C, 350 °C and 360 °C for 2 h at each step. A mixture of analytes (comprising of both polar and nonpolar) were extracted for 30 min in three replicates using sol-gel poly-THF coated microextraction capillary after each step of thermal conditioning. The extraction results are presented in Table 5.3. As can be seen from Table 5.3, there is no noticeable change in extraction efficiency of the sol-gel poly-THF coated capillary even after heating at 360 °C. The high thermal stability of sol-gel poly-THF coating as evidenced from the experimental data also corroborates with the data obtained from thermogravimetric analysis.
Table 5.3 Thermal stability of sol-gel poly-THF coated microextraction capillaries through GC peak areas of the solutes extracted after conditioning at different temperatures*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Thermal conditioning temperature</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>300 °C</td>
<td>320 °C</td>
<td>340 °C</td>
<td>350 °C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A x 10^-5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonanal (100 ppb)</td>
<td></td>
<td>1.26</td>
<td>1.15</td>
<td>1.13</td>
<td>1.15</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol (400 ppb)</td>
<td></td>
<td>1.26</td>
<td>1.29</td>
<td>1.29</td>
<td>1.24</td>
</tr>
<tr>
<td>Heptanophenone (40 ppb)</td>
<td></td>
<td>3.19</td>
<td>3.03</td>
<td>3.13</td>
<td>3.19</td>
</tr>
<tr>
<td>Phenanthrene (20 ppb)</td>
<td></td>
<td>1.74</td>
<td>1.76</td>
<td>1.79</td>
<td>1.81</td>
</tr>
<tr>
<td>Pyrene (40 ppb)</td>
<td></td>
<td>2.96</td>
<td>3.04</td>
<td>2.98</td>
<td>3.06</td>
</tr>
</tbody>
</table>

*Capillary Microextraction-GC analysis of nonanal (100 ppb), 2,4,6-trichlorophenol (400 ppb), heptanophenone (40 ppb), phenanthrene (20 ppb), and pyrene (40 ppb) using sol-gel poly-THF coated capillary. Extraction time, 30 min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, 320 °C, 340 °C, 350 °C and 360 °C, programmed at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C.

A= Actual computer output for peak area in arbitrary unit.
5.3.5 Applications of sol-gel poly-THF coating

Sol-gel poly-THF coated capillaries were used to extract analytes of environmental, biomedical, and ecological importance, including polycyclic aromatic hydrocarbons (PAHs), aldehydes, ketones, alcohols, and phenols. The extracted compounds were further analyzed by GC.

5.3.5.1 Polycyclic aromatic hydrocarbons

PAHs are ubiquitous environmental pollutants that present potential health hazards because of their toxic, mutagenic, and carcinogenic properties [44, 45]. Because of this, Environmental Protection Agency (EPA) has promulgated 16 unsubstituted PAHs in its list of 129 priority pollutants [46a]. Table 5.4 lists pertinent physico-chemical properties of 5 EPA promulgated PAHs which were extracted and analyzed using sol-gel poly-THF coated capillaries and GC-FID. Capillary microextraction results are presented in Table 5.5 and 5.6. Calculated limit of detections for each tested PAHs are presented in Table 5.7. Figure 5.10 shows the gas chromatogram representing CME-GC analysis of the listed unsubstituted polyaromatic hydrocarbons from EPA priority list. They were extracted from an aqueous solution (each at 10 ppb) by capillary microextraction using a sol-gel poly-THF coated capillary. As can be seen from the data presented in Table 5.5 and 5.6, run-to-run and capillary-to-capillary repeatability in peak area obtained in CME-GC-FID experiments was quite satisfactory. For all PAHs, the RSD values were under
6%. Moreover, parts per quadrillion (ppq) level detection limits were obtained for PAHs in the CME-GC-FID using sol-gel poly-THF microextraction capillaries. These detection limits are significantly lower than those reported by others [46b] via SPME-GC-FID (e.g., 260 ppt for pyrene) using 100 µm thick PDMS coated commercial SPME fiber.
Table 5.4 Chemical structures and pertinent physical properties of polyaromatic hydrocarbons (PAHs) extracted using sol-gel poly-THF coated capillaries.

<table>
<thead>
<tr>
<th>Name of the analyte</th>
<th>Chemical structure</th>
<th>Molecular weight (g/mol)</th>
<th>Melting point (°C)</th>
<th>Boiling point (°C)</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthene</td>
<td><img src="image1.png" alt="Chemical Structure" /></td>
<td>152.20</td>
<td>92.5</td>
<td>150.0</td>
<td>0.8987</td>
</tr>
<tr>
<td>Fluorene</td>
<td><img src="image2.png" alt="Chemical Structure" /></td>
<td>166.22</td>
<td>114.8</td>
<td>295.0</td>
<td>1.203</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td><img src="image3.png" alt="Chemical Structure" /></td>
<td>178.23</td>
<td>99.2</td>
<td>340</td>
<td>0.9820</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td><img src="image4.png" alt="Chemical Structure" /></td>
<td>202.26</td>
<td>107.8</td>
<td>384</td>
<td>1.252</td>
</tr>
<tr>
<td>Pyrene</td>
<td><img src="image5.png" alt="Chemical Structure" /></td>
<td>202.26</td>
<td>156</td>
<td>404</td>
<td>1.271</td>
</tr>
</tbody>
</table>
Table 5.5 Run-to-run peak area reproducibility for PAHs in capillary microextraction using sol-gel poly-THF coating*

<table>
<thead>
<tr>
<th>Compounds name</th>
<th>A x 10^4</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthene</td>
<td>12.47</td>
<td>11.93</td>
<td>13.19</td>
<td>12.53</td>
<td>5.04</td>
<td></td>
</tr>
<tr>
<td>Fluorene</td>
<td>11.18</td>
<td>11.35</td>
<td>10.76</td>
<td>11.01</td>
<td>2.75</td>
<td></td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>14.46</td>
<td>13.67</td>
<td>13.72</td>
<td>13.95</td>
<td>3.16</td>
<td></td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>14.01</td>
<td>13.35</td>
<td>13.48</td>
<td>13.63</td>
<td>2.74</td>
<td></td>
</tr>
<tr>
<td>Pyrene</td>
<td>9.57</td>
<td>9.38</td>
<td>9.51</td>
<td>9.49</td>
<td>1.07</td>
<td></td>
</tr>
</tbody>
</table>

* Capillary Microextraction-GC analysis of PAHs (20 ppb each) using sol-gel poly-THF coated capillary. Extraction time, 30min. GC analysis conditions: 10 m x 250 μm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) Acenaphthene, (2) Fluorene, (3) Phenanthrene, (4) Fluoranthene, and (5) Pyrene.

A = Actual computer output for peak area in arbitrary unit.
Table 5.6 Capillary-to-capillary peak area for PAHs in capillary microextraction using sol-gel poly-THF coated capillaries*

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>A x 10^-4</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthene</td>
<td>13.92</td>
<td>13.84</td>
<td>13.38</td>
<td>13.71</td>
<td>2.13</td>
<td></td>
</tr>
<tr>
<td>Fluorene</td>
<td>12.09</td>
<td>12.03</td>
<td>11.50</td>
<td>11.88</td>
<td>2.73</td>
<td></td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>14.72</td>
<td>14.07</td>
<td>15.26</td>
<td>14.69</td>
<td>4.02</td>
<td></td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>13.92</td>
<td>15.63</td>
<td>13.83</td>
<td>14.46</td>
<td>7.05</td>
<td></td>
</tr>
<tr>
<td>Pyrene</td>
<td>9.18</td>
<td>9.50</td>
<td>8.29</td>
<td>8.99</td>
<td>6.96</td>
<td></td>
</tr>
</tbody>
</table>

*Capillary Microextraction- GC analysis of PAHs (20 ppb each) using sol-gel poly-THF coated capillary. Extraction time, 30 min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) Acenaphthene, (2) Fluorene, (3) Phenanthrene, (4) Fluoranthene, and (5) Pyrene. A=Actual computer output for peak area in arbitrary unit.
Table 5.7 Limits of detection (LOD) for PAHs in CME-GC-FID using sol-gel poly-THF microextraction capillaries*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (ppb)</th>
<th>Peak height (µV) (H x 10^-4)</th>
<th>Limit of detection (S/N 3), ppq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthene</td>
<td>20</td>
<td>3.05</td>
<td>625</td>
</tr>
<tr>
<td>Fluorene</td>
<td>20</td>
<td>2.93</td>
<td>460</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>20</td>
<td>3.27</td>
<td>400</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>20</td>
<td>3.94</td>
<td>260</td>
</tr>
<tr>
<td>Pyrene</td>
<td>20</td>
<td>2.47</td>
<td>750</td>
</tr>
</tbody>
</table>

* Capillary Microextraction-GC analysis of PAHs (20 ppb each) using sol-gel poly-THF coated capillary. Extraction time, 30 min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) Acenaphthene, (2) Fluorene, (3) Phenanthrene, (4) Fluoranthene, and (5) Pyrene.

H= Actual computer output for peak height in µV.
Figure 5.10 Capillary Microextraction-GC analysis of PAHs (20 ppb each) using sol-gel poly-THF coated capillary. Extraction time, 30min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C.

Peaks: (1) Acenaphthene, (2) Fluorene, (3) Phenanthrene, (4) Fluoranthene, and (5) Pyrene.
5.3.5.2 Aldehydes

Aldehydes and ketones (carbonyl compounds) are of increasing concern due to their potential adverse health effects and environmental prevalence [47-49]. Aldehydes and ketones can form in water by the photodegradation of dissolved natural organic matter [50]. They may also form as disinfection by-products due to chemical reactions of chlorine and/or ozone (frequently used to disinfect water) with natural organic matter present in water [51]. Many of these by-products have been shown to be carcinogens or carcinogen suspects [52]. This is, in part, due to the high polarity and reactivity of carbonyl compounds in water matrices [51,53,54]. Table 5.8 lists chemical structure and important physico-chemical properties of 5 environmentally important aldehydes. Figure 10 represents a gas chromatogram of the mixture of 5 underivatized aldehydes that were extracted from an aqueous solution containing 20 ppb of each analyte. Run-to-run and capillary-to-capillary microextraction data have been presented in Tables 5.9 and 5.10, respectively. Low RSD values for both run-to-run (ranges from 2.45% to 7.24 %) and capillary-to-capillary (ranges from 3.89 % to 9.31%) microextraction are definitely indicative of high system reproducibility of sol-gel poly-THF coated microextraction capillaries and the extraction method itself.
Table 5.8 Chemical structures and pertinent physical properties of aldehydes extracted using sol-gel poly-THF coated capillaries

<table>
<thead>
<tr>
<th>Name of the analyte</th>
<th>Chemical structure</th>
<th>Molecular weight (g/mol)</th>
<th>Melting point (°C)</th>
<th>Boiling point (°C)</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Undecanal</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>142.2406</td>
<td>63</td>
<td>93 at 23 mm Hg</td>
<td>0.823</td>
</tr>
<tr>
<td>n-Decanal</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>156.2674</td>
<td>7</td>
<td>207-209</td>
<td>0.825</td>
</tr>
<tr>
<td>n-Undecanal</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>170.2942</td>
<td>-</td>
<td>109-115 at 5 mm Hg</td>
<td>0.827</td>
</tr>
<tr>
<td>n-Dodecanol</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>184.321</td>
<td>12</td>
<td>185 at 100 mm Hg</td>
<td>0.829</td>
</tr>
</tbody>
</table>
Table 5.9 Run-to-run peak area reproducibility for aldehydes in capillary microextraction using sol-gel poly-THF coating*

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>A x 10^{-4}</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Run 1</td>
<td>Run 2</td>
</tr>
<tr>
<td>n-Nonanal</td>
<td>7.71</td>
<td>7.78</td>
</tr>
<tr>
<td>n-Decanal</td>
<td>9.02</td>
<td>10.28</td>
</tr>
<tr>
<td>n-Undecanal</td>
<td>7.11</td>
<td>6.67</td>
</tr>
<tr>
<td>n-Dodecanal</td>
<td>5.31</td>
<td>4.79</td>
</tr>
</tbody>
</table>

*Capillary microextraction- GC analysis of aldehydes at 20 ppb concentration using poly-THF coated capillary. Extraction time, 30 min. GC analysis conditions: 10 m x 250 μm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, programmed at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) n-Nonanal, (2) n-Decanal, (3) n-Undecanal, (4) n-Dodecanal.

A= Actual computer output for peak area in arbitrary unit.
Table 5.10 Capillary-to-capillary peak area reproducibility for aldehydes in capillary microextraction using sol-gel poly-THF coating*

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>A x 10^-4</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Nonanal</td>
<td></td>
<td>8.32</td>
<td>7.71</td>
<td>8.13</td>
<td>8.06</td>
<td>3.89</td>
</tr>
<tr>
<td>n-Decanal</td>
<td></td>
<td>9.92</td>
<td>10.76</td>
<td>10.03</td>
<td>10.24</td>
<td>4.47</td>
</tr>
<tr>
<td>n-Undecanal</td>
<td></td>
<td>8.01</td>
<td>7.12</td>
<td>7.85</td>
<td>7.66</td>
<td>6.25</td>
</tr>
<tr>
<td>n-Dodecanal</td>
<td></td>
<td>6.53</td>
<td>5.53</td>
<td>6.53</td>
<td>6.20</td>
<td>9.31</td>
</tr>
</tbody>
</table>

*Capillary microextraction-GC analysis of aldehydes at 20 ppb concentration using poly-THF coated capillary. Extraction time, 30 min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, programmed at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) n-Nonanal, (2) n-Decanal, (3) n-Undecanal, (4) n-Dodecanal.

A= Actual computer output for peak area in arbitrary units.
Table 5.11 Limits of detection (LOD) for aldehydes in CME-GC-FID using sol-gel poly-THF microextraction capillaries*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (ppb)</th>
<th>Peak height (µV) ( (H \times 10^{-4}) )</th>
<th>Limit of detection (S/N 3), ppq</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Nonanal</td>
<td>20</td>
<td>1.93</td>
<td>1000</td>
</tr>
<tr>
<td>n-Decanal</td>
<td>20</td>
<td>2.43</td>
<td>625</td>
</tr>
<tr>
<td>n-Undecanal</td>
<td>20</td>
<td>1.86</td>
<td>750</td>
</tr>
<tr>
<td>n-Dodecanal</td>
<td>20</td>
<td>1.42</td>
<td>940</td>
</tr>
</tbody>
</table>

*Capillary microextraction- GC analysis of aldehydes at 20 ppb concentration using poly-THF coated capillary. Extraction time, 30 min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, programmed at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) n-Nonanal, (2) n-Decanal, (3) n-Undecanal, (4) n-Dodecanal.

H= Actual computer output for peak height in µV.
Figure 5.11 Capillary microextraction-GC analysis of aldehydes in water at 20 ppb concentration using poly-THF coated capillary. Extraction time, 30 min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, programmed at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) n-Nonanal (2) n-Decanal, (3) n-Undecanal and (4) n-Dodecanal.
The data presented in Table 5.11 indicate that a sol-gel poly-THF coated capillary can extract free aldehydes from aqueous media to provide a limit of detection (LOD) which is comparable with, or lower than that achieved through derivatization [53]. For example, LOD for decanal has been reported as 200 ppt [53] (in SPME-GC-ECD) on a 65 µm DVB-PDMS coating after derivatization with o- (2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) whereas in the present work a significantly lower detection limit (625 ppq) was achieved for the same analyte using a sol-gel poly-THF coated capillary in hyphenation with GC-FID, even though ECD often provides higher sensitivity than FID for oxygenated compounds. The same trend has also been observed for other analytes. It should be pointed out that derivatization of these analytes, especially when they are present in trace concentration, may complicate the analytical process, thus compromising quantitative accuracy.

5.3.5.3. Ketones

Figure 5.12 represents a gas chromatogram of a mixture of 5 underivatized ketones (20 ppb each) extracted from an aqueous solution. The selected ketones are listed in Table 5.12 along with their chemical structures and pertinent physico-chemical properties. Excellent peak shapes (Figure 5.12) and run-to-run (Table 5.13) and capillary-to-capillary extraction reproducibility (Table 5.14) are indicative of preserved separation efficiency in CME-GC analysis and versatility of the sol-gel coating procedure used to prepare the extraction capillaries and the used GC column.
Table 5.12 Chemical structures and pertinent physical properties of ketones extracted using sol-gel poly-THF coating

<table>
<thead>
<tr>
<th>Name of the analyte</th>
<th>Chemical structure</th>
<th>Molecular weight (g/mol)</th>
<th>Melting point (°C)</th>
<th>Boiling point (°C)</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrophenone</td>
<td><img src="image" alt="Butyrophenone Structure" /></td>
<td>148.2042</td>
<td>11-13</td>
<td>220-222</td>
<td>0.988</td>
</tr>
<tr>
<td>Valerophenone</td>
<td><img src="image" alt="Valerophenone Structure" /></td>
<td>162.231</td>
<td>-9</td>
<td>105-107 at 5 mm Hg</td>
<td>0.988</td>
</tr>
<tr>
<td>Hexanophenone</td>
<td><img src="image" alt="Hexanophenone Structure" /></td>
<td>176.2578</td>
<td>25-26</td>
<td>265.1</td>
<td>0.958</td>
</tr>
<tr>
<td>Heptanophenone</td>
<td><img src="image" alt="Heptanophenone Structure" /></td>
<td>190.2846</td>
<td>17</td>
<td>155 at 15 mm Hg</td>
<td>0.946</td>
</tr>
<tr>
<td>Decanophenone</td>
<td><img src="image" alt="Decanophenone Structure" /></td>
<td>232.37</td>
<td>36-39</td>
<td>168 at 5 mm Hg</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 5.13 Run-to-run peak area reproducibility for ketones in capillary microextraction using sol-gel poly-THF coating*

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>A x 10^-4</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrophenone</td>
<td>10.81</td>
<td>11.14</td>
<td>11.27</td>
<td>11.07</td>
<td>2.12</td>
<td></td>
</tr>
<tr>
<td>Valerophenone</td>
<td>10.25</td>
<td>10.79</td>
<td>10.85</td>
<td>10.63</td>
<td>3.09</td>
<td></td>
</tr>
<tr>
<td>Hexanophenone</td>
<td>12.64</td>
<td>12.54</td>
<td>11.00</td>
<td>12.06</td>
<td>7.62</td>
<td></td>
</tr>
<tr>
<td>Heptanophenone</td>
<td>11.96</td>
<td>12.51</td>
<td>12.98</td>
<td>12.48</td>
<td>4.12</td>
<td></td>
</tr>
<tr>
<td>Decanophenone</td>
<td>7.87</td>
<td>7.51</td>
<td>8.47</td>
<td>7.95</td>
<td>6.06</td>
<td></td>
</tr>
</tbody>
</table>

*Capillary microextraction-GC analysis of ketones at (20 ppb) using sol-gel poly-THF coated capillary. Extraction time, 30min. GC analysis conditions: 10 m x 250 μm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, program rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) Butyrophenone, (2) Valerophenone, (3) Hexanophenone, (4) Heptanophenone, and (5) Decanophenone.

A= Actual computer output for peak area in arbitrary unit.
Table 5.14 Capillary-to-capillary peak area reproducibility for ketones in capillary microextraction using sol-gel poly-THF coating*

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrophenone</td>
<td>11.23</td>
<td>11.74</td>
<td>12.10</td>
<td>11.69</td>
<td>3.73</td>
</tr>
<tr>
<td>Valerophenone</td>
<td>11.93</td>
<td>12.03</td>
<td>12.51</td>
<td>12.16</td>
<td>2.55</td>
</tr>
<tr>
<td>Hexanophenone</td>
<td>15.01</td>
<td>15.88</td>
<td>14.80</td>
<td>15.23</td>
<td>3.773</td>
</tr>
<tr>
<td>Heptanophenone</td>
<td>16.66</td>
<td>15.35</td>
<td>15.49</td>
<td>15.83</td>
<td>4.53</td>
</tr>
<tr>
<td>Decanophenone</td>
<td>1.24</td>
<td>10.77</td>
<td>10.93</td>
<td>11.37</td>
<td>8.07</td>
</tr>
</tbody>
</table>

*Capillary microextraction- GC analysis of ketones at (20 ppb) using sol-gel poly-THF coated capillary. Extraction time, 30min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, program rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) Butyrophenone, (2) Valerophenone, (3) Hexanophenone, (4) Heptanophenone, and (5) Decanophenone.

A= Actual computer output for peak area in arbitrary unit.
Table 5.15 Limits of detection (LOD) for ketones in CME-GC-FID using sol-gel poly-THF microextraction capillaries*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (ppb)</th>
<th>Peak height (µV) ((H \times 10^{-4}))</th>
<th>Limit of detection ((S/N , 3)), ppq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrophenone</td>
<td>20</td>
<td>2.91</td>
<td>1000</td>
</tr>
<tr>
<td>Valerophenone</td>
<td>20</td>
<td>3.00</td>
<td>460</td>
</tr>
<tr>
<td>Hexanophenone</td>
<td>20</td>
<td>3.47</td>
<td>600</td>
</tr>
<tr>
<td>Heptanophenone</td>
<td>20</td>
<td>3.05</td>
<td>340</td>
</tr>
<tr>
<td>Decanophenone</td>
<td>20</td>
<td>2.03</td>
<td>1000</td>
</tr>
</tbody>
</table>

*Capillary microextraction-GC analysis of ketones at (20 ppb) using sol-gel poly-THF coated capillary. Extraction time, 30 min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, program rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) Butyrophenone, (2) Valerophenone, (3) Hexanophenone, (4) Heptanophenone, and (5) Decanophenone

H= Actual computer output for peak height in µV.
Figure 5.12 Capillary microextraction-GC analysis of ketones at (20 ppb) using sol-gel poly-THF coated capillary. Extraction time, 30 min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, program rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) Butyrophenone, (2) Valerophenone, (3) Hexanophenone, (4) Heptanophenone, and (5) Decanophenone.
5.3.5.4. Chlorophenols

Chlorophenols (CPs) represent an important class of contaminants in environmental waters and soils due to their widespread use in industry, agriculture, and domestic purposes. Chlorophenols have been widely used as preservatives, pesticides, antiseptics, and disinfectants [55]. They are also used in producing dyes, plastics and pharmaceuticals. In the environment, chlorophenols may also form as a result of hydrolysis, oxidation and microbiological degradation of chlorinated pesticides. Chlorine-treated drinking water is another source of chlorophenols [56]. As a result, chlorophenols are often found in waters [57, 58], soils [59], and sediments [59]. Chlorophenols are highly toxic, poorly biodegradable, carcinogenic and recalcitrant [60]. Owing to their carcinogenicity and considerable persistence, five of the chlorophenols (2-chlorophenol; 2,4-dichlorophenol; 2,4,6-trichlorophenol; 4-chloro-3-methylphenol and pentachlorophenol) have been classified as priority pollutants by the US EPA [61]. Since chlorophenols are highly polar, it is quite difficult to extract them directly from polar aqueous media. Derivatization, pH adjustment, and/or salting-out are often used to facilitate the extraction [3]. To reduce the analytical complexity due to derivatization, HPLC-UV is frequently used for the analysis of phenolic compounds [58] but at the expense of detection sensitivity. Figure 13 represents CME-GC analysis of five underivatized chlorophenols extracted from an aqueous medium using a sol-gel poly-THF coated capillary. We did not have to use derivatization, pH adjustment or salting out effect to extract chlorophenols from aqueous medium. Still, we have achieved a lower detection limit (e.g., 18 ppt for pentachlorophenol, by CME-GC-FID) compared to other reports in the literature (1.4 ppb for the same compound, by SPME-GC-FID) [3].
Table 5.16 Chemical structures and pertinent physical properties of chlorophenols (CPs) analyzed using sol-gel poly-THF coating

<table>
<thead>
<tr>
<th>Name of the analyte</th>
<th>Chemical structure</th>
<th>Molecular weight (g/mol)</th>
<th>Melting point (°C)</th>
<th>Boiling point (°C)</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Chlorophenol</td>
<td><img src="#" alt="Structural formula" /></td>
<td>128.5579</td>
<td>7</td>
<td>125.6</td>
<td>1.241</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td><img src="#" alt="Structural formula" /></td>
<td>163.003</td>
<td>45</td>
<td>210</td>
<td>1.383</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td><img src="#" alt="Structural formula" /></td>
<td>197.448</td>
<td>69.5</td>
<td>244.5</td>
<td>1.49</td>
</tr>
<tr>
<td>4-Chloro-3-methylphenol</td>
<td><img src="#" alt="Structural formula" /></td>
<td>142.584</td>
<td>67</td>
<td>235</td>
<td>-</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td><img src="#" alt="Structural formula" /></td>
<td>266.3383</td>
<td>174</td>
<td>310</td>
<td>1.979</td>
</tr>
</tbody>
</table>
Table 5.17 Run-to-run peak area reproducibility for chlorophenols in capillary microextraction using sol-gel poly-THF Coating*

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Chlorophenol</td>
<td>7.53</td>
<td>6.60</td>
<td>7.71</td>
<td>7.28</td>
<td>7.32</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>11.13</td>
<td>12.14</td>
<td>10.92</td>
<td>11.30</td>
<td>5.71</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>14.40</td>
<td>13.71</td>
<td>13.36</td>
<td>13.82</td>
<td>3.83</td>
</tr>
<tr>
<td>4-Chloro-3-methylphenol</td>
<td>16.51</td>
<td>17.00</td>
<td>17.29</td>
<td>16.93</td>
<td>2.35</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>33.01</td>
<td>33.54</td>
<td>31.10</td>
<td>32.55</td>
<td>3.94</td>
</tr>
</tbody>
</table>

*Capillary microextraction-GC analysis of chlorophenols using poly-THF coated capillary. Extractions were carried out from a solution containing 2-chlorophenol (1 ppm); 2,4-dichlorophenol (50 ppb); 2,4,6-trichlorophenol (50 ppb); 4-chloro, 3-methylphenol (100 ppb); and pentachlorophenol (50 ppb). Extraction time, 30min. GC analysis conditions: 10 m x 250 μm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, programmed at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) 2-Chlorophenol, (2) 2,4-Dichlorophenol, (3) 2,4,6-Trichlorophenol, (4) 4-Chloro-3-methylphenol, and (5) Pentachlorophenol.

A= Actual computer output for peak area in arbitrary unit.
Table 5.18 Capillary-to-capillary peak area reproducibility for chlorophenols in capillary microextraction using sol-gel poly-THF coating*

<table>
<thead>
<tr>
<th>Compounds name</th>
<th>A x 10^{-3}</th>
<th></th>
<th></th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Run 1</td>
<td>Run 2</td>
<td>Run 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>4.95</td>
<td>4.22</td>
<td>4.43</td>
<td>4.53</td>
<td>8.74</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>8.84</td>
<td>8.74</td>
<td>8.21</td>
<td>8.60</td>
<td>3.96</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>10.10</td>
<td>9.62</td>
<td>11.10</td>
<td>10.27</td>
<td>7.02</td>
</tr>
<tr>
<td>4-Chloro-3-methylphenol</td>
<td>14.40</td>
<td>13.52</td>
<td>13.27</td>
<td>13.73</td>
<td>4.50</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>27.83</td>
<td>28.38</td>
<td>29.56</td>
<td>28.38</td>
<td>3.22</td>
</tr>
</tbody>
</table>

*Capillary microextraction-GC analysis of chlorophenols using poly-THF coated capillary. Extractions were carried out from a solution containing 2-chlorophenol (1 ppm); 2,4-dichlorophenol (50 ppb); 2,4,6-trichlorophenol (50 ppb); 4-chloro-3-methylphenol (100 ppb); and pentachlorophenol (50 ppb). Extraction time, 30min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, programmed at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) 2-Chlorophenol, (2) 2,4-Dichlorophenol, (3) 2,4,6-Trichlorophenol, (4) 4-Chloro-3-methylphenol, and (5) Pentachlorophenol.

A= Actual computer output for peak area in arbitrary unit.
Table 5.19 Limits of detection (LOD) for chlorophenols in CME-GC-FID using sol-gel poly-THF microextraction capillaries*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (ppb)</th>
<th>Measured peak height (µV) (H x 10⁻³)</th>
<th>Limit of detection (S/N 3), ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Chlorophenol</td>
<td>1 000</td>
<td>3.93</td>
<td>150</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>50</td>
<td>5.23</td>
<td>85</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>50</td>
<td>5.45</td>
<td>81</td>
</tr>
<tr>
<td>4-Chloro-3-methylphenol</td>
<td>100</td>
<td>7.35</td>
<td>30</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>50</td>
<td>11.34</td>
<td>18</td>
</tr>
</tbody>
</table>

*Capillary microextraction-GC analysis of chlorophenols using poly-THF coated capillary. Extractions were carried out from a solution containing 2-chlorophenol (1 ppm); 2,4-dichlorophenol (50 ppb); 2,4,6-trichlorophenol (50 ppb); 4-chloro, 3-methylphenol (100 ppb); and pentachlorophenol (50 ppb). Extraction time, 30 min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, programmed at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) 2-Chlorophenol, (2) 2,4-Dichlorophenol, (3) 2,4,6-Trichlorophenol, (4) 4-Chloro-3-methylphenol, and (5) Pentachlorophenol.

H= Actual computer output for peak height in µV.
Figure 5.13 Capillary microextraction-GC analysis of chlorophenols using poly-THF coated capillary. Extractions were carried out from a solution containing 2-chlorophenol (1 ppm); 2,4-dichlorophenol (50 ppb); 2,4,6-trichlorophenol (50 ppb); 4-chloro, 3-methylphenol (100 ppb); and pentachlorophenol (50 ppb). Extraction time, 30min. GC analysis conditions: 10 m x 250 μm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 30 °C, final 300 °C, programmed at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C, at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) 2-Chlorophenol, (2) 2,4-Dichlorophenol, (3) 2,4,6-Trichlorophenol, (4) 4-Chloro-3-methylphenol, and (5) Pentachlorophenol.
5.3.5.4 Alcohols

Figure 5.14 represents a gas chromatogram for a mixture of alcohols. Being highly polar compounds, alcohols demonstrate higher affinity for water and were extracted from aqueous samples using sol-gel poly-THF capillaries without exploiting any derivatization, pH adjustment or salting-out effects. Run-to-run and capillary-to-capillary microextraction data are presented in Table 5.20 and 5.21 respectively. Limit of detection data are presented in Table 5.22.
### Table 5.20 Run-to-run peak area reproducibility for alcohols in capillary microextraction using sol-gel poly-THF coating*

<table>
<thead>
<tr>
<th>Compounds name</th>
<th>A x 10^-4</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Run 1</td>
<td>Run 2</td>
</tr>
<tr>
<td>1-Heptanol</td>
<td>3.83</td>
<td>4.01</td>
</tr>
<tr>
<td>1-Octanol</td>
<td>8.06</td>
<td>8.31</td>
</tr>
<tr>
<td>1-Nonanol</td>
<td>9.90</td>
<td>9.87</td>
</tr>
<tr>
<td>1-Decanol</td>
<td>13.78</td>
<td>13.81</td>
</tr>
<tr>
<td>1-Undecanol</td>
<td>17.33</td>
<td>16.81</td>
</tr>
<tr>
<td>1-Dodecanol</td>
<td>14.93</td>
<td>14.21</td>
</tr>
<tr>
<td>1-Tridecanol</td>
<td>22.58</td>
<td>21.74</td>
</tr>
</tbody>
</table>

*Capillary microextraction-GC analysis of alcohols (100 ppb each) using sol-gel poly-THF coated capillary. Extraction time, 30 min. GC analysis conditions: 10 m x 250 μm i.d. sol-gel PEG column; splitless injection; injector temperature, initial 30 °C, final 300 °C, programmed at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 280 °C at a rate of 20 C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) 1-Heptanol, (2) 1-Octanol, (3) 1-Nonanol, (4) 1-Decanol, (5) 1-Undecanol, (6) 1-Dodecanol, and (7) 1-Tridecanol.

A= Actual computer output for peak area in arbitrary unit.
Table 5.21 Capillary-to-capillary peak area reproducibility for alcohols in capillary microextraction using sol-gel poly-THF coating*

<table>
<thead>
<tr>
<th>Compounds Name</th>
<th>A x 10^{-4}</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Run 1</td>
<td>Run 2</td>
</tr>
<tr>
<td>Heptanol</td>
<td>2.95</td>
<td>3.42</td>
</tr>
<tr>
<td>Octanol</td>
<td>7.01</td>
<td>7.04</td>
</tr>
<tr>
<td>Nonanol</td>
<td>8.42</td>
<td>8.31</td>
</tr>
<tr>
<td>Decanol</td>
<td>11.29</td>
<td>12.33</td>
</tr>
<tr>
<td>Undecanol</td>
<td>14.83</td>
<td>16.05</td>
</tr>
<tr>
<td>Tridecanol</td>
<td>18.92</td>
<td>17.54</td>
</tr>
</tbody>
</table>

*Capillary microextraction-GC analysis of alcohols (100 ppb each) using sol-gel poly-THF coated capillary. Extraction time, 30min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PEG column; splitless injection; injector temperature, initial 30 °C, final 300 °C, programmed at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 280 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) 1-Heptanol, (2) 1-Octanol, (3) 1-Nonanol, (4) 1-Decanol, (5) 1-Undecanol, (6) 1-Dodecanol, and (7) 1-Tridecanol.

A= Actual computer output for peak area in arbitrary unit.
Table 5.22 Limits of detection (LOD) for alcohols in CME-GC-FID using sol-gel poly-THF microextraction capillaries*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (ppb)</th>
<th>Measured peak height (µV) (H x 10^{-3})</th>
<th>Limit of detection (S/N 3), ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Heptanol</td>
<td>100</td>
<td>17.14</td>
<td>13</td>
</tr>
<tr>
<td>1-Octanol</td>
<td>100</td>
<td>37.16</td>
<td>5</td>
</tr>
<tr>
<td>1-Nonanol</td>
<td>100</td>
<td>42.51</td>
<td>0.75</td>
</tr>
<tr>
<td>1-Decanol</td>
<td>100</td>
<td>60.63</td>
<td>0.61</td>
</tr>
<tr>
<td>1-Undecanol</td>
<td>100</td>
<td>80.32</td>
<td>0.59</td>
</tr>
<tr>
<td>1-Dodecanol</td>
<td>100</td>
<td>73.92</td>
<td>1.15</td>
</tr>
<tr>
<td>1-Tridecanol</td>
<td>100</td>
<td>76.94</td>
<td>1.15</td>
</tr>
</tbody>
</table>

*Capillary microextraction-GC analysis of alcohols (100 ppb each) using sol-gel poly-THF coated capillary. Extraction time, 30 min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PEG column; splitless injection; injector temperature, initial 30 °C, final 300 °C, programmed at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 280 °C at a rate of 20 C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) 1-Heptanol, (2) 1-Octanol, (3) 1-Nonanol, (4) 1-Decanol, (5) 1-Undecanol, (6) 1-Dodecanol, and (7) 1-Tridecanol.

H= Actual computer output for peak height in µV.
Figure 5.14 Capillary microextraction-GC analysis of alcohols (100 ppb each) using sol-gel poly-THF coated capillary. Extraction time, 30 min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PEG column; splitless injection; injector temperature, initial 30 °C, final 300 °C, programmed at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 280 °C at a rate of 20 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) 1-Heptanol, (2) 1-Octanol, (3) 1-Nonanol, (4) 1-Decanol, (5) 1-Undecanol, (6) 1-Dodecanol, and (7) 1-Tridecanol.
The presented data indicate excellent affinity of the sol-gel poly-THF coating for these highly polar analytes that are often difficult to extract from aqueous media in underivatized form using commercial coatings. Moreover, high detection sensitivity (Table 5.22) and excellent symmetrical peak shapes also demonstrate outstanding performance of the sol-gel poly-THF coating and excellent deactivation characteristics of the sol-gel PEG column used for GC analysis, respectively.

5.3.5.6 Mixture of polar and moderately polar and nonpolar compounds

Finally, a mixture containing analytes from different chemical classes representing a wide polarity range was extracted from an aqueous sample using a sol-gel poly-THF coated capillary. As is revealed from the chromatogram (Figure 5.15), a sol-gel poly-THF coated capillary can be effectively used to simultaneously extract nonpolar, moderately polar, and highly nonpolar compounds from an aqueous matrix.
Figure 5.15 Capillary microextraction-GC analysis of a mixture of nonpolar, moderately polar and highly polar compounds using poly-THF coated capillary. Extractions were carried out from an aqueous sample containing 2-chlorophenol (1 ppm); 2,4,6-trichlorophenol (50 ppb); pentachlorophenol (50 ppb); valerophenone (10 ppb); hexanophenone (10 ppb); nonanal (10 ppb); decanal (10 ppb); fluoranthene (10 ppb); pyrene (10 ppb). Extraction time, 30min. GC analysis conditions: 10 m x 250 µm i.d. sol-gel PDMS column; splitless injection (desorption of analyte in splitless mode); injector temperature, initial 30 °C, final 300 °C, programmed at a rate of 100 °C/min; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C/min; helium carrier gas; FID temperature 350 °C. Peaks: (1) 2-Chlorophenol, (2) Nonanal, (3) Decanal, (4) 2,4,6-Trichlorophenol, (5) Valerophenone, (6) Hexanophenone, (7) Pentachlorophenol, (8) Fluoranthene, and (9) Pyrene.
This may be explained by the existence of different polarity domains [62] (organic and inorganic) in the sol-gel poly-THF coating.

Run-to-run repeatability and capillary-to-capillary reproducibility are two important characteristics for CME as a microextraction technique and for the sol-gel coating technique used for their preparation. These parameters were evaluated from experimental data involving replicate measurements carried out on the same capillary under the same set of conditions (run-to-run) or on a number of sol-gel poly-THF coated capillaries prepared using the same protocol (capillary-to-capillary). The run-to-run repeatability and capillary-to-capillary reproducibility for sol-gel capillary microextraction were evaluated through peak area relative standard deviation (RSD) values for the extracted analytes. For nonpolar and moderately polar analytes (e.g., PAHs, aldehydes, ketones), these parameters had values in the range of 2.19-7.48% and 4.35-10.31, respectively. In the case of polar analytes (phenols and alcohols), these values were less than 7.32% and 11.77 %, respectively. For a sample preparation technique, these peak area RSD values can be regarded as indicative of good consistency in CME performance of the microextraction capillaries as well as the good reproducibility in the method used for their preparation.

5.3.6. Possibility of automation

In the present work, sol-gel CME-GC operation was performed manually which is not convenient from a practical point of view. For wide acceptance of the technique, the inconvenience associated with manual installation of the microextraction capillary in the
GC system needs to be overcome. There are various possibilities to solve this problem, including the use of a robotic arm equipped with devices necessary for performing CME, desorbing the analytes, and transferring the desorbed analytes into the separation column.

In our opinion, sol-gel capillary microextraction technique described in the present dissertation has a great potential for automated operation in hyphenation with both gas-phase and liquid-phase separation techniques. Because of the tubular format of the extraction device combined with high thermal and solvent stability of the surface-bonded sol-gel extraction coating, sol-gel capillary microextraction can be expected to offer high degree of versatility in automated operation.

5.4 Conclusion

Novel sol-gel poly-THF coating was developed for high-performance capillary microextraction to facilitate ultra-trace analysis of polar and nonpolar organic compounds. Parts per quadrillion level detection limits were achieved using Poly-THF coated microextraction capillaries in conjunction with GC-FID. To the best of our knowledge, we are the first to report [70] on the use of sol-gel poly-THF sorbent in analytical microextraction. Sol-gel Poly-THF coatings showed extraordinarily high sorption efficiency for both polar and nonpolar compounds, and proved to be highly effective in providing simultaneous extraction of nonpolar, moderately polar, and highly polar analytes from aqueous media. Sol-gel poly-THF coated microextraction capillaries showed excellent thermal and solvent stability, making them very suitable for hyphenation with both gas-phase and liquid-phase separation techniques, including GC,
HPLC, and CEC. In CME-HPLC and CME-CEC hyphenations, sol-gel poly-THF coated microextraction capillaries have the potential to provide new levels of detection sensitivity in liquid-phase trace analysis, and to extend the analytical scope of CME to thermally labile-, high molecular weight-, and other types of compounds that are not amenable to GC. Further sensitivity enhancement should be possible through the use of monolithic microextraction capillaries with sol-gel poly-THF based hybrid organic-inorganic sorbents. This could open up new possibilities in ultra-trace analysis of organic pollutants in aqueous media.
5.5 References for Chapter Five


Appendices
Capillary Electrophoresis and Fluorescence Anisotropy for Quantitative Analysis of Peptide–Protein Interactions Using JAK2 and SH2-Bβ as a Model System

Peilin Yang, Rebecca J. Whelan, Emily E. Jameson, Jason H. Kurzer, Lawrence S. Argetsinger, Christin Carter-Su, Abuzar Kabir, Abdul Malik, and Robert T. Kennedy

Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109

Fluorescence anisotropy capillary electrophoresis (FACE) and affinity probe capillary electrophoresis (APCE) with laser-induced fluorescence detection were evaluated for analysis of peptide–protein interactions with rapid binding kinetics. The Src homology 2 domain of protein SH2-Bβ (SH2-Bβ (525–670)) and a tyrosine-phosphorylated peptide corresponding to the binding sequence of JAK2 were used as a model system. For peptide labeled with fluorescein, the $K_D = 82 \pm 7$ nM as measured by fluorescence anisotropy (FA). APCE assays had a limit of detection (LOD) of $100 \text{ nM}$ or 12 amol injected for SH2-Bβ (525–670). The separation time of $4 \text{ s}$, achieved using an electric field of $2860 \text{ V/cm}$ on $7$-cm-long capillaries, was on the same time scale as complex dissociation allowing $K_D (101 \pm 12 \text{ nM})$ in good agreement with FA measurements and dissociation rate ($k_{\text{off}} = 0.95 \pm 0.02 \text{ s}^{-1}$) corresponding to a half-life of $0.73 \text{ s}$ to be determined. This measurement represents a 30-fold higher rate of complex dissociation than what had previously been measurable by nonequilibrium CE analysis of equilibrium mixtures. Using FACE, the protein was detected with an LOD of $300 \text{ nM}$ or $7.5 \text{ mol}$ injected. FACE was not used for determining $K_D$ or $k_{\text{off}}$, however, this method provided better separation resolution for multiple forms of the protein than APCE. Both methods were found suitable for analysis of cell lysates. These results demonstrate that FACE and APCE may be useful complements to existing techniques for exploring binding interactions with rapid kinetics.

Cellular chemistry is controlled by affinity interactions between biomolecules. Quantitative analysis of such interactions is important in developing an understanding of how reactions are organized within cells and for developing drugs or chemical probes of cellular processes. A variety of affinity methods based on capillary electrophoresis (CE) have emerged as potentially useful instrumental approaches for quantitative analysis of nonequilibrium interactions between biomolecules. The objective of this work was to extend the utility of CE methods to binding systems that have rapid on–off kinetics. As many cellular chemistry interactions rely on such rapid binding, these improved methods are expected to have utility in signal transduction studies.

Affinity CE methods can be classified as nonequilibrium methods, in which binding partners are mixed and then free and bound components separated by electrophoresis, or equilibrium methods, in which one of the binding partners is added to the separation buffer and the other binding partner injected and separated. In the latter case, the binding interaction is detected by a migration time shift or by a change in fluorescence property. The nonequilibrium methods include competitive assays, nonequilibrium CE of equilibrium mixtures (NEECEM), and affinity probe CE (APCE). Equilibrium methods include affinity CE (ACE), affinity CE with laser-induced fluorescence polarization detection, and fluorescence anisotropy CE (FACE). When compared to other methods such as surface plasmon resonance and fluorescence anisotropy (FA), the CE techniques offer the advantage of utilizing separations, which allows analysis in complex mixtures and analysis of multiple interactions or binding partners at one time, in binding assays, CE methods are also inherently miniaturized and therefore useful.


2492 Analytical Chemistry, Vol. 77, No. 8, April 15, 2005

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Appendix A (Continued)

![Chemical structures](image)

Figure 1. Structure of BODIPY-JAK2 813 peptide (A) and Fluor-JAK2 813 peptide (B).

...much less sample than other techniques, which is especially important in biochemical studies where limited quantities are available.

While equilibrium CE methods have been used for rapid binding systems, nonequilibrium CE methods have only been used for binding reactions that have relatively low dissociation rates, on the order of min⁻¹, because for reactions with high dissociation rates the separation time is too long to detect complex. The development of techniques for rapid CE separations, along with mathematical tools for analyzing electrophoretic data, has opened the possibility of using nonequilibrium methods, like APCE, for analyzing interactions with more rapid kinetics; however, this has yet to be demonstrated. In this work, we test the hypothesis that nonequilibrium separation analysis can be used for systems with more rapid dissociation rates (e⁻² s⁻¹) when using conditions suitable for rapid CE.

The model system used in this study is a Src homology 2 (SH2) domain protein binding a phosphorylated peptide. SH2 domain proteins play an important role in cellular signal transduction by binding to proteins at phosphorylated tyrosine residues resulting in cascades of cellular chemistry changes. The three residues immediately following the phosphotyrosine are a key determinant of specificity for SH2 binding; therefore, small peptides encompassing this sequence can be used for assays. SH2 domain protein and phosphopeptide binding has been reported to have rapid binding kinetics with off rates of 0.1–1 s⁻¹, making them a good model system for this study.

The protein used is the SH2 domain of SH2-B, an adaptor protein that binds JAK2, a member of the Janus family of cytoplasmic tyrosine kinases involved in cytokine signaling. The SH2 domain of SH2-B (amino acids 525–670) binds to JAK2 at tyrosine 813 after that site is autophosphorylated in response to growth hormone. Binding experiments were performed using an oligopeptide, corresponding to the expected binding sequence of JAK2, which was labeled with BODIPY FL or 5-carboxyfluorescein (Figure 1) on the N-terminus. Binding assays were performed using FACE, APCE, and FA assays to allow comparison of the techniques. We demonstrate that although the protein–peptide complex has a half-life of less than 1 s, it can be readily detected by the CE methods. Furthermore, the first quantitative measurements of binding equilibria and kinetics are determined for the SH2-B/JAK2 interaction.

EXPERIMENTAL METHODS

Chemicals. Tris–glycine buffer (1×) was purchased from Bio-Rad laboratories (Hercules, CA). All solutions were prepared with deionized water from an E-Pure water purification system (Barnstead International Co., Dubuque, IA). 5-carboxyfluorescein, succinimidyl ester (5-FAM, SE), 6,6-difluoro-5,7-dimethyl-4-bora-3a,4a-diazas-indacene-3-propionic acid hexanoic acid succinimidyl ester (BODIPY FL, SE) and rhodamine 110 were purchased from Molecular Probes (Eugene, OR). Dulbecco’s Modified Eagle Medium (DMEM) was from Invitrogen (Carlsbad, CA). Aprotinin and Leupeptin were from Roche. JAK2 tyrosine 813-phosphorylated peptide with fluorescein label (FluorJAK2 813 peptide) and BODIPY label (BODIPY-JAK2 813 peptide) were synthesized and labeled by the Protein Core of the Michigan Diabetes Research and Training Center (see Figure 1 for structures of both peptides). SH2-B (525–670) was expressed and purified as a fusion protein with glutathione-S-transferase (GST) as previously described. The GST tag was incorporated for purification by glutathione-agarose beads.

COS-7 Cell Culture and Cell Lysis. A 100 mm × 20 mm cell culture dish (Fisher Scientific) of COS-7 cells were grown to confluence in DMEM supplemented with 1 mM L-glutamine, 100 units of penicillin/mL, 100 μg of streptomycin/mL, 0.25 μg of amphotericin/mL, and 8% fetal bovine serum. Cells were then washed three times in chilled phosphate-buffered saline (10 mM sodium phosphate, 137 mM NaCl, 1 mM Na₂VO₃, pH 7.4) and solubilized in lysis buffer (30 mM Tris, pH 7.5, 0.1% Triton X-100, 150 mM NaCl, 2 mM ethylenediamine-tetraacetic acid (EDTA), 1 mM NaVO₃, pH 7.5), containing 1 mM phenylmethylsulfonyl fluoride, aprotinin (10 μg/mL), and leupeptin (10 μg/mL). Cell lysates (1.25 × 10⁶ cells/mL) were centrifuged at 16,000 × g for 10 min, and the supernatant was saved for further analysis.


Analytical Chemistry, Vol. 77, No. 6, April 15, 2005 2483
Appendix A (Continued)

Fluorescence Anisotropy Experiments. FA measurements were performed on a Perkin-Elmer FPA3200 Universal Microplate Analyzer (Packard Instrument Co., Meriden, CT). Fluorescence was excited at 485 ± 10 nm and emission collected with a 535 ± 12 nm band-pass filter. The factory default G value (0.9) was used for all assays. For protein–peptide binding assays, samples were made to the desired concentration (0.01 mM BODIPY-JAK2 813 peptide or 20 nM Fluor-JAK2 813 peptide with 0–700 nM GST–SH2/B) in 25 mM Tris, 192 mM glycine buffer at pH 8.5. FA was measured immediately after mixing. To determine nonspecific binding, 10 nM unlabeled JAK2 813 peptide was added to each of the above protein–peptide mixtures, other conditions being the same. Specific binding was determined by subtracting nonspecific binding from total binding. The dissociation constant ($K_d$) was determined by fitting data to

$$r = r_0 + \frac{K_d}{[I]} [I] + \frac{K_d}{[I]} [I]$$

where $r$ is the measured anisotropy, $[I]$ is the initial protein concentration, and $r_0$ and $K_d$ are anisotropy values of the complex and free ligand, respectively. We assumed $r_0$ to be the measured anisotropy when protein concentration was zero.

FACE Assays. FACE assays were performed using an experimental setup similar to that described previously. Electrophoresis was performed using a high-voltage power supply (Spellman CZE 1000, Poughkeepsie, NY) applying 400 V/cm across an eCAP neutral capillary (90 mm i.d., 375 mm o.d., total length 652 cm, length from inlet to detector 33 cm, Beckman-Coulter, Fullerton, CA). Vertically polarized 488-nm light from a Kr+ laser was used for excitation. Fluorescence emission was collected at 90° to the excitation and spatially filtered before being split into its parallel and perpendicular components by a polarizing beam splitter cube. Each polarized component was filtered by a 580 ± 10 nm band-pass filter and detected by PMT. Anisotropy was calculated from the signals acquired at both PMTs. Data acquisition and calculation of anisotropy were performed using LabView (National Instruments, Austin, TX) program written in-house running on a personal computer equipped with a National Instruments AT-MIO-16 data acquisition card.

Electrophoresis buffers contained 25 mM Tris, 192 mM glycine, and the desired concentration of pH 8.5. Samples containing purified GST–SH2/B (525–670) or cell lysate and Fluor- or BODIPY-JAK2 813 peptide were diluted before separation. All dilutions were made in Tris–glycine buffer. For sample loading, the capillary inlet was elevated to a height of 10 cm for 20 s to allow sample to be injected by gravity. At the beginning of each experiment, the separation capillary (eCAP neutral capillary) was rinsed with 3% acetic acid, water, and separation buffer for 5 min each.

APCE-LIF Assays. The flow-gated CE-LIF instrument used for APCE assays was described previously. An unmodified fused-silica capillary (10 μm i.d., 300 μm o.d., total length 7 cm, inlet to detector length 3.5 cm) was used as the separation capillary. All samples were introduced onto the capillary by electrolytic injection via a flow gate interface at 2 kV for 0.2 s and separated at 20 kV except for APCE-LIF assays with BODIPY-JAK2 813 peptide in which 15 kV was applied. Tris–glycine buffer was continuously delivered to the flow gate at a flow rate of 1.0 mL/min by a Series 1 HPLC pump (LabAlliance, Fisher Scientific, Pittsburgh, PA).

Samples containing Fluor- or BODIPY-JAK2 813 peptide and GST–SH2/B (525–670) were made to the desired concentration in Tris–glycine buffer and immediately assayed using flow-gated CE-LIF. The 10 mM rhodamine 110 was added to all samples as internal standard.

The $K_d$ was determined by

$$K_d = \frac{[I]_0}{[A_1] + [I]} \frac{[A_1]}{[A_2] + [A_3]}$$

where $[I]_0$ is the initial ligand concentration and $[A_1]$, $[A_2]$, and $[A_3]$ are areas of free peptide peak, complex peak, and exponential part of the electropherogram, respectively. The dissociation rate constant ($k_{off}$) was determined using

$$k_{off} = \frac{[I]_0}{t_{1/2}}$$

where $t_{1/2}$ is the migration time of the complex. Half-life was calculated from $t_{1/2} = \ln 2/k_{off}$.

RESULTS AND DISCUSSION

Anisotropy Measurements. Initial experiments were aimed at using FA to characterize binding of the fluorescent JAK2 813 peptides and GST–SH2/B (525–670). Figure 2 compares the effect of protein concentration on FA of the two peptides. (No significant fluorescence intensity change was observed upon binding.) The $K_d$ for fluoroecein and BODIPY-labeled peptide were determined from these curves to be $82 ± 7$ (n = 3) and $467 ± 10$ nM (n = 3), respectively, using eq 1. Thus, the BODIPY-labeled peptide has lower affinity and a lower net anisotropy change than the fluorescein-labeled peptide (compare anisotropy

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2484  Analytical Chemistry, Vol. 77, No. 8, April 15, 2005
positive anisotropy feature that increases with increasing protein concentration results from detection of a protein-peptide complex that has high anisotropy compared to background peptide. With 20 nM peptide in the electrophoresis buffer, the limit of detection (LOD) for GST-8H2-B@ (525–670) was 300 nM. The precision of individual assays depended upon protein concentration so that at 8 µM protein the relative standard deviation (RSD) of multiple measurements was 0.3% (n = 8) but at 300 nM the RSD was 19%. (n = 8).

The concentration of fluorescent peptide in the electrophoresis buffer had large effects on both peak shape and S/N for FACE assays. Figure 3B shows that the complex peak tends to narrow with increasing peptide concentration. The effect on anisotropy has previously been observed with ACE measurements and is due to the effect of equilibration of the protein between bound and unbound states, each of which migrates at a different rate.16 Although the peak narrowed with higher concentration, resolution actually appeared better at moderate peptide concentrations. The presence of multiple peaks was surprising given that the protein was purified for these experiments. This result suggests that multiple forms of the protein are produced in the cells, perhaps due to different conformations or posttranslational modifications of the protein. It is also possible that the protein degrades after purification.

Figure 3B also illustrates that the anisotropy signal tended to decrease with increasing peptide signal, consistent with the theoretical model established previously.14 Because anisotropy is linearly additive, the contribution of bound and free fluorescent peptide to total anisotropy is determined by both the fraction bound and the anisotropy of each species. As peptide concentration increases, the amount of complex formed increases however, the fraction bound is less and the net anisotropy tends to approach that of the free species since a large molecule is due to the free species. Although the signal increases with lower peptide concentration in electrophoresis buffer, noise in the anisotropy trace increased as fluorophore concentration decreased. Because of these competing effects, the S/N had an optimum as illustrated in Figure 3C. We estimate the detection limit for the protein at the optimal peptide concentration to be 100 nM. (Detection limit was determined as the concentration that would yield S/N of 3 where the noise was the root mean square of the baseline signal.) The effect of noise at low concentrations was not considered in the previous model of FACE.14

To demonstrate the capability of FACE for assays in complex mixtures, lysates from Escherichia coli cells overexpressing GST-8H2-B@ (525–670) and unmodified COS-7 cells were analyzed using Fluor-JAK2 813 peptide added to the electrophoresis buffer (Figure 4). For E. coli cell lysate, the migration time of the complex was identical to that of the purified protein. The protein concentration within the lysate of E. coli overexpressing SH2-B@ (525–670) was estimated from the peak height to be ≈5 µM. Cell lysate contained ~1.2 x 10^8 cells/ml and 25 µl was injected so the signal detected corresponds to 0.4 pmol/cell (~4 x 10^7 cell equivalents injected). A putative SH2-B@ peak was also detectable in lysate from COS-7 cells, a simian kidney cell line expected to naturally express this protein (Figure 4B). The migration time

![Figure 3](image)

**Figure 3.** FACE assays of GST–SH2-B@ (525–670). (A) Electrophoresis buffer contained 20 nM Fluor-JAK2 813 peptide. GST–SH2-B@ (525–670) was increased from 0.3 to 8 µM. (B) Sample contained 2 µM GST–SH2-B@ (525–670). Electrophoresis buffer contained Fluor-JAK2 813 peptide with concentration varied from 5 to 500 nM. Separation conditions of (A) and (B) were as described in the Experimental Section under FACE Affinity Assays. (C) Signal-to-noise ratio (S/N) at peptide concentration from 5 to 500 nM corresponding to electropherograms in (B).

at saturation in Figure 2). The BODIPY construct has a larger spacer between fluorophore and peptide, which could possibly lead to more rotational freedom and lower anisotropy of the fluorophore even when a complex is formed. Given these results, Fluor-JAK2 813 peptide was used for the majority of the FACE and APCE-LIF assays.

**FACE Assays.** We have recently introduced FACE as an equilibrium affinity CE method in which unlabeled proteins are separated by CE using buffer that contains a fluorescent binding partner (peptide in this case) and fluorescence anisotropy is monitored at the detection point.14 As unlabeled protein migrates through the detection zone, it increases the anisotropy signal if it binds the fluorophore. This technique allows, in principle, mixtures to be separated and detected based upon their binding to a fluorescent ligand. Figure 3A illustrates FACE assay results for samples that contained 0.3–8 µM GST–SH2-B@ (525–670) with 20 nM Fluor-JAK2 813 peptide in the electrophoresis buffer. The
was slightly greater than that seen with the overexpressed protein; however, the protein detected in this experiment should be full-length SH2-Bj without a GST tag and therefore would be expected to migrate differently. The detected signal appeared to be due to specific binding to labeled peptide because it could be eliminated by adding 500 nM unlabeled peptide to the electrophoresis buffer (lower trace in Figure 4B). Using calibration with GST–SH2-Bj (525–670), we estimated the concentration of SH2-Bj in COS-7 cell lysate to be 300 nM corresponding to 200 amol/cell. These results demonstrate that SH2 domain proteins can be detected by FACE in complex samples. The method would also appear to be compatible with highly miniaturized analysis as the mass detection limits are low and the volume of injected COS-7 cell lysate corresponds to ~20 COS-7 cells.

**APCE-LIF assays.** We then examined the use of Fluor-JAK2 813 peptide as an affinity probe for detection of GST–SH2-Bj (525–670) by APCE. Because the kinetics of binding were expected to be fast, flow-gated CE with short columns was used to achieve rapid separation. Figure 5A shows the result of an APCE assay of GST–SH2-Bj (525–670). For this assay, 1 nM GST–SH2-Bj (525–670) and 100 nM Fluor-JAK2 813 peptide were premixed and injected electrosynthetically. In trace 1, the separation was completed in 4 s using an electric field of 2800 V/cm and peaks corresponding to complex and free peptide are detected. The complex peak is confirmed by observing that it increased with increasing protein while the free peaks decreased (Figure 5B) and was eliminated by addition of excess unlabeled peptide to the mixture (data not shown). Two peaks for peptide were detected, likely due to separation of two isoforms of fluorophores. In addition to the peaks, a bridge between the peaks was detected that corresponded to peptide that dissociated from the complex during separation, an effect observed when separation occurs on the same timescale as the complex half-life. At 1400 V/cm, with a separation time of 8 s the complex peak was not detected, probably because it had completely dissociated (trace 2, Figure 5A) on this timescale. (Similar results were obtained with longer columns and longer times.) These results indicate the importance of short separation times for detection of rapidly dissociating complexes. Analysis of samples containing different concentrations of protein had RSDs of 3–10% for peptide peak heights (n = 10 for each sample), demonstrating good reproducibility of this method.

The presence of two active peptides could complicate quantitative analysis of binding; however, both peptide peaks decreased with increasing protein concentration to a similar degree, indicating that they bound with comparable affinity (data not shown). Therefore, we used the sum of the two peptide peak areas for the calculation of \( K_d \) and \( k_{cat} \).

Krylov's group recently demonstrated an approach, NECEEM, that uses eqs 2 and 3 to determine \( K_d \) and \( k_{cat} \) from AFCE data when dissociated ligand can be detected, as in Figure 5.5,7 Figure
Appendix A (Continued)

Figure 6. Sample electropherogram used in determination of $K_d$ and $k_{diss}$ by APCe-LIF. Sample contained $0.5 \mu M$ GST–SH2-Bb (525–670), 100 nM Fluor-JAK2 813 peptide, and 10 nM rhodamine 110. Separation conditions were the same as in Figure 5. Arrows marked correspond to bound peptide (A2), peptide that dissociated during the separation (A3), and peptide that was free in the sample solution (A1). Calculations of $K_d$ and $k_{diss}$ are described in the Experimental Section under APCe-LIF Assays.

Figure 7. APCe-LIF assays for purified GST–SH2-Bb (525–670) and cell lysate. Sample contained 100 nM Fluor-JAK2 813 peptide, 10 nM rhodamine 110, and 0.25 $\mu M$ GST–SH2-Bb (525–670) (trace 1) or cell lysate from E. coli overexpressing the protein (trace 2). 15 indicates rhodamine used as internal standard. Protein concentrations were similar in both samples. Separation conditions were the same as in Figure 5.

APCe-LIF and FACE Assays for GST–SH2-Bb (525–670) with BODIPY-JAK2 813 Peptide. We also evaluated the use of FACE and APCe for analyzing the interaction of GST–SH2-Bb (525–670) with the BODIPY-JAK2 813 peptide. Because anisotropy measurements (Figure 2) indicated that BODIPY-JAK2 813 peptide has 5-fold lower affinity for GST–SH2-Bb (525–670) than the fluorescein-labeled peptide, we anticipated a larger $k_{diss}$ and therefore more difficulty in detecting the complex by APCe. Figure 8 compares results of APCe and FACE experiments performed with BODIPY-JAK2 813 peptide similar to those performed with Fluor-JAK2 813 peptide as the fluorescent probe. The complex was not detected by APCe, although some broadening of the peptide peak was observed, likely due to interaction with protein (Figure 8A). We estimate that, with a separation time of 4 s and $K_d$ of 100 nM, the highest $k_{diss}$ that can be determined by APCe is 2 s$^{-1}$ corresponding to a $t_{1/2}$ of 0.5 s. In the FACE assay, the protein was readily detected (Figure 8B) with an LOD of 10 nM when using BODIPY-JAK2 813 peptide at 20 nM. The higher LOD, compared with Fluor-JAK2 813 peptide, is due to the lower fluorescence intensity of the BODIPY label, lower FA change upon binding, and lower binding affinity. From these results, we can conclude that APCe fails to detect complex with extremely fast off-rate whereas FACE, in which separation is performed under equilibrium, is able to detect rapidly dissociating complexes.

Comparison of FA, FACE, and APCe. In these experiments, a protein–peptide binding interaction with fast kinetics has been investigated by FA, FACE, and APCe-LIF. Table 1 compares these techniques, in terms of their multitabyte capability, binding parameter quantification ($K_d$ and $k_{diss}$), molecular size information, LOD, and sample consumption. $K_d$s were easily derived from anisotropy changes as a function of concentration. In principle, $k_{diss}$ could be determined by spiking an excess of unlabeled ligand and recording the decay of the complex by anisotropy; however, high dissociation rates require rapid mixing and recording capability. FA on a plate reader is unable to discern the presence.
Appendix A (Continued)

Table 1. Comparison of Analytical Capabilities for Detection of Noncovalent Interactions of Techniques Utilized in This Work

<table>
<thead>
<tr>
<th>measurement property</th>
<th>FA</th>
<th>FACE</th>
<th>APCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>multiple analytes in one assay</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>$K_d$ determination</td>
<td>yes</td>
<td>potentially yes</td>
<td>yes</td>
</tr>
<tr>
<td>detect complex with rapid dissociation rate</td>
<td>yes</td>
<td>yes</td>
<td>limited by separation time</td>
</tr>
<tr>
<td>analyze crude samples (unpurified)</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>size information of complexes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>sample consumed per assay at detection limit$^a$</td>
<td>2.4 pmol</td>
<td>7.5 fmol</td>
<td>12 amol</td>
</tr>
<tr>
<td>LOD$^a$</td>
<td>50 nM</td>
<td>300 nM</td>
<td>100 nM</td>
</tr>
</tbody>
</table>

$^a$ Sample consumption and LOD were determined from binding of GST–SH2-B by (525–670) and Fluor-JAK2 813 peptide. They can be different for other binding systems.

The main advantages of FACE in these experiments were its ability to partially resolve protein forms that interacted with the peptide and its ability to detect binding partners regardless of the off-rate (at least within the range used in this work) as illustrated by the experiment with the BODIPY-JAK2 813 peptide. FACE is also advantageous in that fluorescence anisotropy detection provides information on the size of the analyte, thus confirming the formation of complexes, and reducing the effect of background fluorescence on analyte detection in real biological samples when compared to APCE analysis. In principle $K_d$ and $k_{on}$ could be determined from FACE data since the anisotropy signal and peak shape depends on these parameters$^a$ however, methods to extract this information have not yet been developed. The detection limit was worse than that obtained by FA; however, this is not an inherent limitation and may reflect instrumental differences in the fluorescence detection systems.

APCE was unique among the methods in demonstrating that multiple forms of the peptide ligand were present in the sample. In principle APCE could also separate multiple protein forms; however, this requires identification of conditions suitable for binding and separation, which is not always feasible. APCE is capable of measuring $K_d$ and $k_{on}$ when using the NDEEM method of analysis. Previous experiments measured off-rates on the order of $10^{-5}$ to $10^{-6}$ s$^{-1}$$^a$ however, the dissociation rate of the BODIPY-JAK2 813 peptide was apparently too fast to be detected. Thus, the time scale accessible by APCE is dependent upon separation speed and may be further improved with even faster separations. The LOD for GST–SH2-B by (525–670) was comparable to that of FACE, 100 nM corresponding to 12 amol injected. This detection limit is higher than previous reports for APCE$^a$ and may be due to the fluorophores, differences in detector design, and loss of complex during separation.

CONCLUSION

In this work, we investigated a binding system with rapid kinetics using two sensitive CE-based techniques, FACE and APCE-LIF. Using these two methods, binding interactions can be quantitatively studied, including interactions with rapid kinetics and within complex biological samples such as cell lysates. FACE and APCE-LIF may be useful complements to existing methods for studying signal transduction events and for discovering novel affinity interactions.

Figure 8. APCE-LIF and FACE assays of GST–SH2-B by (525–670) using BODIPY-JAK2 813 peptide as the probe. (A) FACE-LIF assays. Sample contained 50 nM BODIPY-JAK2 813 peptide, 6 nM rhodamine 110 (marked as IS), and 1 nM BAP (trace 4) or 1 µM BAP (trace 2) GST–SH2-B by (525–670). Separation conditions were as described in Experimental Section under APCE-LIF Assays. (B) FACE assays. Sample contained 5 µM GST–SH2-B by (525–670) and 20 nM BODIPY-JAK2 813 peptide. Electrophoresis buffer contained 20 mM BODIPY-JAK2 813 peptide. Separation conditions were the same as in Figure 5.

of multiple forms of the protein or peptide, and it is unsuitable for assays of cell lysate because of potential interference from viscosity and background fluorescence. The FA measurement provided the lowest concentration LOD but a relatively high mass LOD because a large amount of sample was consumed for a single measurement. An advantage of FA on a plate reader is its capability for high-throughput assays.

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Appendix B

Sol–gel approach to in situ creation of high pH-resistant surface-bonded organic–inorganic hybrid zirconia coating for capillary microextraction (in-tube SPME)

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Abstract

A novel zirconia-based hybrid organic–inorganic sol–gel coating was developed for capillary microextraction (CME) (in-tube SPME). High degree of chemical inertness inherent in zirconia makes it very difficult to covalently bind a suitable organic ligand to its surface. In the present work, this problem was addressed from a sol–gel chemistry point of view. Principles of sol–gel chemistry were employed to chemically bind a hydroxy-terminated silicone polymer (polymethylldiphenylsiloxane, PDMDS) to a sol–gel zirconia network in the course of its evolution from a highly reactive alkoxide precursor undergoing controlled hydrolytic polycondensation reactions. A fused silica capillary was filled with a properly designed sol solution to allow for the sol–gel reactions to take place within the capillary for a predetermined period of time (typically 15–30 min). In the course of this process, a layer of the evolving hybrid organic–inorganic sol–gel polymer got chemically anchored to the silanol groups on the capillary inner walls via condensation reaction. At the end of this in-capillary residence time, the unbonded part of the sol solution was expelled from the capillary under helium pressure, leaving behind a chemically bonded sol–gel zirconia–PDMDS coating on the inner walls. Poly cyclic aromatic hydrocarbons, ketones, and aldehydes were efficiently extracted and preconcentrated from dilute aqueous samples using sol–gel zirconia–PDMDS coated capillaries followed by thermal desorption and GC analysis of the extracted solutes. The newly developed sol–gel hybrid zirconia coatings demonstrated excellent pH stability, and retained the extraction characteristics intact even after continuous rinsing with a 0.1 M NaOH solution for 24 h. To our knowledge, this is the first report on the use of a sol–gel zirconia-based hybrid organic–inorganic coating as an extraction medium in solid phase microextraction (SPME).

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Keywords: Capillary microextraction; In-tube SPME; Sol–gel extraction media; Sol–gel technology; Sol–gel zirconia polymethylldiphenylsiloxane coating; pH stability; Sample preconcentration; Gas chromatography; Hyphenated techniques; Poly cyclic aromatic hydrocarbons; Aldehydes; Ketones

1. Introduction

Solid phase microextraction (SPME) was developed in 1989 by Belardi and Pawliszyn [1] to facilitate rapid sample preparation for both laboratory and field analyses. It provided a simple and efficient solvent-free method for the extraction and preconcentration of analytes from various sample matrices.

In SPME, a sorptive coating (either on the outer surface of a fused silica fiber or on the inner surface of a fused silica capillary) serves as the extraction medium in which the analytes get preferentially sorbed and preconcentrated. Polymeric surface coatings are predominantly used in conventional fiber-based SPME [1–4] as well as in the more recently materialized in-tube SPME [5–8] also referred to as capillary microextraction (CME) [9]. A number of new polymeric coatings have recently been developed [10]. Besides polymeric coatings, SPME fibers have also been prepared by using nonpolymeric materials [11] or by gluing reversed-phase high-performance liquid chromatography (HPLC) particles
onto SPME fiber surface [12]. The sorbent coating plays a fundamentally important role in the SPME analysis, and further development and growth of SPME will greatly depend on new breakthroughs in the areas of sorbent development and coating technology [13].

Sol-gel chemistry offers an effective methodology for the synthesis of macromolecular materials under extraordinarily mild thermal conditions (typically at room temperature). The room temperature operation, inherent in sol-gel chemistry, facilitates the material synthesis process by easing the operational requirements on equipment specification and laboratory safety. This greatly simplifies the job to carry out and/or control sol-gel reactions within small-diameter fused silica capillaries. The sol-gel approach provides a facile mechanism to chemically bind an in situ created sol-gel coating to the inner walls of the capillary made out of an appropriate sol-gel-active material. Thanks to this chemical bonding, sol-gel coatings possess significantly higher thermal and solvent stabilities [14] compared with their conventional counterparts. The sol-gel approach can be applied to create silica-based as well as the newly emerging transition metal oxide-based sorbents. Furthermore, sol-gel chemistry provides an opportunity to create advanced material systems to achieve enhanced performance and selectivity in analytical separations and sample preconcentration [16,15].

Sol-gel organic–inorganic hybrid materials provide desirable sorptive properties that are difficult to achieve by using either purely organic or purely inorganic materials. Because of this unique opportunity to achieve enhanced selectivity, hybrid sol-gel materials have created a great deal of interest in the field of microcolumn separations and sample preparation. In the recent past, silica-based organic–inorganic hybrid stationary phases have been developed in the form of surface coatings [16–18] and monolithic beds [19]. In 1993, Dubrio and co-workers [20] developed a procedure for the preparation of a thin layer of silica gel with chemically bonded C18 moieties on the inner walls of fused-silica capillaries for use as open-tubular columns in reversed-phase high-performance liquid chromatography. Colon and Goe [21] used sol-gel technology to prepare stationary phase coatings for open-tubular liquid chromatography and electrochromatography. Malik and co-workers introduced sol-gel coated columns for capillary GC [22] and sol-gel coated fibers for solid-phase microextraction [13,23]. Subsequently, other groups also got involved in sol-gel research aiming at developing novel sorbents for solid-phase microextraction [24–28] and solid-phase extraction [29,30]. Compared with conventional fibers, sol-gel SPME fibers demonstrated superior performance by exhibiting high thermal stability (up to 380 °C) [24] and solvent stability [25]. This enhanced stability of sol-gel coated fibers is attributed to the chemical bonding between the sol-gel coating and the fiber surface. Compared with the conventionally prepared fibers, in many instances, sol-gel SPME fibers showed better selectivity and extraction sensitivity, [26] less extraction time, [27] and extended lifetime [26]. Recently, sol-gel capillary microextraction was reported by Malik and co-workers [9]. In this format, also known to as in-tube SPME, sample extraction was accomplished using a sol-gel coating created on the inner surface of a fused silica capillary.

The sol-gel microextraction sorbents reported to date are predominantly silica-based. In spite of many attractive material properties (e.g., mechanical strength, surface characteristics, catalytic inertness, surface derivatization possibilities, etc.), silica-based materials have some inherent shortcomings. The main drawback of silica-based sorbents is the narrow range of pH stability. Under extreme pH conditions, silica-based materials become chemically unstable, and their sorptive properties may be compromised. For example, silica dissolves under alkaline conditions, and their dissolution process starts at a pH value of about 8 [31]. Under highly acidic pH conditions, silica-based bonded phases become hydrolytically unstable [32]. Therefore, developing sorbents with a wide range of pH stability is an important research area in contemporary separation and sample preparation technologies. Transition metal oxides (zirconia, titania, etc.) are well-known for their pH stability [33], and appear to be logical candidates for exploration to overcome the above-mentioned drawbacks inherent in silica-based materials.

Zirconia possesses much better alkali resistance than other metal oxides, such as alumina, silica, and titania. It is practically insoluble within a wide pH range (1–14) [36–39]. Zirconia also shows outstanding resistance to dissolution at high temperatures [40,41]. Besides the extraordinary pH stability, excellent chemical inertness and high mechanical strength are two other attractive features that add value to zirconia for being used as a support material in chromatography [34] and membrane-based separations [35].

Extensive research work has been done on zirconia particles and their surface modifications for use as HPLC stationary phases [42,43]. A number of reports have also recently appeared in the literature on the use of zirconia-modified fused silica capillaries in capillary electrophoresis (CE) [44–48]. However, the excessive chemical inertness of zirconia particles remains a difficult hurdle to creating surface-bonded stationary phases.

We approached this problem from a sol-gel chemistry point of view. We took into consideration the fact that contrary to the high inertness of zirconia particles that have already been formed and attained highly stable structural characteristics, zirconium alkoxides are highly reactive sol-gel precursors for zirconia. By using appropriate conditions, it should be possible to utilize the reactivity of such zirconia precursors to create organic–inorganic zirconia materials with covalently bonded organic ligands. In this paper, we report the preparation of zirconia-based hybrid organic–inorganic sol-gel sorbents from a highly reactive precursor, zirconium butoxide, and a sol-gel-active organic polymer (hydroxyl-terminated PDMDPS). The covalent bonding of the organic ligand to the sol-gel zirconia network structure was accomplished via condensation reaction in the course of controlled hydrolytic polycondensation reactions taking place in the sol.
Appendix B (Continued)

2. Experimental

2.1. Equipment

All CME-GC experiments were performed on a Shimadzu Model 14A capillary GC system equipped with a flame ionization detector (FID) and a split-splitless injector. On-line data collection and processing were done using ChromPerfect (version 3.5) software (Justice Laboratory Software, Denver, NJ). A Fisher Model G-560 Vortex Genie 2 system (Fisher Scientific, Pittsburgh, PA) was used for thorough mixing of various sol solution ingredients. A Microcentrifuge model APO 5760 microcentrifuge (Accurate Chemical and Scientific Corp., Westbury, NY) was used to separate the sol solution from the precipitate (if any) at 13,000 rpm (15,682 x g). A Nicolet model Avatar 320 FT-IR instrument (Thermo Nicolet, Madison, WI) was used to acquire infrared spectra of the prepared sol-gel materials. A Barnstead Model 04741 Nanopure deionized water system (Barnstead/Thermolyne, Dubuque, IA) was used to obtain ~16.0 MΩ water. Stainless steel mini-juicers (SGE Inc., Austin, TX) were used to connect the fused silica capillary GC column with the microextraction capillary, as made of fused silica. An in-house-designed liquid sample dispenser (Fig. 1) was used to facilitate gravity-fed flow of the aqueous sample through the sol-gel microextraction capillary. A homebuilt, gas pressure-operated capillary filling/purging device [49] was used to perform a number of operations: (a) rinse the fused silica capillary with solvents; (b) fill the extraction capillary with the sol solution; (c) expel the sol solution from the capillary at the end of sol-gel coating process; and (d) purge the capillary with helium after treatments like rinsing, coating, and sample extraction.

2.2. Chemicals and materials

Fused-silica capillary (320 and 250 μm, i.d.) with a protective polyimide coating was purchased from Polymicro Technologies Inc. (Phoenix, AZ). Naphthalene and HPLC-grade solvents (methylene chloride, methanol) were purchased from Fisher Scientific (Pittsburgh, PA). Hexamethyldisilazane (HMDSO), poly(methylhydroxilsiloxane) (PMHS), ketones (valerophenone, hexanophenone, heptanophenone, and decanophenone), aldehydes (methylaldehyde, n-decaldehyde, undecyl aldehyde, and dodecanal), polyethylene-based hydrocarbons (PEMs) (octylsilane, decyldimethylsilane, hexylsilane, and heptyldimethylsilane), were purchased from Aldrich (Milwaukee, WI). Two types of silanol-terminated poly(dimethylsilylsiloxane) (PDMDPS) copolymers (with 2-3% and 14-18% contents of the diphenyl-containing component) were purchased from United Chemical Technologies Inc. (Bristol, PA).

2.3. Preparation of sol-gel zirconia-PDMDPS coating

A carefully designed sol solution was used to create the coating. The key ingredients of the sol solution used are listed in Table 1. The sol solution was prepared in a clean polypropylene centrifuge tube by dissolving the following ingredients in mixed solvent system consisting of methylene chloride and butanol (250 μL each): 10–15 μL of zirconium(IV) butoxide (80% solution in 1-butanol), 85 μg of silanol-terminated poly(dimethylsilyl)oxane copolymer, 70 μg of poly(methylhydroxilsiloxane), 10 μL of 1,1,1,3,3-hexamethyldisilazane, and 2-4 μL of glacial acetic acid. The dissolution process was aided by thorough vortexing. The sol solution was then centrifuged at 13,000 rpm (15,682 x g) to remove the precipitate (if any). The top clear sol solution was transferred to a clean vial and was further used in the coating process. A hydrothermally treated fused silica capillary (2 mm) was filled with the clear sol solution, using pressurized helium (50 psi) in the filling/purging device [49]. The sol solution was allowed to stay inside the capillary for a controlled period of time (typically 15–30 min) to facilitate the formation of a sol-gel coating, and its chemical bonding to the capillary inner walls. After that, the free portion of the solution was expelled from the capillary, leaving behind a surface-bonded sol-gel coating.
within the capillary. The sol–gel coating was then dried by purging with helium. The coated capillary was further conditioned by temperature programming from 40 to 150 °C at 1 °C/min and held at 150 °C for 300 min. Following this, the conditioning temperature was raised from 150 to 320 °C at 1 °C/min and held at 320 °C for 120 min. The extraction capillary was further cleaned by rinsing with 3 mL of methylene chloride and conditioned again from 40 to 320 °C at 4 °C/min. While conditioning, the capillary was constantly purged with helium at 1 mL/min. The conditioned capillary was then cut into 10 cm long pieces that were further used to perform capillary microextraction.

2.4. Preparation of the samples

PAHs, ketones, and aldehydes were dissolved in methanol or tetrahydrofuran to prepare 0.1 mg/L stock solutions in sterilized glass vials. For extraction, fresh samples with ppb level concentrations were prepared by diluting the stock solutions with deionized water.

2.5. Gravity-fed sample dispenser for capillary microextraction

The gravity-fed sample dispenser for capillary microextraction (Fig. 1) was constructed by in-house modification of a Chromatex AQ column (Kontes Glass Co., Vineland, NJ) consisting of a thick-walled glass cylinder coaxially placed inside an acrylic jacket. The inner surface of the thick-walled cylindrical glass column was deactivated by treating with a 5% (v/v) solution of HMDS in methylene chloride followed by overnight heating at 100 °C. The column was then cooled to ambient temperature, thoroughly rinsed with methanol and liberal amounts of deionized water, and dried in a helium flow. The entire Chromatex AQ column was subsequently reassembled.

2.6. Sol–gel capillary microextraction-GC analysis

To perform capillary microextraction, a previously conditioned sol–gel zirconia-PDMPS coated microextraction capillary (10 cm × 320 μm i.d. or 10 cm × 250 μm i.d.) was vertically connected to the bottom end of the empty sample dispenser (Fig. 1). The aqueous sample (50 mL) was then placed in the dispenser from the top, and allowed to flow through the microextraction capillary under gravity. While passing through the extraction capillary, the analyte molecules were sorbed by the sol–gel zirconia-PDMPS coating residing on the inner walls of the capillary. The sample flow through the capillary was allowed to continue for 30–40 min for an extraction equilibrium to be established. After this, the microextraction capillary was purged with helium at 25 kPa for 1 min and connected to the top end of a vertically placed two-way mini-union connecting the microextraction capillary with the inlet end of the GC column. Approximately, 6.5 mm of the extraction capillary remained tightly inserted into the connector, as did the same length of GC column from the opposite side of the mini-union facing each other within the connector. The installation of the capillary was completed by providing a leak-free connection at the bottom end of the GC injection port so that top 9 cm of the extraction capillary remained inside the injection port. The extracted
Appendix B (Continued)


3. Result and discussion

Capillary microextraction [9] uses a sorbent coating on the inner surface of a capillary, and thereby overcomes a number of deficiencies inherent in conventional fiber-based SPME such as susceptibility of the sorbent coating to mechanical damage due to scraping during operation, fiber breakage, and possible sample contamination. In CME, the sorbent coating is protected by the fused silica tubing against mechanical damage. The capillary format of SPME also provides operational flexibility and convenience during the microextraction process since the protective polymer coating on the outer surface of fused silica capillary remains intact. Inner surface-coated capillaries provide a simple way to perform extraction in conjunction with a gravity-fed sample dispenser (Fig. 1), and thus avoid typical drawbacks of fiber-based SPME, including the need for sample agitation during extraction as well as the sample loss and contamination problems associated with this.

The sol–gel process is a straightforward route to obtaining homogeneous gels of desired compositions. In recent years, it has received increased attention in analytical separations and sample preparations due to its outstanding versatility and excellent control over properties of the created sol–gel materials that proved to be promising for use as stationary phases and extraction media.

A general procedure for the creation of sol–gel stationary phase coating on the inner walls of fused silica capillary GC columns was first described by Malik and co-workers [22]. In the present work, a judiciously designed sol solution ingredients (Table 1) was used to create the sol–gel zirconia-PMDPS coating on the fused silica capillary inner surface. Zirconium(IV) butoxide (80% solution in 1-butanol) was used as a sol–gel precursor and served as a source for the inorganic component of the sol–gel organic–inorganic hybrid coating.

The sol–gel Zirconia-PMDPS coating presented here was generated via two important reactions: (1) hydrolysis of a sol–gel precursor, zirconium(IV) butoxide; and (2) polycondensation of the precursor and it hydrolysis products between themselves and with other sol–gel-active ingredients in the coating solution, including silanol-terminated PMDPS. The hydrolysis of the zirconium(IV) butoxide precursor is represented by Scheme 1 [59].

Condensation of the sol–gel polymer growing in close vicinity of the capillary walls with silanol group on the capillary surface led to the formation of an organic–inorganic coating chemically anchored to the capillary inner walls (Scheme 2A).

A major obstacle to preparing zirconia-based sol–gel materials using zirconium alkoxide precursors (e.g., zirconium butoxide) is the very rapid sol–gel reaction rates for these precursors. Even if the solution of zirconium alkoxide is stirred vigorously, the rates of these reactions are so high that large agglomerated zirconia particles precipitate out immediately when water is added [51]. Such fast precipitation makes it difficult to reproducibly prepare zirconia sol–gel materials. Ganguli and Kunda [52] addressed the fast precipitation problem by dissolving zirconium propoxide in a non-polar dry solvent like cyclohexane. The hydrolysis was performed by exposing the coatings prepared from the solution to atmospheric moisture. Heating to 450°C was necessary to obtain transparent films. The hydrolysis rates of zirconium alkoxides can also be controlled by chelating with ligand-exchange reagents. Acetic acid [53,54], valeric acid [55], B-diketones [56–58], triethanolamine [59], and 1,5-diaminopentane [60] have been used as chelating reagents for zirconia sol–gel reactions. In general, chelation occurs when the added reagent replaces one or more alkoxy groups forming a strong bond. The formation of this bond reduces the hydrolysis rate by decreasing the number of available alkoxy groups [60].

In the present work, we controlled the hydrolysis rate of zirconium butoxide by using glacial acetic acid [61] as a chelating agent as well as a source of water released slowly through the esterification with 1-butanol [62,63]. Two Silanol-terminated poly (dimethylsiloxane)siloxane) copolymers (with 23% and 14–18% diphenyl-containing blocks) were used as sol–gel-active organic components to be chemically incorporated in the sol–gel network through polycondensation reactions with the zirconium butoxide.

![Scheme 1. Hydrolysis of zirconium(IV) butoxide precursor.](image-url)
precursor and its hydrolysis products. An IR spectrum of
the pure co-polymers (the one with 2-3% phenyl-containing
block) is presented in Fig. 2A where a small stretching at
3068 cm⁻¹ indicates the presence of phenyl groups.

![IR spectra](image)

**Fig. 2.** IR spectra representing: (A) pure silanol-terminated PDMODPS copolymer with 2-3% diphenyl-containing component; and (B) sol–gel zirconia-PDMODPS material prepared using PDMODPS copolymer with 14-18% diphenyl-containing component.

This advantageous chemical incorporation of an organic
component into the sol–gel network is responsible for the
formation of an organic–inorganic hybrid material system
that can be conveniently used for in situ creation of surface
coating on a substrate like the inner walls of a fused sil-
ica capillary. Besides, the organic groups help to reduce the
shrinkage and cracking of the sol–gel coating [64,65]. Fur-
furthermore, sol–gel process can be used to control the porosity
and thickness of the coating and to improve its mechanical
properties [66]. Poly(methylhydroxiloxane) and 1,1,1,3,3,3-
hexamethyldisilazane that were used in the sil solution,
served as deactivation reagents to perform chemical derivat-
ization of the strongly adsorptive residual hydroxyl groups on
the resulting sol–gel material. The purpose of these reactions
was to minimize the strong adsorptive interactions between
polar solutes and the sol–gel sorbent that may lead to sample
loss, peak tailing, sample carry-over and other deleterious
effects. In the presented method for the preparation of the
sol–gel zirconia coated microextraction capillary, the deact-
viation reactions were designed to take place mainly during
thermal conditioning of the capillary following the sol–gel
coating procedure.

Hydroytic polycondensation reactions for sol–gel-active
reagents are well established in sol–gel chemistry [67–70],
and constitute the fundamental mechanism in sol–gel syn-
thesis. The condensation between sol–gel-active zirconia and
silicon compounds is also well documented [71–73]. According
to published literature data, the characteristic IR band
for Si-O-Si bonds is located in the vicinity of 945–980 cm⁻¹.
**Fig. 2B shows an IR spectrum of sol–gel zirconia PDMODPS**
**material prepared by using a PDMODPS polymer containing**
Appendix B (Continued)

![Cross-sectional view (1000x)](image1) ![Coating thickness (10,000x)](image2)

Fig. 3. Scanning electron microscopic images of a 0.32 mm i.d. Sol-gel zirconia-PDMPS coated microextraction capillary. (A) Illustrates cross-sectional view (1000x) of roughened surface obtained by sol-gel coating process. (B) Illustrates the coating thickness (10,000x).

approximately eight times higher amounts of the phenyl group than that presented in Fig. 2A. The presence of the stretching at 954 cm⁻¹ indicates the presence of Zr-O-Si bonds in the prepared sol-gel material [74].

Metal-bound hydroxyl groups on the coated sol-gel coating represent strong adsorptive sites for polar solutes. In the context of analytical microextraction or separation, presence of such groups is undesirable, and may lead to a number of deleterious effects including sample loss, reproducibility problems, sample carryover problems, and peak distortion and tailing. Therefore, appropriate measures need to be taken to deactivate these adsorptive sites. This may be accomplished by chemically reactivating the hydroxyl groups with suitable derivatization reagents. Like silica-based sol-gel coatings, the surface hydroxyl groups of sol-gel zirconia coating can be derivatized using reactive silicon hydride compounds such as alkyl hydrosilanes [76,77] and hexamethyldisilazane [78]. In this work, we used a mixture of polymethylhydrosiloxane and hexamethyldisilazane for this purpose: the underlying chemical reactions are

<table>
<thead>
<tr>
<th>Chemical class</th>
<th>Name</th>
<th>Peak area repeatability (n=4)</th>
<th>R.S.D. (%)</th>
<th>Mean t₂ (min)</th>
<th>R.S.D. (%)</th>
<th>Detection limit (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAHs</td>
<td>Naphthalene</td>
<td>11692.42</td>
<td>7.25</td>
<td>15.32</td>
<td>0.11</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Acenaphthene</td>
<td>23560.38</td>
<td>4.58</td>
<td>17.12</td>
<td>0.05</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Fluorene</td>
<td>39970.90</td>
<td>2.45</td>
<td>17.23</td>
<td>0.12</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Phenanthrene</td>
<td>9010.92</td>
<td>3.46</td>
<td>18.76</td>
<td>0.10</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Pyrene</td>
<td>55905.40</td>
<td>2.78</td>
<td>20.22</td>
<td>0.22</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Naphthocean</td>
<td>22378.12</td>
<td>5.42</td>
<td>21.44</td>
<td>0.14</td>
<td>0.05</td>
</tr>
<tr>
<td>Aldehyde</td>
<td>1-Butanol</td>
<td>150.102.25</td>
<td>1.29</td>
<td>15.27</td>
<td>0.03</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>1-Decanal</td>
<td>22906.68</td>
<td>5.45</td>
<td>15.04</td>
<td>0.16</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Undecanal</td>
<td>39413.15</td>
<td>5.08</td>
<td>16.61</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Dodecanal</td>
<td>32182.70</td>
<td>3.72</td>
<td>17.22</td>
<td>0.11</td>
<td>0.05</td>
</tr>
<tr>
<td>Ketones</td>
<td>Valerophene</td>
<td>63712.88</td>
<td>3.39</td>
<td>16.79</td>
<td>0.06</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Hexanophene</td>
<td>13780.88</td>
<td>1.24</td>
<td>17.40</td>
<td>0.07</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Heptanophene</td>
<td>47398.87</td>
<td>1.24</td>
<td>18.19</td>
<td>0.27</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Octanophene</td>
<td>83156.67</td>
<td>2.20</td>
<td>19.44</td>
<td>0.11</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Tricosalphene</td>
<td>60546.25</td>
<td>5.57</td>
<td>19.82</td>
<td>0.03</td>
<td>0.57</td>
</tr>
</tbody>
</table>
Appendix B (Continued)

One of the most important undertakings in CME is the creation of a stable, surface-bonded sorbent coating on the inner walls of a fused silica capillary. Fig. 3 represents scanning electron microscopic images of a sol-gel Zirconia-PDMPS coated fused silica capillary prepared in the present work. The SEM images A and B were obtained at a magnification of 3,000 x. The images show the uniform coating and the smooth inner walls of the capillary.

Table 3
Capillary-to-capillary and run-to-run peak area repeatability for mixture of PAHs, aldehydes, and ketones in four replicate measurements by CME-GC using sol-gel Zirconia-PDMPS coated extraction capillaries.

<table>
<thead>
<tr>
<th>Name of the analyte</th>
<th>Peak area repeatability (n=4)</th>
<th>Run-to-run</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Capillary-to-capillary</td>
<td>Run-to-run</td>
</tr>
<tr>
<td></td>
<td>Mean peak area (arb. unit)</td>
<td>R.S.D. (%)</td>
</tr>
<tr>
<td>Undecenal</td>
<td>47040.0</td>
<td>4.60</td>
</tr>
<tr>
<td>Hexanal</td>
<td>27538.4</td>
<td>1.61</td>
</tr>
<tr>
<td>Fluorene</td>
<td>53280.7</td>
<td>5.40</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>51399.9</td>
<td>4.91</td>
</tr>
</tbody>
</table>
Fig. 6. CM5-GC analysis of ketones using a sol–gel zirconia–PDMS-coated extraction capillary. Extraction parameters: 10 cm × 0.32 mm i.d. microextraction capillary; extraction time, 40 min (gravity fed at room temperature). Other conditions: 10 cm × 0.25 mm i.d. Sol–gel PDMS GC column; splitless desorption; injector temperature rose from 30 to 300 °C; column temperature program from 30 to 300 °C at rate of 20 °C/min; helium carrier gas; FID 350 °C. Peaks: (1) valerophenone; (2) hexanophenone; (3) heptanophenone; (4) octanophenone; and (5) nonanophenone.
of 1000 and 10,000×, respectively. The microstructural details revealed in these images clearly show that the created sol-gel zirconia coating possesses a porous make-up which substantially differs from that obtained by us for sol-gel titania coating [79].

Sol-gel zirconia-PDMDPS-coated capillaries allowed the extraction of analytes belonging to various chemical classes. Experimental data highlighting CME-GC analysis of polycyclic aromatic hydrocarbons using a sol-gel zirconia-PDMDPS coated capillary is shown in Fig. 4.

CME-GC experiments were performed on an aqueous sample with low ppb level analyte concentrations. Experimental data presented in Table 2 shows that CME-GC with a sol-gel zirconia-PDMDPS coating provides excellent run-to-run repeatability in solvent peak areas (3–7%) and the used sol-gel GC column provided excellent repeatability in retention times (less than 0.2%). It should be pointed out that the column used for GC analyses was also prepared in-house using a sol-gel method described by us in a previous publication [22].

The reproducibility of the newly developed method for the preparation of sol-gel hybrid organic-inorganic zirconia coated capillaries was evaluated by preparing three sol-gel zirconia PDMDPS-coated capillaries in accordance with the new procedure and following their performance in CME-GC analysis of different classes of analytes extracted from aqueous samples. The GC peak area obtained for an extracted analyte was used as the criterion for capillary-to-capillary reproducibility which ultimately characterizes the capillary preparation method reproducibility. The results are presented in Table 3. For each analyte, four replicate extractions were made on each capillary and the mean of the four measured peak areas was used in Table 3 for the purpose of capillary-to-capillary reproducibility. The presented data show that the capillary-to-capillary reproducibility is characterized by an RSD value of less than 5.5% for all three classes of compounds used for this evaluation. For a sample preparation method, a less than 5.5% R.S.D. is indicative of excellent reproducibility.

Fig. 5 illustrates a gas chromatogram of several free aldehydes extracted from an aqueous sample using a sol-gel zirconia-PDMDPS coated capillary. Here, the concentrations of the used aldehydes were in 80–500 ppb range. The extraction was carried out on a 10 cm × 0.32 mm i.d. Sol-gel zirconia-PDMDPS coated microextraction capillary for 30–40 min. The extraction of the analytes was performed at room temperature. Aldehydes are known to have toxic and carcinogenic properties, and therefore, their presence in the environment is of great concern because of their adverse effects on public health and vegetation [80]. Aldehydes are major disinfection byproducts formed as a result of chemical reaction between disinfectant (ozone or chlorine) and organic compounds in drinking water [81]. Therefore, accurate analysis of trace-level contents of aldehyde in the environment and in drinking water is important [82]. Aldehydes are polar compounds that are often derivatized [83] for GC analysis to avoid undesirable adsorption that causes peak tailing. Sol-gel zirconia-PDMDPS coated capillary provided highly efficient extraction of the aldehydes, and the used sol-gel GC column provided excellent peak shapes which is also indicative of high quality of deactivation in the used sol-gel GC column. This also demonstrates effective focusing of the analytes at the column inlet after their thermal desorption from the microextraction capillary. For the aldehydes, sol-gel CME-GC with the zirconia-PDMDPS coated capillary provided excellent repeatability in peak area (R.S.D. <5%) and retention time (R.S.D. <0.16%).

Fig. 6 shows a gas chromatogram illustrating CME-GC analysis of several ketones extracted from an aqueous sam-

![Fig. 7: CME-GC analysis of mixture of PAHs, aldehydes and ketones using a sol-gel zirconia-PDMDPS coated extraction capillary. Extraction parameters: 10 cm × 0.32 mm i.d. microextraction capillary; extraction time, 40 min (gravity fed at room temperature). Other conditions: 10 cm × 0.25 mm i.d. Sol-gel PDMS GC column; splitless injection; injector temperature rose from 30 to 300 °C; column temperature program from 30 to 300 °C at rate of 20 °C/min; helium carrier gas; FID. 350 °C. Peaks: (1) napthalene; (2) n-decylaldehyde; (3) homohexylaldehyde; (4) valerenaldehyde; (5) dodecaldehyde; (6) hexamethine; (7) fluorine; (8) heptamethine; (9) phtharathine; (10) pyrene; and (11) napthalene.](image-url)
Appendix B (Continued)


Like aldehydes, there was no need for derivatization of the ketones, either during extraction or GC analysis. Sharp and symmetrical GC peaks, evident from the chromatograms, show the effectiveness of the used CMU-GC system, as well as the practical utility of the mini-union metal connector providing leak-free connection between the extraction capillary and the GC column. Excellent reproducibility was achieved in CME-GC of ketones using sol–gel zirconia–PDMDS coated capillary as shown in Table 2. The peak area RSD% values for ketones were less than 5.6% and their retention time repeatability on used sol–gel PDMDS column was characterized by R.S.D. values of less than 0.27%.

Fig. 7 shows a gas chromatogram illustrating CME-GC analysis of an aqueous sample containing different classes of compounds including PAHs, aldehydes and ketones, and shows that the sol–gel zirconia–PDMDS extraction capillary can provide simultaneous extraction of polar and non-polar compounds present in the aqueous sample, and demonstrates the advantage over conventional SPME coatings that often do not allow such effective extraction of both polar and non-polar analyte from the same sample.

In capillary microextraction technique, the amount of analyte extracted into the sorbent coating depends not only on the polarity and thickness of the coated phase, but also on the extraction time. Fig. 8 illustrates the kinetic profile for the extraction of fluorine (a non-polar analyte), heptanophenone and undecylic aldehyde (both are moderately polar analytes) on a sol–gel zirconia–PDMDS-coated microextraction capillary. The CME experiments were carried out using aqueous samples of individual test analytes. The extraction equilibrium for fluorine reached in 10 min, which is much shorter than extraction equilibrium time for heptanophenone and undecylic aldehyde (both approximately 30 min). This is because fluorine exhibits hydrophobic behavior that has higher affinity toward the non-polar PDMDS-based sol–gel zirconia coating than toward water. On the other hand, heptanophenone and undecylic aldehyde, being more polar and hydrophilic than fluorine showed a slower extraction by the coated non-polar sol–gel zirconia-PDMDS sorbent.

Sol–gel zirconia–PDMDS coating showed high pH stability, and retained excellent performance after rinsing with 0.1 M NaOH (pH 13) for 24 h. Chromatograms in Fig. 9a and b show CME-GC analysis of four PAHs before (Fig. 9a) and after (Fig. 9b) zirconia–PDMDS extraction capillary was rinsed with 0.1 M NaOH solution.

As is evident from Fig. 9, the extraction performance of the sol–gel zirconia–PDMDS capillary remained practically unchanged after rinsing with NaOH as it can be seen in Table 4.

Fig. 8. Extraction kinetics of aqueous undecylic aldehyde, heptanophenone, and fluorine on a sol–gel zirconia–PDMDS microextraction coated capillary. Extraction parameters: 10 cm × 0.32 mm i.d. microextraction capillary; Other conditions: 10 cm × 0.25 mm i.d. Sol–gel GC PDMDS column; splitless desorption; injector temperature from 30 to 200 °C; column temperature program from 30 to 300 °C at rate of 20 °C/min; helium carrier gas: FID 350 °C.

Fig. 9. CME-GC analysis of PAHs using a sol–gel zirconia–PDMDS coated microextraction capillary, before (a) and after (b) rinsing the microextraction capillary with 0.1 M NaOH solution for 24 h. Extraction parameters: 10 cm × 0.25 mm i.d. microextraction capillary; coating thickness 30 mm; extraction time: 30 min (gravity fed at room temperature). Other conditions: 10 cm × 0.25 mm i.d. Sol–gel GC PDMDS column; splitless desorption; injector temperature rise from 30 to 300 °C; column temperature program from 30 to 280 °C at rate of 20 °C/min; then from 280 to 300 °C at rate of 2 °C/min; helium carrier gas FID 350 °C. Peaks: (1) naphthalene; (2) fluorine; (3) phenanthrene; and (4) pyrene.
Table 4
Peak area repeatability data for ppb level concentrations of PAHs before and after extraction capillary treated with 0.1 M NaOH

<table>
<thead>
<tr>
<th>Name of the analyte</th>
<th>Peak area repeatability before rinsing with 0.1 M NaOH</th>
<th>Peak area repeatability after rinsing with 0.1 M NaOH</th>
<th>Relative change in peak area $\left[ \frac{(A_2 - A_1)}{A_1} \times 100(%) \right]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthene</td>
<td>30880.91 correct unit</td>
<td>28412.76 correct unit</td>
<td>3.12</td>
</tr>
<tr>
<td>Fluorene</td>
<td>35425.63 correct unit</td>
<td>33428.86 correct unit</td>
<td>5.64</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>47547.31 correct unit</td>
<td>46525.33 correct unit</td>
<td>2.15</td>
</tr>
<tr>
<td>Pyrene</td>
<td>33859.61 correct unit</td>
<td>35636.56 correct unit</td>
<td>5.17</td>
</tr>
</tbody>
</table>

For comparison, the same experiment was conducted using a 10 cm piece of a conventionally coated commercial PDMIDPS-based GC column as the microextraction capillary. The results are shown in Fig. 10. A drastic loss in extraction sensitivity after rinsing the conventionally coated silica-based microextraction capillary with 0.1 M NaOH solution is obvious (Fig. 10).

These data suggest that the created hybrid sol-gel zirconia-based coatings have significant pH stability advantage over conventional silica-based coatings, and that such coatings have the potential to extend the applicability of capillary microextraction and related techniques to highly basic samples, or analytes that require highly basic condition for the extraction and/or analysis.

4. Conclusion

Sol-gel zirconia-based hybrid organic-inorganic sorbent coating was developed for use in microextraction. Principles of sol–gel chemistry was employed to chemically bind a hydroxy-terminated silicone polymer (polydimethyl(3,5-diphenyloxiane) to a sol-gel zirconia network in the course of its evolution from highly reactive alkoxide precursor (zirconium tetrabutoxide) undergoing controlled hydrolytic polycondensation reactions. For the first time, sol–gel zirconia-PDMIDPS coating was employed in capillary microextraction. The newly developed sol-gel zirconia-PDMIDPS coating demonstrated exceptional pH stability: its extraction characteristics remained practically unchanged after rinsing with a 0.1 M solution of NaOH (pH 13) for 24 h. Solventless extraction of analytes was carried out simply by passing the aqueous sample through the sol-gel extraction capillary for approximately 30 mm. The extracted analytes were efficiently transferred to a GC column via thermal desorption, and the desorbed analytes were separated by temperature programmed GC. Efficient CME-GC analyses of diverse range of solutes was achieved using sol–gel zirconia-PDMIDPS capillaries. Parts per trillion (ppt) level detection limits were achieved for polar and non-polar analytes in CME-GC-FID experiments. Sol-gel zirconia-PDMIDPS coated microextraction capillaries showed remarkable run-to-run repeatability (R.S.D. < 0.27%) and produced peak area R.S.D. values in the range of 1.24–7.25%.
Appendix B (Continued)
Appendix B (Continued)


Appendix C

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JOURNAL OF CHROMATOGRAPHY A


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High pH-resistant, surface-bonded sol–gel titania hybrid organic–inorganic coating for effective on-line hyphenation of capillary microextraction (in-tube solid-phase microextraction) with high-performance liquid chromatography

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Abstract

Sol–gel titania–poly(dimethylsiloxane) (TiO2–PDMS) coating was developed for capillary microextraction (CME) to perform on-line preconcentration and HPLC analysis of trace impurities in aqueous samples. A method is presented describing in-situ preparation of the titania-based sol–gel PDMS coating and its immobilization on the inner surface of a fused silica microextraction capillary. To perform CME–HPLC, the sol–gel TiO2–PDMS capillary was installed in the HPLC injection port as an external sampling loop, and a conventional ODS column was used for the liquid chromatographic separation. The target analytes were extracted on-line by passing the aqueous sample through this sampling loop. The sol–gel titania–PDMS coated capillaries were used for on-line extraction and HPLC analysis of polycyclic aromatic hydrocarbons, ketones, and alkylbenzenes. The extracted analytes were then transferred to the HPLC column using an organic-rich mobile phase followed by HPLC separation via gradient elution. To our knowledge, this is the first report on the use of sol–gel titania–PDMS coating to perform in-tube solid-phase microextraction. The newly developed sol–gel titania–PDMS capillary demonstrated excellent pH stability and enhanced extraction capability over the commercial GC coatings that are conventionally used for the same purpose. Extraction characteristics of a sol–gel titania–PDMS capillary remained practically unchanged after continuous rinsing with a 0.1 M NaOH solution (pH 13) for 12 h.

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Keywords: Sol–gel; Capillary microextraction; In-tube solid-phase microextraction; Gradient elution; Titania–poly(dimethylsiloxane); Polycyclic aromatic hydrocarbons; Ketones; Alkylbenzenes

1. Introduction

Solid-phase microextraction (SPME), a solvent-free sample preparation technique, was developed by Pawliszyn and co-workers [1–3] using a fused-silica fiber externally coated with a polymeric sorbent covering a small segment of it at one of the ends. Analytes present in the sample medium were directly extracted and preconcentrated by the coated sorbent in the process of reaching an extraction equilibrium with the sample matrix. The preconcentrated analytes were then desorbed into a GC instrument for analysis.

In conventional fiber-based SPME, still there exist a number of shortcomings that need to be overcome. These include inadequate thermal and solvent stability of conventionally prepared sorbent coatings [4], low sample capacity, difficulties associated with the immobilization of thick coatings, susceptibility of the fiber (especially the coated end) to mechanical damage [5,6], and technical difficulties...
associated with the hyphenation of fiber-based SPME with liquid-phase separation techniques [7,8].

Capillary microextraction (CMIE) [9] also called in-tube SPME [10,11] presents a convenient format for coupling SPME to HPLC and for automated operation of SPME-HPLC. Hyphenation of CMIE to HPLC is especially important for the analysis of a wide range of less volatile or thermally labile compounds [12] that are not amenable to GC separation. In the open tubular format of CME, a sorbent coating is applied to the inner surface of a capillary. This alternative format provides an effective solution to the problem associated with the mechanical damage of sorbent coating frequently encountered in conventional fiber-based SPME where the coating is applied to the outer surface of the fiber. In this new format of SPME, a segment of wall-coated capillary GC column is commonly used [10–12] for the direct extraction of organic analytes from an aqueous medium. To perform HPLC analysis, the extracted analytes are transferred to the HPLC column by desorbing them with an appropriate mobile phase.

Capillary microextraction has great prospects in liquid-phase trace analysis. However, to achieve its full analytical potential, the technology needs further improvements in a number of areas. First, segments of GC columns that are commonly used for sample preconcentration have thin coatings that limit the sorption capacity, and hence, the extraction sensitivity of in-tube SPME. Second, the sorbent coatings in such microextraction capillaries usually are not chemically bonded to capillary inner walls, which limits their thermal and solvent stabilities. Third, conventionally prepared GC coatings that are used in in-tube SPME capillaries inherently possess poor pH stability. This places serious limitations on the range of applications amenable to CME-HPLC analysis. Low pH stability of in-tube SPME coatings practically excludes the applicability of the technique to high-pH samples or analytes that require high-pH solvent systems for desorption from the microextraction capillary. Therefore, development of methodologies for the creation of high pH- and solvent-resistant sorbent coatings is an important area in the future development of in-tube SPME, and is expected to play a major role in effective hyphenation of this sample preconcentration technique with liquid-phase separation techniques that commonly use organic-aqueous mobile phases with a wide range of pH conditions [13].

Sol-gel chemistry has been recently applied to solid-phase microextraction [4,14–17] and capillary microextraction [9] to create silica-based hybrid organic-inorganic capillary coatings. The sol-gel technique provided chemically bonded coatings on the inner surface of fused-silica capillaries, and easily solved the coating stability problems described above.

Although sol-gel technique helped overcome some significant shortcomings of SPME, in-tube SPME techniques by providing an effective means of chemical immobilization for sorbent coatings, an important problem inherent in silica-based material systems (commonly used in SPME or CME) still remains to be solved: silica-based materials possess a narrow window of pH stability [18]. In the context of SPME, it pertains to the stability of silica-based fibers and coatings. The development of alternative materials possessing superior pH stability and better mechanical strength should provide SPME with additional ruggedness and versatility.

Recently, titania has attracted interest in separation science due to its superior pH stability and mechanical strength compared with silica [19–23]. Several studies have been conducted on the application of titania in chromatographic separations. Tani and Suzuki [21] reported the preparation of titania-based packing materials for HPLC by sol-gel method, and investigated their properties. Tsai et al. [22] prepared silica-capillaries coated with titania or alumina for capillary electrophoresis (CE) separation of proteins. Fujimoto [23] used a thermal decomposition technique to create titania coatings on the inner surface of fused-silica capillaries for capillary zone electrophoresis (CZE) and capillary electrophromatography (CEC) applications. The titania-coated capillaries were found to possess a bi-directional electroosmotic flow (EOF) and low solubility in aqueous solutions within a pH range of 3–12. Pesek et al. [24] reported the surface derivatization of titania with triethoxysiline to prepare titania-based stationary phases via silanization-hydrolysis. Some other groups [25,26] reported preparations of silica-coated titania monolayers for faster and more efficient coating, which is important for further preparation of nanocomposites.

To date, very little (if any) research has been done on the development and application of titania-based coatings in analytical microextraction techniques. In this paper, we report the preparation of sol–gel TiO2-PDMS coated capillaries and show the possibility of on-line CME-HPLC operation using sol–gel TiO2–PDMS microextraction capillaries to provide a significant improvement in pH stability and extraction sensitivity.

2. Experimental

2.1. Equipment

On-line CME-HPLC experiments were carried out on a Micro-Tech Scientific (Vista, CA) Ultra Plus HPLC system with a variable wavelength UV detector (Linear UVIS 2000). A Nicolet model Avatar 320 FT-IR (Thermo Nicolet, Madison, WI) was used for FT-IR measurements. A reversed-phase ODS column (25 cm × 4.6 mm i.d., 5 μm dp) was used for HPLC separation of the extracted analytes. A Fisher model G-560 Vortex Genie 2 system (Fisher Scientific, Pittsburgh, PA) was used for thorough mixing of the sol solutions. A Microcentrifuge model APO 5760 centrifuge (Accurate Chemical and Scientific Corp., Westbury, NY) was used for centrifugation of sol solutions. A Barnstead model 04741 Nanopure deionized water system (Barnstead/Thermolyne, Dubuque, IA) was used to obtain 16.0 MΩ cm water. On-line data collection and processing were done using
2.2. Chemicals and materials

Fused silica capillary (250 and 320 μm i.d.) was purchased from Polymicro Technologies Inc. (Phoenix, AZ). A commercial polysiloxane-based GC column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used for comparison with sol-gel titania-PDMS-based microextraction capillary in pH stability studies. Titanium (IV) isopropoxide (99.999%), 1-butanol (99.4%), polystyrene-based hydrocarbons (PSHs), 1,1,1,3,3,3-hexamethyldisilazane (HMDS), trifluoroacetic acid (TFA), polymeric aromatic hydrocarbons (PAHS) (acenaphthylene, fluorene, phenanthrene, fluoranthene, chrysene, naphthalene, and alkylbenzenes (toluene, ethylbenzene, xylene, propylbenzene, butylbenzene, amylbenzene) were purchased from Aldrich (Milwaukee, WI). Hydroxy-terminated poly(dimethylsiloxane) (PDMS) was purchased from United Chemical Technologies Inc. (Bristol, PA). HPLC-grade solvents (acetonitrile, methylene chloride, and methanol) were purchased from Fisher Scientific.

2.3. Preparation of the sol solution

The sol solution was prepared by thoroughly vortexing the following reagents in a 2 mL polypropylene centrifuge tube: a sol-gel active organic component (hydroxymethylsilane, 50 mg), two solvents (methanol, chloroform, and 1-butanol, 200 μL each), a mixture of two surface deactivation reagents (HMDS, 8 μL and PMHS, 2 μL), and a sol-gel chelating agent (27% TFA in H2O, 18 μL). The content of the tube was centrifuged for 5 min at 13,000 rpm (15,682 × g). The top clear solution was transferred to another clean vial by decantation, and was used for coating the fused silica microextraction capillary.

2.4. Preparation of sol-gel TiO2-PDMS coated microextraction capillaries

A 1 mL hydrothermally treated [27] fused silica capillary (250 or 320 μm i.d.) was installed on an in-house built gas pressure-operated capillary filling/purging device [28], and the capillary was filled with the prepared sol solution under 10 psi helium pressure. After filling, the sol solution was kept inside the capillary for 15 min to facilitate the creation of a surface-bonded coating due to sol-gel reactions taking place in the coating solution inside the capillary. Following this, the unbonded portion of the sol solution was expelled from the capillary under helium pressure (20 psi), and the capillary was further purged with helium for 30 min. The coated capillary was then conditioned in a GC oven by programming the temperature from 40 °C to 320 °C at 1 °C/min under helium purge. The capillary was held at 320 °C for 180 min. Finally, the capillary was cooled down to room temperature and rinsed with methylene chloride and methanol (2 mL each). Following this, the capillary was installed in the GC for drying and further thermal conditioning under temperature-programmed heating as described above, except that this time the capillary was held at the final temperature for 30 min.

2.5. Capillary microextraction (CME) and on-line CME-HPLC analysis

A schematic of the CME-HPLC setup for on-line capillary microextraction and HPLC analysis is presented in Fig. 1. An ODS column (25 cm × 4.6 mm i.d., 5 μm dP) was previously installed in the HPLC system and pre-equilibrated with the mobile phase consisting of a mixture of acetonitrile and water (85:15, v/v). A 40 cm segment of the sol-gel TiO2-PDMS coated microextraction capillary was mounted on the injection port as an external sampling loop. Analytes were pre-concentrated in the sol-gel TiO2-PDMS coating by passing the aqueous sample from a gravity-fed dispenser [9] through this sol-gel titania-PDMS coated microextraction capillary for 40 min. Using a syringe, the sampling loop was flushed out with deionized water to remove the sample matrix. The analytes extracted in the sol-gel TiO2-PDMS coating of the sampling loop were then transferred into the HPLC column by desorbing with 100% acetonitrile for 30 s. This was accomplished by simply switching the injection valve from the "load" to "inject" position. The injected analytes were then separated on the ODS column under gradient elution conditions by programming acetonitrile composition in the organo-aqueous mobile phase from 80% (v/v) to 100% in 15 min.

2.5.1. Treatment of coated capillaries with 0.1 M NaOH solution

A 40 cm segment of the sol-gel TiO2-PDMS coated capillary was directly installed on the gravity-fed sample dispenser, and continuously rinsed with 0.1 M NaOH solution (pH 13) for 12 h. The capillary was then flushed out with deionized water for 30 min, and mounted back on the HPLC injection port. The target analytes (PAHs) were extracted online for 40 min, followed by their HPLC analyses as described in Section 2.5. Using the same procedure, a 40 cm segment of the commercial PDMS-based GC capillary was treated with a 0.1 M NaOH solution. CME performances of the used capillaries were evaluated both before and after the alkaline treatment to explore pH stability of the used coatings.

2.6. Safety precautions

The presented work involved the use of various chemicals (organic and inorganic) and solvent that might be environmentally hazardous with adverse health effects. Proper safety measures should be taken in handling strong bases...
Appendix C (Continued)

3. Results and discussion

The goal of this research was to develop high pH-resistant, surface-bonded sol-gel titania coatings for capillary microextraction to facilitate effective hyphenation of CME with HPLC. Judicious utilization of unique attributes of sol-gel chemistry allowed us to create a surface-bonded hybrid organic–inorganic titania coating on the inner walls of a fused silica capillary providing an opportunity to exploit advanced material properties of titania-based sorbents [29,30] in capillary microextraction. Unlike the conventional multi-step coating technologies [31–34], the sol-gel approach involves a single-step procedure to accomplish the sorbent coating, its chemical immobilization, and deactivation [35].

As sol-gel precursors, titanium alkoxides differ significantly from silicon alkoxides in terms of their chemical reactivity and complex-forming ability. These differences dictate the adoption of different strategies for the creation of titania-based sol-gel sorbents compared with those for silica-based analogs. While sol-gel reactions in a silica-based system are rather slow and often require the use of catalysts to accelerate the process [36], titania-based (transition metal oxide-based in general) sol-gel reactions are very fast. This is explained by the fact that titanium alkoxides are very reactive toward nucleophilic reagents like water [37]. They readily undergo hydrolysis, which results in a very fast sol-gel process. Because of this, titania-based sol-gel reactions need to be decelerated by a suitable means to allow for the sol-gel process to be conducted in a controlled manner. This is usually accomplished through the use of suitable chelating agents that form complexes with the sol-gel precursors (or replace the reactive alkox group with a less reactive group), thus hindering their participation in the sol-gel reactions. Without such a chelating agent, the gelation takes place instantaneously as the sol-gel solution ingredients are mixed together. Chelating agents such as acetate acid [38,39], trifluoroacetic acid [40], or metal β-diketonates [41] are often used for this purpose.

In the present work, sol-gel TiO₂ PDMS coated capillaries were prepared through hydrolytic polycondensation reactions performed within fused silica capillaries followed by thermal condensation of the created coatings to achieve fine porous structures. Here, TFA served as a chelating agent [40], and decelerated the gelation process for the creation of TiO₂ PDMS coating. It has been shown by infrared spectra that the acetate ion can serve as a bidentate ligand (chelating and bridging) to the transition metal alkoxides, such as Ti(OR)₄ or Zr(OR)₄ [38,40,42].

Fig. 2 represents two scanning electron micrographs (SEM) showing the fine structural features of a 520 μm i.d. fused silica capillary with sol-gel TiO₂ PDMS coating on the inner surface. As is evident from these images, the sol-gel TiO₂ PDMS coating in the microextraction capillary acquires a porous structure, providing enhanced surface area and sorption ability. Based on the SEM data, the
Appendix C (Continued)

The thickness of the sol-gel TiO$_2$–PDMS coating was estimated at 0.5 µm.

The sol-gel process for the generation and chemical immobilization of the coating involves: (A) hydrolysis of the titanium alkoxide precursor [43], (B) polycondensation of the hydrolysis products into a three-dimensional sol-gel network [44,45], (C) chemical incorporation of hydroxy-terminated PDMS in the sol-gel network [50,47], and (D) chemical anchoring of the sol-gel hybrid polymer to the inner walls of the capillary [44,45]. Scheme 1 illustrates the hydrolysis and polycondensation reactions of the sol-gel precursor, titanium (IV) isopropanoxide, and Scheme 2 represents the final structure of the sol-gel TiO$_2$–PDMS coating on the inner surface of a fused silica capillary.

The formation of Ti–O–Si bonds in the prepared sol-gel sorbent was examined by FT-IR. The FT-IR experiments were performed by passing IR radiation through a thin layer of sol-gel titania coating material that was used in the fused silica capillary. This was done in separate experiments outside the fused silica capillary. It has been reported [48,49] that a characteristic IR band representing Si–O–Ti bonds is located at 940–960 cm$^{-1}$. Fig. 3 shows FT-IR spectra of the sol-gel TiO$_2$–PDMS coating with a specific band at 952.63 cm$^{-1}$. This is indicative of the presence of Si–O–Ti bonds in the sol-gel sorbent used in the fused silica microextraction capillaries to perform on-line CMS–HPLC analysis.

Deactivation of the sol-gel coatings can be expected to take place mainly during thermal conditioning of the capillary, through derivatization of the free hydroxyl groups in the coating structure with HMDS [50] and PMHS [25,51] incorporated in the sol solution. To control the gelation time and to obtain a transparent gel, it was essential to find an optimum ratio (v/v) of HMDS and PMHS. In the present study, this ratio was found to be 4:1 (HMDS:PMHS, v/v).

Sol–gel technology is quite versatile, and allows for the control of coating thickness either by manipulating the reaction time or composition of the sol solution. Zeng et al. [16] has recently reported the preparation of 70 µm thick silica-based sol-gel coating on conventional SPME fiber. It should be possible to create such thick coatings (either silica-based or transition metal oxide-based) on the inner surface of fused silica capillaries as well. Use of thicker coatings should enhance the sample capacity and extraction sensitivity in CMCE with titania-based sol-gel coatings.

![Fig. 2. Scanning electron microscopic images of a 320 µm id. fused silica capillary with sol-gel TiO$_2$–PDMS coating: (A) cross-sectional view (500x) and (B) surface view (10000x).](image)

![Fig. 3. FT-IR spectra of the sol-gel TiO$_2$–PDMS coating.](image)
Appendix C (Continued)

Fig. 4. Chromatograms representing capillary microextraction (ME) analysis of PCBs using solid-liquid adsorbent (PDMS-coated and PDMS-coated commercial PDMS-based GC) and aqueous solution (a) and calibration curve of liquid-liquid microextraction with a trimethylsilyl (TMS) solution (b). Column: Zorbax SB-C18, 4.6 μm, 250 mm × 4.6 mm ID. Injection: 1 μL of 1 mg/mL solution of PCBs in 100% methanol for 1 min. Flow rate: 0.5 mL/min, ambient temperature: 35 °C. Aqueous solution: 2.5 μg/mL (a), and 48 μg/mL (b). TMS solution: 0.25 μg/mL (c) and 0.5 μg/mL (d).

Peak assignments: (1) phenol (50 ppb), (2) anisole, (3) pchlorophenol (25 ppb), and (4) p-chlorophenol (100 ppb).
Appendix C (Continued)

Table 1

<table>
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<tr>
<th>Extracted PAHs</th>
<th>Peak area repeatability (n = 3)</th>
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<th>Percent change in peak area (A_f - A_i) / A_i (× 100%)</th>
<th>Detection limits (ppb) (S/N = 3)</th>
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<td></td>
<td>Mean peak area (A_i) (arbitrary unit)</td>
<td>R.S.D. (%)</td>
<td>Mean peak area (A_f) (arbitrary unit)</td>
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<td>5.2</td>
<td>16.3</td>
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</table>

a Extraction conditions: 40 cm × 0.25 mm i.d. × 0.5 μm sol-gel TiO_2–PDMS-coated capillary; extraction time: 40 min; HPLC conditions: 25 cm × 4.6 mm i.d. ODS column (5 μm), gradient elution 0–20% ACN/water (100:60) for 20 min; 1 mL/min flow rate; UV detection at 254 nm.

The sol-gel titania–PDMS coatings demonstrated excellent pH stability profiles conventionally created coatings like those used in commercial GC capillary columns. Fig. 4B illustrates the CME performance of a TiO_2–PDMS-coated microextraction capillary (250 μm i.d.) in CME-HPLC analysis of PAHs before (Fig. 4A) and after (Fig. 4B) rinsing the capillary with 0.1 M NaOH solution (pH 13) for 12 h. Analogously, obtained data for a piece of commercial PDMS-based GC column are presented in Fig. 4C and 4D, respectively. Chromatogram of Fig. 4B was obtained on the sol-gel TiO_2–PDMS coated microextraction capillary after it was thoroughly rinsed with deionized water. The extraction of PAHs was performed under the same set of conditions as in Fig. 4A. From the comparison of peak profiles and peak heights in Fig. 4C and 4D, it is evident that the sol-gel TiO_2–PDMS coating in the microextraction capillary remained unaffected even after the prolonged rinsing with 0.1 M NaOH solution of pH 13.

Fig. 5 Capillary microextraction–HPLC analysis of PAHs. Extraction conditions: 40 cm × 0.25 mm i.d. × 0.5 μm sol-gel TiO_2–PDMS-coated capillary; extraction time: 40 min (gravitation); other conditions: 25 cm × 4.6 mm i.d. ODS column (5 μm), gradient elution with mobile phase composition programmed from 0% to 100% acetonitrile/water for 15 min, 1 mL/min flow rate; UV detection at 254 nm; ambient temperature. Peaks: (1) naphthalene (1 ppm), (2) acenaphthylene (1 ppm), (3) fluorene (500 ppb), and (4) phenanthrene (500 ppb).

Fig. 6 Capillary microextraction–HPLC analysis of alkylbenzenes. Extraction conditions are the same as in Fig. 5. Other conditions: 25 cm × 4.6 mm i.d. ODS column (5 μm), gradient elution with mobile phase composition programmed from 0% to 100% acetonitrile/water for 15 min, 1 mL/min flow rate; UV detection at 205 nm; ambient temperature. Peaks: (1) toluene (600 ppb), (2) ethylbenzene (200 ppb), (3) xylenes (50 ppb), (4) propylbenzene (50 ppb), (5) butylbenzene (30 ppb), and (6) amylbenzene (50 ppb).
appendix C (Continued)

Table 2

<table>
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<th>Detection limits (ppb)</th>
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<td></td>
<td>Amylene</td>
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* Extraction conditions: 40 cm × 0.32 mm i.d., 0.5 μm sol-gel TiO₂–PDMS-coated capillary; extraction time: 40 min; HPLC conditions: 25 cm × 4.6 mm i.d., CDS column (5 μm dₕ); gradient elution from 30/50 (v/v) ACN/water to 100% ACN for 15 min (20 mm for PAHs); 1 mL/min flow rate; UV detection at 254 nm (205 nm for alkylbenzenes).

On the other hand, the PDMS-based stationary phase coating in the commercial GC capillary showed significantly less extraction sensitivity as is evident from peak heights in Fig. 4c. It also failed to survive the harsh conditions of rinsing with 0.1 M NaOH solution, which is evidenced by a dramatic decrease in the extraction sensitivity after the NaOH treatment (compare Fig. 4c and 4d). These results show that a sol-gel TiO₂–PDMS coated capillary possesses excellent pH stability and retains its extraction ability under extreme pH conditions, while conventionally prepared PDMS-based GC coatings were found to be unstable under such extreme pH conditions [52,53].

Table 1 shows repeatability and detection limit data for CME-HPLC analysis using sol-gel TiO₂–PDMS coated microextraction capillaries. For a 0.25 mm i.d. sol-gel titania–PDMS microextraction capillary, the R.S.D. value in peak area remained within 9.2%, and detection limits in the range of 0.25–5.37 ppb were achieved using UV detection.

Fig. 5 presents a chromatogram illustrating CME-HPLC analysis of moderately polar aromatic ketones extracted from an aqueous sample using a 0.32 mm i.d. sol-gel coated TiO₂–PDMS capillary.

Compared to PAHs samples, ketones needed higher analyte concentrations (100 ppb–1 ppm) for CME-HPLC analysis. This may be explained by the nonpolar nature of the sol-gel TiO₂–PDMS coating, higher solubility of ketones in water due to higher polarity, and the working principles of UV detection. In this case, the run-to-run peak area repeatability was less than 8% R.S.D. Detection limits for the extracted ketones ranged between 2.47 ppb for heptanophane to 11.60 ppb for valeterophane in conjunction with UV detection. From the presented results it is evident that sol-gel TiO₂–PDMS coating is able to extract both nonpolar and moderately polar analytes with good extraction sensitivity. Such an ability of the used sol-gel coating may be due to the presence of two different types of domains (a nonpolar organic domain based on PDMS and a more polar inorganic domain based on sol-gel titania materials) in such coatings [54].

Fig. 6 illustrates on-line CME-HPLC analysis of alkylbenzenes using a TiO₂–PDMS coated capillary. Excellent detection limits were also achieved for these analytes (0.65–5.45 ppb), using UV detection. Like PAHs, alkylbenzenes are less polar analytes than aromatic ketones, and they are well extracted by a sol-gel TiO₂–PDMS extraction capillary with low-ppb and sub-ppb level detection limits. Table 2 summarizes the peak area repeatability and detection limit data for PAHs, ketones, and alkylbenzenes.
Appendix C (Continued)

Fig. 7 illustrates the extraction kinetic profile for: (A) fluorene (nonpolar analyte) and (B) hexamethyleneterephthalate (moderately polar analyte) on a sol-gel TiO$_2$-PDMS coated microextraction capillary. Experimental data for these curves representing extraction kinetic profiles were obtained by individually performing capillary microextraction for each of the solutes. The microextraction experiments were performed using aqueous samples containing 100 and 300 ppb concentrations of fluorene and hexamethyleneterephthalate, respectively. A series of capillary microextraction experiments were conducted to vary the extraction time for each of the two analytes that were extracted from their standard solutions. Three replicate extractions of each analyte were performed for 1, 5, 10, 20, 30, 40, 50, and 60 min. The average peak was then plotted against the extraction time to obtain Fig. 7. For both fluorene and hexamethyleneterephthalate, extraction equilibrium was reached within 40 min as is evidenced by the plateau on the extraction curve. Since PDMS has no polar characteristics, the TiO$_2$-PDMS coating tends to extract a nonpolar analyte, in this case fluorene, better than a more polar analyte, hexamethyleneterephthalate, which has higher affinity for the aqueous medium.

Further optimization of capillary preparation method and operation conditions may be necessary to exploit full analytical potential of the sol-gel titania coated extraction capillaries. It will also be interesting to use TiO$_2$-PDMS extraction capillary in CME-GC to achieve better detection limits, since CME-GC will allow for the use of highly sensitive flame ionization detector. Such an assumption stems from the fact that sol-gel TiO$_2$-PDMS coatings have already shown good extraction capabilities for CME-HPLC equipped with a UV detector, which is much less sensitive than the FID. The use of wider bore capillaries with thicker sol-gel coatings or monolithic extraction beds [55] should further enhance the extraction sensitivity.

4. Conclusions

To the best of our knowledge, this is the first report on the creation and use of a sol-gel TiO$_2$-PDMS coating in solid-phase microextraction. Sol-gel TiO$_2$-PDMS coated microextraction capillaries possess excellent pH stability and retain their extraction characteristics intact even after prolonged treatment with highly alkaline (pH 13) NaOH solution. Direct chemical bonding of the coating to capillary inner walls provides these coatings with excellent solvent resistance, and make sol-gel TiO$_2$-PDMS coated capillaries very much suitable for on-line sample preconcentration in CME-HPLC analysis. The newly developed sol-gel TiO$_2$-PDMS coating was effectively used for the extraction of different classes of analytes with good extraction sensitivity, and run-to-run repeatability. Low ppb and sub-ppb level (0.15–11.66 ppb) detection limits were achieved for PAHs, ketones, and alkylbenzenes in CME-HPLC analysis using the newly constructed sol-gel TiO$_2$-PDMS coated microextraction capillary in conjunction with UV detection. Through proper optimization of experimental conditions for sol-gel coating and the capillary microextraction processes it should be possible to further enhance the extraction sensitivity. For volatile and thermally unstable analytes, use of sol-gel TiO$_2$-PDMS coated capillaries in CME-GC should provide significant enhancement in sensitivity.

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References

Parts per quadrillion level ultra-trace determination of polar and nonpolar compounds via solvent-free capillary microextraction on surface-bonded sol–gel polytetrahydrofuran coating and gas chromatography–flame ionization detection

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Abstract

Sol–gel polytetrahydrofuran (poly-THF) coating was developed for high-sensitivity sample preconcentration by capillary microextraction (CME). Parts per quadrillion (ppq) level detection limits were achieved for both polar and nonpolar analytes through sample preconcentration on sol–gel poly-THF coated microextraction capillaries followed by gas chromatography (GC) analysis of the extracted compounds using a flame ionization detector (FID). The sol–gel coating was in situ created on the inner walls of a fused silica capillary using a sol solution containing poly-THF as an organic component, methyltrimethoxysilane (MTMOS) as a sol–gel precursor, trifluoroacetic acid (TFA, 5% water) as a sol–gel catalyst, and hexamethyldisilazane (HMDS) as a deadendating reagent. The sol solution was introduced into a hydrothermally-treated fused silica capillary and the sol–gel reactions were allowed to take place inside the capillary for 60 min. A wall-bonded coating was formed due to the condensation of silanol groups residing on the capillary inner surface with those on the sol–gel network fragments evolving in close vicinity of the capillary walls. Poly-THF is a medium polarity polymer, and was found to be effective in carrying out simultaneous extraction of both polar and nonpolar analytes. Efficient extraction of a wide range of trace analytes from aqueous samples was accomplished using sol–gel poly-THF coated fused silica capillaries for further analysis by GC. The test analytes included polycyclic aromatic hydrocarbons (PAHs), aldehydes, ketones, chlorophenols, and alcohols. To our knowledge, this is the first report on the use of a poly-THF based sol–gel material in analytical microextraction. Sol–gel poly-THF coated CME capillaries showed excellent solvent and thermal stability (≤320 °C).

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Keywords: Sol–gel; Capillary microextraction; In-tube solid-phase microextraction; Polytetrahydrofuran; Polynuclear aromatic hydrocarbons; Aldehydes; Ketones; Chlorophenols; Alcohols

1. Introduction

Solid-phase microextraction (SPME) [1] is an excellent solventless alternative to the traditional sample preparation techniques like liquid-liquid extraction (LLE), Soxhlet extraction, solid-phase extraction (SPE), etc. It is a simple, sensitive, time-efficient, cost-effective, reliable, easy-to-automate, and portable sample preparation technique.

In SPME, analyte enrichment is accomplished by using a sorbent coating in two different formats: (a) conventional fiber-based format [1] and; (b) the more recently developed "in-tube" format [2]. In its conventional format, SPME uses a sorbent coating on the external surface of a fused silica fiber (typically 100–200 μm in diameter) covering a short segment at one of the ends. In the in-tube format, the sorbent coating is applied to the inner surface of a capillary. SPME completely eliminates the use of organic solvents in sample preparation, and effectively integrates a number of critically important analytical steps such as sampling, extraction,
Appendix D (Continued)

preconcentration, and sample introduction for instrumental analysis. Thanks to these positive attributes, SPME has experienced an explosive growth over the last decade.

Despite rapid advancements in the area of SPME applications, a number of problems still remain to be solved. First, existing SPME coatings are designed to extract either polar or nonpolar analytes from a given matrix. For example, being a nonpolar stationary phase, polydimethylsiloxane (PDMS) shows excellent selectivity towards nonpolar analytes. The polar polycarbonate coating, on the other hand, demonstrates excellent selectivity towards polar compounds. Such an approach is not very convenient for samples where both polar and nonpolar analytes are present and both need to be analyzed. For such applications, it is important to have a coating that can extract both polar and nonpolar compounds with high extraction sensitivity needed for trace analysis. Second, in conventional SPME only a short length of the fiber is coated with sorbent. The short length of the coated segment on the fiber translates into low sorbent loading which in turn leads to low sample capacity. This imposes a significant limitation on the sensitivity of the classical fiber-based SPME. Improving sensitivity is still a major challenge in SPME research. This is particularly important for analyzing ultra-trace contaminants that are present in the environment. One possible way of improving extraction sensitivity in SPME is by increasing the coating thickness [3, 4]. However, equilibration time rapidly increases with the increase in coating thickness because of the dynamic diffusion-controlled nature of the extraction process [3]. As a consequence, both extraction and subsequent desorption processes become slower, resulting in longer total analysis time.

Moreover, immobilization of thicker coating on fused silica surface is difficult to achieve by conventional approaches [5] indicating the necessity of an alternative approach to effective immobilization of thick coatings. Third, low thermal and solvent stability of SPME coatings represents a major drawback of conventional SPME technology, and is a direct consequence of the poor quality of sorbent immobilization. With a very few exceptions, SPME fibers have been coated by mere physical deposition of the stationary phase. The absence of chemical bonding of the sorbent coating to the fused silica surface is considered to be the main reason for low thermal and solvent stability of SPME fibers [6]. Low thermal stability of thick coatings forces one to use low desorption temperatures to preserve coating integrity, which in turn, leads to incomplete sample desorption and sample carryover problems. Besides, low solvent stability of the coating poses a significant obstacle to reliable hyphenation of in-tube SPME with liquid-phase separation techniques (e.g., high-performance liquid chromatography (HPLC)) that employ organic or organo-aqueous mobile phases [3]. It is evident that future advancements in SPME would greatly depend on new developments in the areas of sorbent chemistry and coating technology that will allow preparation of chemically immobilized coatings from advanced material systems providing desired selectivity and performance in SPME.

One possible approach to address most of the problems described above is to use sol-gel technology to create sorbent coatings [6-9]. Sol-gel chemistry provides a simple and convenient pathway leading to the synthesis of advanced material systems that can be used to prepare surface coatings [10, 11]. In the context of fused silica fiber/capillary-based SPME, major advantages offered by sol-gel technology are as follows: (1) it combines surface treatment, deactivation, coating, and stationary phase immobilization into a single-step procedure making the whole SPME fiber/capillary manufacturing process very efficient and cost-effective; (2) it creates chemical bonds between the fused silica surface and the created sorbent coating; (3) it provides surface-coatings with high operational stability ensuring reproducible performance of the sorbent coating under operation conditions involving high temperature and/or organic solvents, and thereby it expands the SPME application range towards both higher-boiling as well as thermally labile analytes; (4) it provides the possibility to combine organic and inorganic material properties in extraction sorbents providing tunable selectivity; (5) it offers the opportunity to create sorbent coatings with a porous structure which significantly increases the surface area of the extracting phase and provides acceptable stationary phase loading and sample capacity using thinner coatings.

A number of shortcomings inherent in conventional SPME originate from the design and physical construction of the fiber and the syringe-like SPME device. These include susceptibility of fiber to breakage during coating or operation, mechanical damage of the coating due to scraping, and operational uncertainties due to needle bending. In-tube SPME [2], also termed capillary microextraction [7], is practically free from these inherent format-related shortcomings of conventional SPME. It uses a fused silica capillary (generally a small piece of GC column) with a stationary phase coating on the inner surface to perform extraction. The protective polyimide coating outside the capillary remains intact and provides reliable protection against breakage. Moreover, this format provides a simple, easy, and convenient way to couple SPME to high-performance liquid chromatography.

Despite numerous advantageous features, in-tube SPME still has several inherent shortcomings that originate mainly from the deficiency of the coating technique used to prepare the extraction capillary. Conventional static coating technique, commonly employed to prepare GC capillary columns (short segments of which are used in in-tube SPME), is not suitable for generating thick coatings necessary for enhanced extraction sensitivity in SPME. Besides, in general, a conventionally prepared coating is not chemically bonded to the fused silica capillary surface. As a consequence, such coatings exhibit low thermal and solvent stability. Recently, sol-gel capillary microextraction (CMF) has been proposed [7] to address the above mentioned problems through in situ creation of surface-bonded coatings via sol-gel technology, which is suitable for creating both thick and thin coatings on the capillary inner wall.
Appendix D (Continued)

In both conventional SPME and CME, the sorbent coating plays a critically important role in the extraction process. To date, several types of sorbent coatings have been developed and used for extraction. These coatings can be broadly divided into two major types: (1) single-phase and (2) composite coatings. Single-phase SPME coatings include polydimethylsiloxane (PDMS) [12], polyacrylate [3], carbopack [13], polyimide [14], polypyrrole [15], and molecularly imprinted materials [16,17]. Among the composite coatings are carboxyl/divinybenzene (CW/DVB) [18], polydimethylsiloxane/divinybenzene (PDMS/DVB) [19], polydimethylsiloxane/carbohydrate (PDMS/carbohydrate) [20], and carboxyl/aqueous solution (CW/PR) [21].

In recent years, sol-gel SPME coatings [6,7,21-27] have drawn wide attention due to their inherent advantageous features and performance superiority over traditional coatings (both non-bonded and cross-linked types). Sol-gel PDMS [6,7,22,28] coatings possess significantly higher thermal stability ($>$300°C) than their conventional counterparts for which the upper temperature limit generally remains within 200-270°C [29]. High thermal and solvent stability have been demonstrated for other sol-gel stationary phases: sol-gel PEG [23] (320°C), sol-gel crown ethers [25] (340°C), sol-gel hydroxyfullerene [27] (360°C), sol-gel poly(methylenophenylinosiloxane) [26] (350°C).

Sol-gel PEG coating [23] has been recommended for polar analytes. Sol-gel crown ether [24,25] demonstrated higher extraction efficiencies for aromatic amines compared to CW/DVB fiber. Gburi et al. [21] described the preparation of sol-gel octyl coatings for SPME-HPLC analysis of organometallic compounds from aqueous solutions. Compared with the commercial SPME coatings, a hydroxyfullerene-based sol-gel coating [27] showed higher sensitivity, faster mass transfer rate for aromatic compounds, and possessed molecular planarity recognition capability for polychlorinated biphenyls (PCBs). Yang et al. [26] prepared sol-gel poly(methylenophenylinosiloxane) (PMPVS) coating using sol-gel technology that provided very high extraction efficiency for aromatic compounds.

Poly-THF (also called polytetramethylene oxide, PTMO) is a hydroxyl-terminated polar material that has been used as an organic component to synthesize organic-inorganic hybrid materials [30-35]. Sol-gel poly-THF has been used as bioactive bone repairing material [36], and as a proton conducting solid polymer electrolyte that might allow the operation of high temperature fuel cells [37]. Little work has been devoted to explore the potential of the sol-gel poly-THF material for use as an extraction medium in analytical chemistry. In the present work, we describe a sol-gel chemistry-based approach to in situ creating poly-THF based hybrid organic-inorganic stationary phase coatings on the inner walls of fused silica capillaries and demonstrate the possibility of using such coatings to extract parts per trillion (ppt) and parts per quadrillion (ppq) level concentrations of both polar and nonpolar analytes from aqueous sample matrices.

2. Experimental

2.1. Equipment

Capillary microextraction-gas chromatography (CME-GC) experiments with sol-gel poly-THF coated capillaries were carried out on a Shimadzu model 17A GC system (Shimadzu Corporation, Kyoto, Japan) equipped with a programmed temperature vaporizer (PTV injector) and a flame ionization detector (FID). An in-house designed liquid sample dispenser (Fig. 1) was used to perform CME via gravity-fed flow of the aqueous samples through the sol-gel poly-THF coated capillary. A Fisher Model G-560 Genie 2 Vortex (Fisher Scientific, Pittsburgh, PA) was used for thorough mixing of sol solution ingredients. A Microcentur model A5050 microcentrifuge (Accurate Chemical and Scientific Corporation, Westbury, NY) was used for centrifugation at 13,000 rpm, 15,682 x g of sol solutions made for coating the microextraction capillaries. An Avatar model 320 FTIR System ( Nicolet Analytical Instruments, Madison, WI) was used to obtain the IR spectra of poly-THF, sol-gel solution having all ingredients except poly-THF and sol-gel poly-THF sorbent. A JEOL model JSM-35 scanning electron microscope (SEM) was used for the investigation of the coated capillary surface. A homebuilt, gas pressure-operated filling/purging device [38] was used to fill the ex-

Fig. 1. Schematic of a gravity-fed sample dispensing unit used in capillary microextraction with a sol-gel poly-THF coated capillary.
traction capillary with the solution, to expel the solution from the capillary after predetermined period of in-capillary residence, as well as to purge the microextraction capillary with helium. Ultra pure (17.2 MQ) water was obtained from a Barnsted Model 04741 Nanopure de-ionized water system (Barnsted-Thermolyne, Dubuque, IA). ChromPerfect (Version 3.5 for Windows) computer software (Justice Laboratory Software, Denville, NJ) was used for on-line collection, integration, and processing of the experimental data.

2.2. Chemicals and materials

Fused silica capillary (250μm i.d.) with a protective polyimide coating on the external surface was purchased from Polymicro Technologies Inc. (Phoenix, AZ). Poly-THF 250 was a gift from BASF Corporation (Parsippany, NJ). Acanthophene, fluorene, phenanthrene, fluoranthene, pyrene, n-noradene, undecanol, dodecan, tridecan, valerophenone, hexamethylenophenone, decafluorophenone, 2,4,4-dichlorophenol, 2,4,6-trichlorophenol, 4-chloro, 3-methyl phenol, and pentachlorophenol were purchased from Aldrich (Milwaukee, WI); n-decyl aldehyde, 1-nonenol, 1-decanol, 1-undecanol, and 1-tridecanol were purchased from Acros Organics (Pittsburgh, PA). Lauryl alcohol was purchased from Sigma (St. Louis, MO). HPLC-grade methanol and dimethyl chloride and all borosilicate glass vials were purchased from Fisher Scientific (Pittsburgh, PA).

2.3. Preparation of sol-gel poly-THF coated microextraction capillaries

Sol-gel poly-THF coated microextraction capillaries were prepared by using a modified version of a previously described procedure [8]. Briefly, a sol solution was prepared by dissolving 250 mg of poly-THF 250, 250 μL of methyltrimethoxysilane (sol-gel precursor), 20 μL of 1,1,1,3,3,3-hexamethyldisilazane (surface deactivation reagent), and 100 μL of trifluoroacetic acid (5% H2O) (sol-gel catalyst) in 400 μL of methylene chloride. The mixture was then vortexed (3 min), centrifuged (5 min) and the clear supernatant of the sol solution was transferred to another clean vial. Following this, a piece of cleaned and hydrothermally treated fused silica capillary (5 m) was filled with the sol solution using a helium pressure-operated filling/purging device [38]. The sol solution was kept inside the capillary for 60 min to facilitate the formation of a surface-bonded sol-gel coating. On completion of the in-capillary residence time, the unbonded portion of the sol solution was expelled from the capillary under helium pressure (50 psi) and the coated capillary was purged with helium for an hour. The sol-gel poly-THF coated capillary was further conditioned in a GC oven using temperature-programmed heating (from 40 to 320°C at 1°C/min, held at 320°C for 5 h under helium purge). Before using for extraction, the sol-gel poly-THF coated capillary was rinsed sequentially with methylene chloride and methanol followed by drying in a stream of helium under the same temperature-programmed conditions as above, except that the capillary was held at the final temperature for 30 min. The sol-gel poly-THF coated capillary was then cut into 12.5 cm long pieces that were further used to perform microextraction.

2.4. Preparation of sol-gel PDMS and sol-gel PEG columns for GC analyses

The GC capillary columns used to analyze the extracted compounds were also prepared in-house by sol-gel technique. For nonpolar and moderately polar analytes, a sol-gel PDMS column was used. For polar analytes, a sol-gel PEG capillary column was employed. The sol-gel PDMS and sol-gel PEG columns were prepared by procedures described by Wang et al. [8] and Shende et al. [39], respectively.

2.5. Cleaning and deactivation of glassware

To avoid any contamination of the standard solutions from the glassware, all glassware used in the current study was thoroughly cleaned with Sparkle detergent followed by rinsing with copious amounts of deionized water and drying at 150°C for 2 h. To silanize the inner surface of the dried glassware, they were treated with a 5% (v/v) solution of HMDS in dimethyl chloride followed by heating in an oven at 250°C for 5 h under helium purge. The silanized glassware was then rinsed sequentially with methylene chloride and methanol and dried in an oven at 100°C for 1 h. Prior to use, all glassware were rinsed with generous amounts of deionized water and dried at room temperature in a flow of helium.

2.6. Preparation of standard solutions for CME on sol-gel poly-THF coated capillaries

All stock solutions were prepared by dissolving 50 mg of each analyte in 5 mL of methanol in a deactivated amber glass vial (10 mL) to obtain a solution of 10 mg/mL. The solution was further diluted to 0.1 mg/mL in methanol. The final aqueous solution was prepared by further diluting this solution with water to achieve 0.1 μg/mL to 0.1 mg/mL level concentrations depending on the compound class. Freshly prepared aqueous solutions were used for extraction.

2.7. Gravity-fed sample dispenser for capillary microextraction

A gravity-fed sample dispenser was used for capillary microextraction (Fig. 1). It was built by modifying a Chromatex A3 QM column (Kontes Glass Co., Vineland, NJ), which consists of a three-walled Pyrex glass cylinder concentrically placed in an acrylic jacket. Since glass surfaces tend to adsorb polar analytes, the inner surface of the glass cylinder was deactivated by treating with HMDS solution as described before. The cylinder was then cooled down to ambient temperature, thoroughly rinsed with methanol and deionized water, and
Appendix D (Continued)

2.8. Extraction of analytes on sol–gel poly-THF coated capillaries

A 12.5 cm long segment of the sol–gel poly-THF coated capillary (250 μm i.d.) was conditioned under helium purge in a GC oven using a temperature program from 40 to 320 °C at 10 °C/min, held at the final temperature for 30 min. The conditioned capillary was then vertically connected to the lower end of the gravity-fed sample dispenser (Fig. 1) using a plastic connector. A 50 mL volume of the aqueous sample containing trace concentrations of the target analytes was added to the inner glass cylinder through the sample inlet located at the top of the dispenser. The solution was passed through the capillary for 30 min to facilitate the extraction equilibrium to be established. The capillary was then detached from the dispenser and purged with helium for 1 min to remove residual water from the capillary walls.

2.9. Thermal desorption of extracted analytes and CME-GC analysis

For GC analysis, the sol–gel poly-THF coated capillary containing the extracted analytes was installed in the GC injection port and interfaced with the GC capillary column. Before carrying out the installation, both the injection port and the GC oven were cooled down to 30 °C and the glass wool was removed from the injection port liner. One end of the capillary was then introduced into the glass liner from the bottom end of the injection port so that 8 cm of the capillary remained inside the injection port. A graphite ferrule was used to secure an airtight connection between the capillary and the injection port. Interfacing of the extraction capillary with the GC column was accomplished by using a deactivated two-way press-fit quartz connector. Installation and interfacing of the extraction capillary with the GC column were followed by thermal desorption of extracted analytes from the installed sol–gel poly-THF coated microextraction capillary. For this, the temperature of the PTV injection port was rapidly raised to 300 °C @ 100 °C/min while keeping the GC oven temperature at 30 °C (5 min). Under these temperature program conditions, the extracted analytes were effectively desorbed from the sol–gel poly-THF coating and were transported to the cooler coupling zone consisting of the lower end segment of the microextraction capillary and/or to the front end of the GC column both located inside the GC oven and maintained at 30 °C. As the desorbed analytes reached the cooler interface zone (30 °C), they were focussed into a narrow band. On completion of the 5 min desorption and focusing period, the analytes in this narrow band were analyzed by GC using temperature-programmed operation as follows: from 30 to 500 °C at 20 °C/min with a 10 min hold time at the final temperature.

3. Results and discussion


Polytetrahydrofuran (poly-THF) [42] is a medium polarity polymer with terminal hydroxyl groups that can be utilized to bind this polymer to a sol–gel network via polycondensation reaction. It consists of tetramethylene oxide repeating units, and is synthesized through cationic ring opening polymerization of tetrahydrofuran using various initiators [43].

Table 1 lists the chemical ingredients used in this work to prepare the sol solution for creating a sol–gel poly-THF coated capillary.

The in situ creation of a highly stable, deactivated sol–gel coating involved the following processes: (1) catalytic hydrolysis of the alkoxide precursors; (2) polycondensation of

<table>
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<th>Name</th>
<th>Function</th>
<th>Structure</th>
</tr>
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<tr>
<td>Methyltrimethoxysilane (MTMOS)</td>
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<tr>
<td>Polytetrahydrofuran</td>
<td>Organic ligand</td>
<td>H – O – [(CH₂)₄ – O]ₖ – H</td>
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<td>CH₃COOH</td>
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<td>Methylene chloride</td>
<td>Solvent</td>
<td>CH₂Cl₂</td>
</tr>
<tr>
<td>Hexamethyldisilazane</td>
<td>Deactivating reagent</td>
<td>H₆Si–N–NH–Si–H₆</td>
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</tbody>
</table>
the hydrolyzed precursor with other sol-gel-active components of the sol solution; (3) chemical bonding of poly-THF to the evolving sol-gel network; (4) chemical anchoring of the evolving hybrid organic-inorganic polymer to the inner walls of the capillary; and (5) derivatization of residual silanol groups on the coating by HMDS.

In order to create the sol-gel poly-THF coating in situ, the sol solution was kept inside the capillary for 60 min to allow for the hydrolytic polycondensation reactions to take place in the sol solution located inside the capillary. In presence of the sol-gel catalyst (TFA), the sol-gel precursor (MTMOS) undergoes hydrolysis reaction. The hydrolysis products can then take part in polycondensation reactions in a variety of ways to create a three-dimensional sol-gel network (Scheme 1).

During this polycondensation process, the growing sol-gel network can chemically incorporate the poly-THF molecules resulting an organic-inorganic hybrid network

Scheme 2. Surface-bonded sol-gel poly-THF network on the fused-silica capillary inner walls.

structure. Fragments of this network located in close vicinity of the fused silica capillary walls have the opportunity to become chemically bonded to the capillary inner surface as a result of condensation reaction with the silanol groups on the capillary walls. This leads to the formation of a surface-bonded sol-gel coating on the inner walls of the capillary. HMDS, used in the coating solution, deactivates the residual silanol groups on the sorbent coating during the post-coating thermal conditioning of the capillary.

A simplified scheme of the surface-bonded sol-gel poly-THF network on the fused-silica capillary inner walls is presented in Scheme 2.

Fig. 2. IR spectra of pure polytetrahydrofuran (top), sol solution having all ingredients except polytetrahydrofuran (middle), sol-gel polytetrahydrofuran coating (bottom).
Appendix D (Continued)

Fig. 3. Scanning electron microscopic image of a 320 μm i.d. sol–gel poly-THF coated fused silica capillary used in capillary microextraction. (a) Illustrating uniform coating thickness on the inner surface of the fused silica capillary, magnification: 15,000 x. (b) Illustrating porous network of the poly-THF coating obtained by sol–gel coating technology, magnification: 10,000 x.

Fig. 2 represents three FTIR spectra representing pure poly-THF (top), sol solution having all ingredients except poly-THF (middle), sol–gel poly-THF sorbent (bottom). The bottom spectrum contains an IR band at 1045 cm⁻¹, which is characteristics of Si–O–C bonds and is indicative of the successful chemical incorporation of polytetrahydrofuran in the silica-based sol–gel network [35].

Fig. 3 represents scanning electron micrographs of a sol–gel poly-THF coated capillary at two different orientations using two different magnifications: 15,000 x (3a) and 10,000 x (3b).

From Fig. 3a, the coating thickness was estimated at 0.5 μm. As can be seen from the image, sol–gel poly-THF coating is remarkably uniform in thickness. Fig. 3b represents the surface view of the coating obtained at a magnification of 10,000 x. It reveals the underlying porous structure of the sol–gel poly-THF coating. Due to the porosities nature, the sol–gel poly-THF extraction media possesses enhanced surface area, an advantageous feature to achieve enhanced sample capacity. The porous structure also facilitates efficient mass transfer through the coating, which in turn, translates into reduced equilibrium time during extraction.

CME is a non-exhaustive extraction technique. Quantitation by CME is based on solute extraction equilibrium established between the sample solution and the coating. Therefore, the time required to reach the equilibrium is particularly important. Fig. 4 illustrates the CME kinetic profiles of two nonpolar analytes (fluoranthene and pyrene), two moderately polar analytes (heptamethylcyclopentane and dodecane) and a highly polar analyte (pentachlorophenol) extracted on a sol–gel poly-THF coated capillary. Experiments were carried out using aqueous solutions of fluoranthene (10 ppb), pyrene (10 ppb), dodecane (20 ppb), heptamethylcyclopentane (20 ppb), and pentachlorophenol (50 ppb). As can be seen, both nonpolar, moderately polar, and highly polar compounds reached respective equilibria within 30 min. This is indicative of the fast diffusion in the sol–gel poly-THF coating. Based on these experimental results, further experiments in this work were carried out using a 30 min extraction time.

Sol–gel poly-THF coated capillaries were used to extract analytes of environmental, biomedical, and ecological importance, including polycyclic aromatic hydrocarbons (PAHs), aldehydes, ketones, alcohols, and phenols. The extracted compounds were further analyzed by GC. The CME GC analysis data for PAHs, aldehydes, and ketones are presented in Table 2, and those for alcohols and phenols are provided in Table 3.

PAHs are ubiquitous environmental pollutants that present potential health hazards because of their toxic, mutagenic, and carcinogenic properties [44,45]. Because of this,
Table 2

<table>
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<tr>
<th>Chemical class of the analysis</th>
<th>Name of the analysis</th>
<th>Peak area repeatability (n = 3)</th>
<th>Retention time (tR) repeatability (n = 3)</th>
<th>Detection limits S/N = 3 (ppb)</th>
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<td>Capillary-to-capsily</td>
<td>Run-to-run</td>
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Environmental Protection Agency (EPA) has promulgated 16 unsubstituted PAHs in its list of 129 priority pollutants [46b]. Fig. 5 shows a gas chromogram representing CME-GC analysis of 5 unsubstituted polyaromatic hydrocarbons from EPA priority list. They were extracted from an aqueous solution (each at 10 ppb) by capillary microextraction using a sol-gel poly-THF coated capillary. As can be seen from the data presented in Table 2, run-to-run and capillary-to-capillary repeatability in peak area obtained in CME-GC-FID experiments was quite satisfactory. For all PAHs, the relative standard deviation (R.S.D.) values were under 6%. Moreover, parts per quadrillion level detection limits were obtained for PAHs in the CME-GC-FID using by sol-gel poly-THF microextraction capillaries. These detection limits are significantly lower than those reported by others [46b] via SPME-GC-FID (e.g. 260 ppt for pyrene) using 100 µm thick PDMS coated commercial SPME fiber.

Aldehydes and ketones (carbonyl compounds) are of increasing concern due to their potential adverse health effects and environmental prevalence [47–49]. Aldehydes and ketones can form in water by the photodegradation of dissolved natural organic matter [50]. They may also form as disinfection-by-products due to chemical reactions of chlorine and/or ozone (frequently used to disinfect water) with natural organic matter present in water [51]. Many of these by-products have been shown to be carcinogenic or carcinogen suspects [52]. This is, in part, due to the high polarity and reactivity of carbonyl compounds in water matrices [51,53,54].

![Graph](image.png)

Fig. 6. Capillary microextraction-GC analysis of aldehydes at 20 ppb concentration using poly-THF coated capillary. Extraction time, 30 min. GC analysis conditions: 10 m × 320 µm i.d. sol-gel PDMS column; splitless injection; injector temperature: initial 300 °C, final 300 °C; at a rate of 100 °C/ min; GC oven temperature programmed from 30 °C to 300 °C at a rate of 20 °C/min; helium carrier gas FID temperature 350 °C. Peaks: (1) n-nonanal; (2) heptanal; (3) undecanal; and (4) dodecanal.

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<th>Table 3</th>
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<th>R.S.D. (ppb)</th>
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Appendix D (Continued)

Fig. 7. Capillary microextraction-GC analysis of ketones at 20 ppb using poly-THF coated capillary. Extraction time, 30 min. GC analysis conditions: 10 m x 0.25 μm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 300 °C, final 500 °C, at a rate of 100 °C/min; GC oven temperature programmed from 300 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas: FID temperature 350 °C; Peaks: (1) butyrophene; (2) valerophene; (3) heptaphene; (4) heptaphenone; and (5) octaphenone.

Fig. 6 represents a gas chromatogram of a mixture of underivatized aldehydes that were extracted from an aqueous solution containing 20 ppb of each analyte. The data presented in Table 2 indicate that a sol-gel poly-THF coated capillary can extract free aldehydes from aqueous media to provide a limit of detection (LOD) which is comparable with, or lower than that achieved through derivatization [53]. For example, LOD for decanal has been reported as 200 ppt [53] (in SPME-GC-EC) on a 65 μm DVB/PDMS coating after derivatization with O-acetylation. However, in the present work, a significantly lower detection limit (25 ppm) was achieved for the same analyte using a sol-gel poly-THF coated capillary in hyphenation with GC-FID, even though ECD is often preferred for higher sensitivity compared with FID, because it is more selective for oxygenated compounds. The same trend has also been observed for other analytes. It should be pointed out that derivatization of these analytes, especially when they are present in trace concentrations, may complicate the analytical process, thus compromising quantitative accuracy.

Fig. 7 represents a gas chromatogram of a mixture of five underivatized ketones (20 ppb each) extracted from an aqueous solution. Excellent peak shapes (Fig. 7) and run-to-run and capillary-to-capillary extraction reproducibility (Table 2) are indicative of preserved separation efficiency in CME-GC analysis and versatility of the sol-gel coating procedure used to prepare the extraction capillaries and the used GC column.

Fig. 8. Capillary microextraction-GC analysis of chlorophenols using poly-THF coated capillary. Extraction time, 30 min. GC analysis conditions: 10 m x 0.25 μm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 300 °C, final 300 °C; at a rate of 100 °C/min; GC oven temperature programmed from 300 °C (hold for 5 min) to 300 °C at a rate of 20 °C/min; helium carrier gas: FID temperature 350 °C; Peaks: (1) 2-chlorophenol; (2) 2, 4-dichlorophenol; (3) 2, 4, 6-trichlorophenol; (4) 4-chloro, 3-methylphenol (100 ppb) and pentachlorophenol (50 ppb).

Chlorophenols (CPs) represent an important class of contaminants in environmental waters and soils due to their widespread use in industry, agriculture, and domestic purposes. Chlorophenols have been widely used as preservatives, pesticides, antiseptics, and disinfectants [55]. They are also used in producing dyes, plastics and pharmaceuticals. In the environment, chlorophenols may also form as a result of hydrolysis, oxidation and microbiological degradation of chlorinated pesticides. Chlorine-treated drinking water is another source of chlorophenols [56]. As a result, chlorophenols are often found in waters [57,58], soils [59], and sediments [59]. Chlorophenols are highly toxic, poorly biodegradable, carcinogenic and recalcitrant [60]. Owing to their carcinogenicity and considerable persistence, five of the chlorophenols (2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 4-chloro-3-methylphenol and pentachlorophenol) have been classified as priority pollutants by the US EPA [61]. Since chlorophenols are highly polar, it is quite difficult to extract them directly from polar aqueous media. Derivatization, pH adjustment, and/or salting-out are often used to facilitate the extraction [3]. To reduce the analytical complexity due to derivatization, HPLC is frequently used for the analysis of phenolic compounds [58]. Fig. 8 represents CME-GC analysis of five underivatized chlorophenols extracted from an aqueous medium using a sol-gel poly-THF coated capillary.

384
Appendix D (Continued)


We did not have to use derivatization, pH adjustment or salting-out effect to extract chlorophenols from aqueous medium. Still, we have achieved a lower detection limit (e.g. 18 ppt for pentachlorophenol, by CME–GC–FID) compared to other reports in the literature (1.4 pptb for the same compound, by SPME–GC–FID) [3].

Fig. 9 represents a gas chromatogram for a mixture of chlorophenols. Being highly polar compounds, chlorophenols demonstrate higher affinity for water and are usually difficult to extract them from an aqueous matrix. In the present study, these highly polar analytes were extracted from aqueous samples using sol–gel poly–THF capillaries without exploiting any derivatization, pH adjustment or salting-out effects. The presented data indicate excellent affinity of the sol–gel poly–THF coating for these highly polar analytes that are often difficult to extract from aqueous media in derivatized form using commercial coatings. Moreover, high detection sensitivity (Table 3) and excellent symmetrical peak shapes also demonstrate outstanding performance of the sol–gel poly–THF coating and excellent deactivation characteristics of the sol–gel PEG column used for GC analysis, respectively.

Finally, a mixture containing analytes from different chemical classes representing a wide polarity range was extracted from an aqueous sample using a sol–gel poly–THF coated capillary. As is revealed from the chromatogram (Fig. 10), a sol–gel poly–THF coated capillary can simultaneously extract nonpolar, moderately polar, and highly polar compounds from an aqueous matrix. This may be explained by the existence of different polarity domains [62] organic and inorganic in the sol–gel poly–THF coating.

Run-to-run repeatability and capillary-to-capillary reproducibility are two important characteristics for CME as a microextraction technique and for the sol–gel coating method used for their preparation. These parameters were evaluated through peak area relative standard deviation values obtained from experimental data involving replicate measurements carried out on the same capillary under one set of conditions (run-to-run repeatability) and on a number of sol–gel coated capillaries prepared using the same protocol (capillary-to-capillary reproducibility). For nonpolar and moderately polar analytes (Table 2), these parameters had values in the range of 2.19–7.48 and 4.35–10.31%, respectively. In the case of polar analytes (Table 3), these values were less than 7.4 and 11.8%, respectively. For a sample preparation technique, such peak area R.S.D. values are quite acceptable and may be regarded as indicative of good consistency in microextraction process as well as satisfactory reproducibility of the sol–gel method used for the preparation of the microextraction capillaries.

Additionally, the retention time (Rt) repeatability data for
sol-gel PDMS (0.04-0.10% R.S.D., Table 2) and sol-gel PEG (0.15-0.20% R.S.D., Table 3) GC columns show outstanding consistency in chromatographic performance of the in-house prepared GC columns used in this study.

In the present work, sol-gel CME-GC operation was performed manually which is not convenient from a practical point of view. For wide acceptance of the technique, the inconvenience associated with manual installation of the microextraction capillary in the GC system needs to be overcome. There are various possibilities to solve this problem, including the use of a robotic arm equipped with devices necessary for performing CMEs, desorbing the analytes, and transferring the desorbed analytes into the separation column.

In our opinion, sol-gel capillary microextraction technique described in the present manuscript has a great potential for automated operation in hyphenation with both gas- and liquid-phase separation techniques. Thanks to the capillary format of the extraction device combined with high thermal and solvent stability of the surface-bonded sol-gel extraction phase, sol-gel capillary microextraction can be expected to offer high degree of versatility in automated operation.

4. Conclusion

Novel sol-gel poly-THF coating was developed for high-performance capillary microextraction to facilitate ultra-trace analysis of polar and nonpolar organic compounds. Parts per quadrillion level detection limits were achieved using poly-THF coated microextraction capillaries in conjunction with GC-FID. To the best of our knowledge, this represents the first report on the use of sol-gel poly-THF sorbent in analytical microextraction. Sol-gel poly-THF coatings showed extraordinarily high sorption efficiency and proved to be highly effective in providing simultaneous extraction of nonpolar, moderately polar, and highly polar analytes from aqueous media. Sol-gel poly-THF coated microextraction capillaries showed excellent thermal and solvent stability, making them very suitable for hyphenation with both gas-phase and liquid-phase separation techniques, including GC, HPLC, and CEC. In CME-HPLC and CME-CEC hyphenations, sol-gel poly-THF coated microextraction capillaries have the potential to provide new levels of detection sensitivity in liquid-phase trace analysis, and to extend the analytical scope of CME to thermally labile, high molecular weight, and other types of compounds that are not amenable to GC. Further sensitivity enhancement should be possible through the use of monolithic microextraction capillaries. This could open up new possibilities in ultra-trace analysis of organic pollutants in aqueous media.

Acknowledgement

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References

Appendix D (Continued)

Appendix E

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Capillary microextraction on sol–gel dendrimer coatings

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Abstract

Sol–gel capillary microextraction (CME) is a new direction in the solventless sample preparation for the preconcentration of trace analytes, and presents significant interest in environmental, pharmaceutical, petrochemical, biomedical, agricultural and flavor, and a host of other important areas. It utilizes advanced material properties of organic-inorganic hybrid sol–gel polymers to perform efficient extraction and preconcentration of target compounds from a wide variety of matrices. In the present work, a novel benzyl-terminated dendron-based sol–gel coating was developed for CME. A detailed investigation was conducted to evaluate the performance of the newly developed sol–gel dendrimer coatings to perform solventless extraction of a wide range of polar and nonpolar analytes. The characteristic branched architecture of dendrons makes them structurally superior extraction media compared to their traditional linear polymeric counterparts. Sol–gel chemistry was used to chemically immobilize dendritic macromolecules on fused silica capillary inner surface. Due to the strong chemical bonding with the capillary inner walls, sol–gel dendron coatings showed excellent thermal and solvent stability in capillary microextraction in hyphenation with chromatographic analysis. Efficient extraction of a wide range of analytes from their aqueous solutions was accomplished using sol–gel dendron coated fused silica capillaries. Lower parts per trillion level detection limits were achieved in CME–GC for both polar and nonpolar analytes including polyaromatic hydrocarbons (PAHs), aldehydes, ketones, phenols, and alcohols.

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Keywords: Capillary microextraction; Sol–gel dendrimer coating; Sol–gel; Solid-phase microextraction; Aldehydes; Ketones; Phenols; Alcohols; Polynuclear aromatic hydrocarbons

1. Introduction

Solid-phase microextraction (SPME) \cite{1} is now considered to be a fairly mature sample preparation technique with a wide variety of applications ranging from environmental to biomedical to agricultural, and a host of other samples of scientific and industrial importance. It successfully overcomes the inherent shortcomings of conventional sample preparation methods by completely eliminating the use of organic solvents and by integrating a number of sample handling operations such as extraction, preconcentration, and sample introduction for instrumental analysis that follows the sample preparation step. In addition, SPME is a simple, inexpensive, easy-to-automate, portable, and time-efficient sample preparation technique. Due to these positive attributes, SPME has experienced an explosive growth since its inception a little over a decade ago.

SPME is based on the distribution of analytes between the sample matrix and the extracting phase coated either on the outer surface of a solid fiber (fiber-based SPME) or on the inner surface of a capillary (in-tube SPME or capillary microextraction (CME) \cite{2}). Various SPME coatings have been successfully used to accomplish solventless extraction of analytes from different matrices. These include polydimethylsiloxane (PDMS) \cite{1}, polyacrylate \cite{3}, carbowax \cite{4}, polynucleus \cite{5}, polypropylene \cite{6}, molecularly imprinted materials \cite{7,8}, carbowax/divinylbenzene (CW/DVB) \cite{9}, polydimethylsiloxane/divinylbenzene (PDMS/DVB) \cite{10}, polydimethylsiloxane/carbowax (PDMS/Carbowax) \cite{11}, carbowax/templated resin (CW/TPR) \cite{11}, sol–gel PDMS \cite{12,13}, sol–gel PEG \cite{2,14}, sol–gel crown ether \cite{15,16}. The extraction affinity is determined by various types of intermolecular and steric interactions between the analyte species and the extracting phase coating. Thus, selective extraction of analytes can be achieved based on their po-
Appendix E (Continued)

larity, hydrophobicity, chemical composition, shape/size, etc. To this end, selective extraction by SPME has often been performed based on solute polarity. However, such an approach is not very effective for samples where both polar and nonpolar contaminants are present, and both need to be analyzed. For such samples, it is very important to have a coating that can extract both polar and nonpolar compounds simultaneously with high extraction sensitivity.

Most of the SPME coatings that have been used so far are based on linear organic polymers. Linear polymers have some inherent shortcomings for their use as SPME coatings in that they possess a wide range of molecular weight distribution responsible for wide variations in their physical properties [17], their wide dispersity makes it difficult to achieve batch-to-batch reproducibility; and moreover, they are highly viscous and poorly soluble in common organic solvents, putting limitations in their effective use as SPME coatings.

Dendrimers [18,19] are highly branched macromolecules that can easily overcome many of the inherent shortcomings of linear polymers. Dendrimers are created in a step-wise fashion using simple branched monomeric units, the nature and functionality of which can be easily controlled and varied. The supramolecular properties of dendrimers can be effectively tailored by the introduction of desired functional groups at either the core [20], the peripheral surface [21], the branching unit [22], or at multiple sites within the dendrimer [23]. Dendritic macromolecules possess physical properties that, in many cases, greatly differ from their linear analogs. For example, monodisperse structure of a dendrimer is built in generations (layer by layer) around a core moiety [24]. In organic solvents, they exhibit high solubility and low viscosity compared with their linear analogs [25]. These discrepancies in physical properties are reflections of the fundamental differences in the molecular architectures of these two types of macromolecules providing drastically different numbers of terminal functional groups [26].

Dendrimers possess open and vacuous structures characterized by channels and pockets which is especially true for higher generations [27]. Unlike first and second generations, the higher generation dendrimers have greater internal surface area compared with the external surface area [28]. Therefore, third and higher generation dendrimers should be well suited for applications where large surface area (both internal and external) is a prerequisite. Because of their tree-like branched architecture, functionalized dendrons are potential candidates for novel sorbents to be used in analytical sample enrichment and separations. This opens new possibilities in achieving enhanced selectivity, sensitivity, and performance in chromatographic separations and sample preparations.

To date, in the area of analytical separations, dendrimers have been used as: (a) pseudo-stationary phases in electrospray chromatography [29–31], (b) bonded stationary phases in capillary electrophoresis [32], (c) chiral stationary phases in HPLC [33], and (d) GC stationary phases [34]. Effective immobilization of the polymeric coating on fused silica fiber or capillary inner surface is a prerequisite for the maximum utilization of its analytical potential. However, it is often difficult to achieve acceptable degree of immobilization of thick SPME coatings through conventional approaches [35]. As has been pointed out by Cühnen et al. [12], the absence of chemical bonds between the polymeric coating and the fused silica fiber surface is responsible for low thermal and solvent stability of conventionally coated SPME fibers. Low thermal stability of thick coatings leads to incomplete sample desorption and sample carryover problems. On the other hand, low solvent stability of coatings presents a significant obstacle to the hyphenation of in-tube SPME (capillary microextraction (CME)) with liquid-phase separation techniques since organic or organo-aqueous liquids are employed for the desorption of analytes from the SPME coating used for extraction [36,37].

Most of the difficulties associated with the creation and immobilization of thick stationary phase coatings on the fused silica surface can be effectively addressed by using sol-gel coating technology [2,12,38,39]. In the context of SPME, sol-gel technology provides a number of significant advantages including single step fiber capillary manufacturing process, material homogeneity at the molecular level, possibility to create hybrid sorbents by effectively combining material properties of organic and inorganic constituents, chemical bonding between the sorbent and the fused silica surface, high thermal and solvent stability of the created sorbent, and porous structure of the hybrid material. In a previous paper [34], we introduced sol-gel dendrimer stationary phase in gas chromatography. To date, we are not aware of any report on the use of sol-gel dendrimer coatings in analytical microextraction. In this paper, we describe a sol-gel approach to in situ creation of dendritic coating on the inner walls of fused silica capillaries, and application of such capillaries to solventless extraction of both polar and nonpolar trace analytes from aqueous samples.

2. Experimental

2.1. Equipment

Sol gel dendrimer CME-GC experiments were carried out on a Shimadzu model 17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector (FID) and a programmed temperature vaporizer (PTV). An in-house built gravity-fed sample dispenser was used to flow the aqueous samples through the sol-gel dendrimer-coated capillary during CME experiments. A Fisher Model G-560 Genie 2 vortex (Fisher Scientific, Pittsburgh, PA) was employed for proper mixing of different solutions. A Microcentrifuge model APO 5750 microcentrifuge (Accurate Chemical and Scientific Corporation, Westbury, NY) was used at 13,000 rpm, 15,682 x g to separate particulates from the sol solutions used for coating.
Appendix E (Continued)


2.2. Chemicals and materials

Fused-silica capillary (250 μm i.d.) with an external protective polyimide coating and two-way fused silica press-fit connectors were purchased from Polymicro Technologies Inc. (Phoenix, AZ). Triethoxysilyl-terminated dendron was synthesized in one of our laboratories following a procedure described elsewhere [34]. Hydroxy-terminated PDMS was purchased from United Chemical Technologies, Inc. (Bristol, PA). Trimethylsilyl-derivatized polyethylene glycol (Mw-1000 and 3400) were obtained from Sherwood Polymers (Huntsville, AL). Aec-naphthene, fluorene, phenanthrene, fluorenathene, pyrene, n-alkyl aldehyde, n-toluic acid, undecyl aldehyde, butylphenone, valerophenone, hexanophenone, heptanophenone, benzophenone, 2-chlorophenol, 3,4-dichlorophenol, 3,5-dimethylphenol, 2,4,6-trichlorophenol were purchased from Aldrich Chemical Co. (Milwaukee, WI) and n-decyl aldehyde was purchased from Sigma Chemical Co. (St. Louis, MO). Methanol (HPLC grade) and all borosilicate glass vials were purchased from Fisher Scientific (Pittsburgh, PA).

2.3. Preparation of sol-gel dendrimer-coated extraction capillaries

Sol-gel dendrimer microextraction capillaries were prepared by using a modified version of a previously described procedures for the preparation of sol-gel dendrimer-coated open tubular GC columns [34]. Briefly, a hydrothermally treated fused silica capillary (3 m × 250 μm i.d.) was filled with a specially designed sol solution using a gas pressure-operated filling-purging device [40]. The sol solution was prepared by dissolving methyltrimethoxysilane (MTMOS; sol-gel precursor, 5 μl), phenyl-terminated dendrimer with a triethoxysilyl containing root (sol-gel-active organic ligand, 50 mg), hexamethyldisilazane (surface deactivation reagent, 10 μl), polymethylhydroxylsloxane (PMHS; surface deactivation reagent, 25 μl), and trifluoroacetic acid (TFA; sol-gel catalyst, 50 μl) in methylene chloride solvent (900 μl). After filling, the sol solution was kept inside the capillary for 30 min to facilitate the formation of a surface-bonded sol-gel dendrimer coating. The free unbonded portion of the sol solution was then expelled from the capillary under helium pressure (50 psi) and the coated capillary was purged with helium for an hour. The sol-gel coated capillary was further conditioned in a GC oven using temperature-programmed heating from 40 to 300 °C at 1 °C/min, and holding the capillary at the final temperature for 5 h under helium purge. Before using for extraction, the coated capillary was rinsed sequentially with methylene chloride and methanol followed by drying in a stream of helium under the same temperature-programmed conditions, except that the capillary was held at the final temperature for 30 min. The capillary was further cooled down to ambient temperature and cut into 13 cm long pieces that were further used to perform microextraction.

2.4. Preparation of sol-gel PDMS- and Sol-gel PEG-coated capillary GC columns

Sol-gel PDMS- and sol-gel PEG-coated capillary GC columns were prepared according to procedures described elsewhere [38,39].

2.5. Gravity-fed sample dispenser for capillary microextraction

A gravity-fed sample dispenser was used for capillary microextraction (Fig. 1).

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Fig. 1. Schematic of a gravity-fed sample dispensing unit used in sol-gel dendrimer capillary microextraction.
Appendix E (Continued)

It was constructed by modifying a Chromatix AQ column (Kontes Glass Co., Vineland, NJ) consisting of a thick-walled Pyrex glass cylinder concentrically placed in an acrylic jacket. Deactivation of the inner surface of the glass cylinder was accomplished by treating with IMDS solution (5% v/v) solution in methylene chloride) followed by heating at 250°C for 1 h in an inert gas environment. The cylinder was then cooled to ambient temperature, thoroughly rinsed with methanol and deionized water, and dried in a flow of helium. The system was then reassembled and was ready for use as a sample delivery device in capillary microextraction.

2.6. Deactivation of glassware

All glassware used in this study was cleaned using Sparkleen detergent, thoroughly rinsed with deionized water followed by drying at 150°C for 2 h. The inner surface of the dried glassware was then treated with a 5% (v/v) solution of IMDS in methylene chloride followed by heating in an oven at 250°C for 8 h under helium flow. The glassware was then rinsed sequentially with methylene chloride and methanol and dried in the oven at 100°C for 1 h. Before use, all glassware was thoroughly rinsed with deionized water and dried at room temperature in a continuous flow of helium.

2.7. Preparation of standard sample solutions for sol-gel dendrimer CME

All stock solutions were prepared by dissolving 50 mg of each analyte in 5 ml of methanol in a 10 ml deactivated amber glass vial to obtain a concentration of 10 mg/ml. The solution was further diluted to 0.1 mg/ml in methanol. The final aqueous sample was prepared by further diluting this solution in water to achieve μg/ml to ng/ml level concentrations depending on the compound class.

2.8. Extraction of analytes on sol-gel dendrimer-coated capillaries

A 13 cm long piece of the sol-gel dendrimer-coated capillary (250 μm i.d.) was conditioned in a GC oven using a temperature program (from 40 to 300°C at 10°C/min, held at the final temperature for 30 min) carried out by simultaneously purging the capillary with helium. The conditioned capillary was vertically connected to the lower end of the gravity-fed sample dispenser using a plastic nut and a ferrule (Fig. 1). A 50 ml volume of the aqueous sample containing trace concentrations of the target analytes was added to the inner glass cylinder of the sample dispenser through the inlet located at the top. A small helium gas pressure (5 psi) was maintained in the system to assist the sample flow. The solution was allowed to pass through the capillary for 30 min. During this time, the analyte molecules were extracted by the sol-gel dendrimer coating as the sample passed through the capillary, and the system moved towards an extraction equilibrium. The capillary was further purged with helium for 1 min to remove residual water from the capillary walls.

2.9. Thermal desorption and GC analysis of the extracted analytes

Thermal desorption of the extracted analytes from the sol-gel dendrimer-coated microextraction capillary was preceded by its installation in the GC injection port, and its secured interfacings with the GC capillary column. To facilitate the installation, both the GC injection port and the oven were first cooled to 30°C, and the quartz wool was removed from the injection port glass liner. The capillary was then introduced into the GC injection port from the bottom end of the port so that its length of the capillary remained inside the injection port. A glass ferrule was used to make an air-tight connection between the capillary and the injection port. The lower end of the capillary (residing inside the GC oven) was connected to the GC capillary column with a deactivated press-fit quartz connector. The temperature of the PTV injection port was then rapidly raised from 30 to 300°C at 100°C/min to desorb the analytes from the extraction capillary into the carrier gas flow, keeping the GC oven temperature at 30°C during the whole desorption process (5 min). Under these conditions, the desorbed analytes were efficiently carried over by helium flow. As soon as the desorbed analytes reached the cooler CME capillary-GC column coupling zone residing inside the GC oven (30°C), the analytes were focused into a narrow band. To facilitate transport of the focused zone through the GC column and its separation into individual components, the GC oven temperature was further programmed as follows: from 30 to 300°C at 15°C/min with a 10 min hold at the final temperature.

3. Results and discussion

The branched architecture of dendrimers makes them promising candidates for use as extraction sorbents with distinct advantages over linear polymers used for the same purpose. The main objective of the present work was to investigate the possibility of using benzyl-terminated dendrimers as a novel extraction medium for solid-phase microextraction. This was accomplished by creating immobilized dendrimer coatings on the fused silica capillary inner surface using principles of sol-gel column chemistry.

Sol-gel column technology [38] provides an elegant single-step procedure for creating organic-inorganic hybrid stationary phase coatings (both thick and thin) inside a fused silica capillary that can be further used to perform capillary microextraction [12] or high-resolution gas chromatographic separations [38,39]. Sol-gel technology also allows the creation of hybrid coatings on the outer surface of a solid fiber [12] that can be used in conventional fiber-based SPME analysis. In both instances, the coating is chemically bonded
Appendix E (Continued)


Scheme 1. Phenyl-terminated dendrimer with a triethoxysilyl root.

to the substrate, and provides high thermal stability required for SPME–GC analysis. Thanks to chemical bonding to the substrate, sol–gel coatings also possess high solvent stability required for hyphenating SPME with liquid-phase separation techniques (e.g., HPLC, MEKC, CEC, etc.) that use organo–aqueous mobile phases.

If an organic polymer or ligand is to undergo sol–gel reaction, it has to be sol–gel-active. The dendrimer used in this study contains ethoxysilyl groups (Scheme 1) in its root, making the dendrimer molecules sol–gel-active. Details of the synthesis of sol–gel-active dendrimers can be found elsewhere [34].

The chemical ingredients used to create the sol–gel dendrimer coating is presented in Table 1. As can be seen in Table 1, methyltrimethoxysilane is the second sol–gel precursor (sol–gel-active dendrimer being the first precursor) used in the coating solution. Under the experimental conditions used, both TMCS and the triethoxysilyl moieties in the benzy1-terminated dendron (Scheme 1) can get hydrolyzed in the presence of the sol–gel catalyst, trifluoroacetic acid (TFA). The hydrolyzed precursors can then undergo polycondensations in a variety of ways to create a sol–gel network. The growing chain of the sol–gel polymer can also undergo polycondensation with hydrolyzed triethoxysilyl root of the dendron to form an organic–inorganic hybrid polymer network with the chemically incorporated dendrimers as an organic constituent. The condensation can also take place with the participation of the silanol groups on the inner surface of the fused silica capillary. The sol–gel dendrimer network developed in the vicinity of the fused silica capillary inner surface gets chemically anchored to the column walls forming a surface-bonded stationary phase film, and remain as such when the sol–gel solution is expelled after 30 min of residence inside the capillary. Both polymethylhydrosiloxane and hexamethyldisilazane (HMDS) used in the sol solution as surface deactivation reagents lack sol–gel-active sites. Therefore, it can be assumed that they rather get physically incorporated in the sol–gel network, and subsequently react with the silanol groups during the thermal conditioning step that follows the coating process. This provides a mechanism for a three-dimensional deactivation process taking place throughout the entire thickness of the sol–gel coating [38] as opposed to traditional two-dimensional deactivation process which is confined only to the capillary surface. Thus the sol–gel technology used for the coating process elegantly combines column deactivation, coating and stationary film immobilization in a simple and effective manner. A simplified scheme of the surface-bonded sol–gel dendrimer network on the fused silica capillary inner walls is presented in Scheme 2.

Table 1
Names, functions and chemical structures of sol–gel dendrimer coating solution ingredients

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<th>Name</th>
<th>Function</th>
<th>Structure</th>
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<tr>
<td>Methyltrimethoxysilane</td>
<td>Sol–gel precursor</td>
<td>CH$_3$</td>
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<tr>
<td>Phenyl-terminated dendron</td>
<td>Sol–gel precursor</td>
<td>CH$_3$O–Si–O(CH$_3$)$_2$</td>
</tr>
<tr>
<td>Phenyl-terminated dendron with a</td>
<td>Sol–gel precursor containing a</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>triethoxysilyl root</td>
<td>dendritic ligand</td>
<td>presents in Scheme 1</td>
</tr>
<tr>
<td>Trifluoroacetic acid/water 95:5</td>
<td>Catalyst</td>
<td>CH$_3$C$_2$H$_2$</td>
</tr>
<tr>
<td>(v/v)</td>
<td>Solvent</td>
<td></td>
</tr>
<tr>
<td>Hexamethyldisilazane (HMDS)</td>
<td>Deactivating reagent</td>
<td>H$_2$O–Si–NH$_2$–Si–CH$_3$</td>
</tr>
<tr>
<td>Polymethylhydrosiloxane (PMHS)</td>
<td>Deactivating reagent</td>
<td>CH$_3$CH$_3$</td>
</tr>
</tbody>
</table>

392
Appendix E (Continued)

Fig. 2 represents two scanning electron micrographs (SEM) of the inner surface of the sol–gel dendrimer-coated capillary. Remarkable uniformity in coating thickness is evident from these SEM images. The coating thickness was estimated at 0.5 μm (Fig. 2a). Moreover, sol–gel dendrimer coating possessed a roughened, porous texture (Fig. 2b) with enhanced surface area which is favorable for extraction.

Fig. 3 illustrates the CME kinetic profile of a nonpolar analyte (phenanthrene) and a polar analyte (2,4,6-trichloro-phenol) extracted on a sol–gel benzyl-terminated dendrimer-coated capillary. Extractions were carried out using aqueous samples containing 1 ppm concentration of each analyte. Both for the polar and nonpolar analytes, extraction equilibria were attained within 30 min (Fig. 3).

Based on these kinetic data, a 30 min extraction time was further used for all samples to ensure attainment of the extraction equilibrium during the extraction process.

Sol–gel dendrimer-coated capillaries were used to extract a wide variety of analytes having different polarity ranges (from nonpolar to highly polar) and of environmental, biomedical and ecological importance. Test analytes included polycyclic aromatic hydrocarbons (PAHs), aldehydes, ketones, alcohols, and phenols. The extracted compounds were further analyzed by GC. The CME–GC analysis data acquired for PAHs, aldehydes, and ketones are presented in Table 2 and those for alcohols and phenols are presented in Table 3.

Polycyclic aromatic hydrocarbons are among the most common environmental pollutants found in air, water, and soil in the USA and other industrialized countries where petroleum products are heavily used. Toxicity, mutagenicity, and carcinogenicity of these compounds in animals [41] has prompted the US Environmental Protection Agency (EPA) to place 16 unsubstituted PAHs in its list of 129 priority pollutants [42]. Fig. 4 represents a gas chromatogram of five unsubstituted polycyclic aromatic hydrocarbons (PAHs) from EPA priority pollutants list. They were extracted from an aqueous sample (each PAH at 10 ppb) using a sol–gel dendrimer-coated microextraction capillary.

As can be seen from the data presented in Table 2, the detection limits obtained for PAHs in CME–GC–FID range between 2.1 and 3.6 ppt. These values are comparable to or better than the detection limits reported in the literature for conventionally coated SPME fibers. For instance, Doong et al. [42] reported a detection limit of 0.25 ng/ml (250 ppt) for fluoranthene obtained by SPME–GC–FID on a commercial 100 μm PDMS coated fiber, which is more than two order of magnitude higher than the value 0.002 ng/ml (2 ppt) obtained in the present work using sol–gel dendrimer CME–GC–FID.

The sol–gel dendrimer-coated CME capillaries were further used to extract trace levels of aldehydes and ketones (carbonyl compounds) in aqueous samples. Carbonyl compounds play an important role in aquatic oxidation processes. In natural waters, these compounds can be produced by the photodegradation of dissolved natural organic matter [43] as well as products of microbiological processes [44]. In recent years, carbonyl compounds are receiving increased attention since they are formed as by-products in the drinking water disinfection processes. Many of these by-products have been shown to be carcinogens or carcinogen suspects [45–47].
Appendix E (Continued)

Table 2
Run-to-run and capillary-to-capillary repeatability (peak area and retention time), and detection limit data for nonpolar and moderately polar analytes in five replicate measurements by CME-GC using sol-gel dendrimer-coated microextraction capillaries.

<table>
<thead>
<tr>
<th>Chemical class of the analyte</th>
<th>Name of the analyte</th>
<th>Peak area repeatability (n = 5)</th>
<th>Retention time (t_R) repeatability (n = 10)</th>
<th>Detection limits, S/N = 3 (ppt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Capillary-to-capillary</td>
<td>Run-to-run</td>
<td>Capillary-to-capillary</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------</td>
<td>------------------------</td>
<td>-----------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Polycyclic aromatic hydrocarbons</td>
<td>Acenaphthene</td>
<td>20001</td>
<td>2.08</td>
<td>22748</td>
</tr>
<tr>
<td></td>
<td>Fluorene</td>
<td>42705</td>
<td>2.58</td>
<td>50171</td>
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<td></td>
<td>Phenanthrene</td>
<td>48103</td>
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<td>Pyrene</td>
<td>65588</td>
<td>2.46</td>
<td>63814</td>
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<td></td>
<td>Acenaphthene</td>
<td>82694</td>
<td>5.72</td>
<td>64783</td>
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<td>Aldehydes</td>
<td>Nonyl aldehyde</td>
<td>32470</td>
<td>9.26</td>
<td>32399</td>
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<tr>
<td></td>
<td>n-Tolualdehyde</td>
<td>96287</td>
<td>6.70</td>
<td>95077</td>
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<tr>
<td></td>
<td>n-Decyl aldehyde</td>
<td>174015</td>
<td>8.37</td>
<td>170101</td>
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<td></td>
<td>Undecyl aldehyde</td>
<td>197549</td>
<td>7.60</td>
<td>213576</td>
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<tr>
<td>Ketones</td>
<td>Butyrophenone</td>
<td>31512</td>
<td>3.70</td>
<td>36832</td>
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<tr>
<td></td>
<td>Valerophenone</td>
<td>69009</td>
<td>5.12</td>
<td>63127</td>
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<td></td>
<td>Hexahydroxyphene</td>
<td>97319</td>
<td>3.81</td>
<td>80990</td>
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<tr>
<td></td>
<td>Heptahydroxyphene</td>
<td>92476</td>
<td>6.45</td>
<td>96529</td>
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<tr>
<td></td>
<td>Benzophenone</td>
<td>68180</td>
<td>2.08</td>
<td>63168</td>
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</table>

pounds in water matrices often impose the need for their derivatization prior to extraction and/or detection by chromatographic techniques [48,49]. However, derivatization of these analytes, especially when present in trace concentrations, may complicate the analytical process, leading to poor accuracy and reproducibility. Fig. 5 is a gas chromatogram representing a mixture of nonderivatized aldehydes that were extracted from an aqueous solution containing 100 ppb of each analyte. The data presented in Table 2 indicates that the detection limits obtained for undervatized aldehydes in CME-GC/FID using a sol-gel dendrimer-coated microextraction capillary range between 3.5 and 19.4 ppt. These values are fairly comparable to the values reported in the literature, which were achieved through derivatiza-

Table 3
Run-to-run and capillary-to-capillary repeatability (peak area and retention time), and detection limit data for polar analytes in five replicate measurements by CME-GC using sol-gel dendrimer-coated microextraction capillaries.

<table>
<thead>
<tr>
<th>Chemical class of the analyte</th>
<th>Name of the analyte</th>
<th>Peak area repeatability (n = 5)</th>
<th>Retention time (t_R) repeatability (n = 10)</th>
<th>Detection limits, S/N = 3 (ppt)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Capillary-to-capillary</td>
<td>Run-to-run</td>
<td>Capillary-to-capillary</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean peak area (arbitrary unit)</td>
<td>R.S.D. (%)</td>
<td>Mean peak area (arbitrary unit)</td>
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<td>27943</td>
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<td>3,4-Dichlorophenol</td>
<td>38078</td>
<td>2.53</td>
<td>39409</td>
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<td>2,4,6-Trichlorophenol</td>
<td>14909</td>
<td>3.87</td>
<td>155662</td>
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<td>4-Chloro, 3-methylphenol</td>
<td>64775</td>
<td>7.37</td>
<td>63088</td>
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<tr>
<td>Alcohols</td>
<td>1-Octanol</td>
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<td>5.58</td>
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<td>1-Nonanol</td>
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<td>143432</td>
<td>4.36</td>
<td>146321</td>
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</table>
Appendix E (Continued)

Fig. 5. Capillary microextraction-GC analysis of Aldihydes at 100 ppb concentration using sol-gel dendrimer-coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 300 °C; program rate 100 °C/min, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C/min; helium carrier gas; FID temperature 350 °C. Peak identification: (1) formaldehyde, (2) m-tolualdehyde, (3) a-decylaldehyde, and (4) amicyclic aldehyde.

Fig. 6. Capillary microextraction-GC analysis of ketones at 100 ppb concentration using sol-gel dendrimer-coated microextraction capillary. Extraction conditions: 13 cm x 0.25 mm i.d. microextraction capillary; extraction time, 30 min. GC analysis conditions: 10 m x 0.25 mm i.d. sol-gel PDMS column; splitless injection; injector temperature, initial 300 °C; program rate 100 °C/min, final 300 °C; GC oven temperature programmed from 30 °C (hold for 5 min) to 300 °C at a rate of 15 °C/min; helium carrier gas; FID temperature 350 °C. Peak identification: (1) butyrophone, (2) terephnone, (3) hexanophene, (4) heptanophene, and (5) benzophene.

...from aqueous media. Derivatization, pH adjustment, and/or salting-out are often used to facilitate the extraction [3,55]. To avoid the analytical complexity due to derivatization, HPLC is frequently used for the analysis of phenolic compounds [56,57], even though it may compromise detection sensitivity. It should be pointed out that the UV detector frequency used in HPLC analysis possesses significantly lower sensitivity than the flame ionization detector commonly used in GC.

In the present study, the extracted phenols were analyzed by GC. No solute derivatization, pH adjustment or salting-out of the aqueous sample was used to extract phenolic compounds from the aqueous medium. Still, sol-gel dendrimer-coated microextraction capillaries allowed to achieve lower detection limits compared to other reported procedures in the literature. For example, in this study we achieved a detection limit of 0.26 ppb for 4-chloro, 3-methylphenol which is lower than the value (1.4 ppb) reported by Buchholz and Pawliszyn [3] obtained on an SPME fiber with 95 μm thick polyacrylate coating. Same trend was also observed for other phenolic compounds. Fig. 7 represents a gas chromatogram of five phenolic compounds obtained in a CME-GC-FID experiment using a sol-gel dendrimer microextraction capillary.

Fig. 8 represents a gas chromatogram of a mixture of alcohols (10 ppb concentration of each). Extraction of these polar compounds was conducted from aqueous samples without any derivatization, pH adjustment or salting-out effects. The presented data shows excellent affinity of the sol-gel dendrimer coating for these highly polar analytes that are often difficult to extract from aqueous media in the derivatized form using commercial coatings. Excellent symmetrical peak shapes and high detection sensitivity (Table 5) are indicative of outstanding performance and deactivation characteristics of sol-gel PEG column used for the GC analysis of the extracted alcohols.

As is revealed from the data presented in Tables 2 and 3, run-to-run and capillary-to-capillary repeatability data for peak area obtained in CME-GC-FID experiments are quite satisfactory. For most solutes, these R.S.D. values were under 5%. For the polar analytes, the R.S.D. values were higher than those for nonpolar analytes. Retention time repeatability data for PAHs, aldehydes, ketones, phenols, and alcohols were characterized by R.S.D. values of less than 0.14%.

Unique molecular architecture of dendrimers and the ability of sol-gel dendrimer coatings to provide efficient and reproducible extraction for both polar and nonpolar compounds with high detection sensitivity make these dendrimer-based materials very promising in analytical extraction technology.
396

Appendix E (Continued)

4. Conclusions

For the first time, sol-gel dendrimer-coated capillaries were used for solventless microextraction and preconcentration in chemical analysis. Both polar and nonpolar analytes were efficiently extracted from aqueous samples on the same sol-gel dendrimer capillary and provided excellent detection sensitivity. Parts per trillion level detection limits were achieved in CME-GC-FID using sol-gel dendrimer-coated extraction capillaries. It should be possible to further enhance the extraction sensitivity by using capillaries with (1) larger inner diameters (e.g., 320 and 520 μm), (2) greater lengths, (3) thicker CME coatings, and (4) sol-gel monolithic extraction beds, or any combination of these factors. Since sol-gel dendrimer extraction phase shows excellent thermal and solvent stability, sol-gel dendrimer-coated microextraction capillaries are suitable for coupling with both GC and HPLC.

Acknowledgements

The authors would like to thank Ms. Betty Loraum of USF Biology Department for technical assistance in scanning electron microscopic experiments as well as the National Science Foundation (GRN-DMR-01-96231) for partial financial support.

References


Fig. 7. Capillary microextraction-GC analysis of Phenols at 10 ppb concentration using sol-gel dendrimer-coated microextraction capillary. Extraction conditions: 33 cm × 0.25 mm i.d., microextraction capillary, extraction time, 10 min. GC analysis conditions: 10 m × 0.25 mm i.d., sol-gel PDMS column, splitless injection; injector temperature, initial 30 °C, program rate 100 °C/min, final 300 °C; GC oven temperature programmed from 50 °C (hold for 2 min) to 300 °C at a rate of 15 °C/min; helium carrier gas; FID temperature 350 °C. Peak identification: (1) 2-chlorophenol, (2) 2,4-dimethoxyphenol, (3) 3,4-dichlorophenol, (4) 2,4,6-trichlorophenol, and (5) 4-chloro, 3-methylphenol.

Fig. 8. Capillary microextraction-GC analysis of alcohols at ppb level concentrations using sol-gel dendrimer-coated microextraction capillary. Extraction conditions: 33 cm × 0.25 mm i.d., microextraction capillary, extraction time, 50 min. GC analysis conditions: 10 m × 0.25 mm i.d., sol-gel PDMS column, splitless injection; injector temperature, initial 30 °C, program rate 100 °C/min, final 300 °C; GC oven temperature programmed from 50 °C (hold for 5 min) to 300 °C at a rate of 15 °C/min; helium carrier gas; FID temperature 350 °C. Peak identification: (1) 1-octanol (500 ppb), (2) 1-propanol (1000 ppb), (3) 1-butanol (30 ppb), (4) 1-pentanol (20 ppb), and (5) 1-hexadecanol (50 ppb).
Appendix E (Continued)

Sol-Gel Poly(ethylene glycol) Stationary Phase for High-Resolution Capillary Gas Chromatography

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A sol-gel chemistry-based method was developed for the preparation of highly stable capillary gas chromatography (GC) columns with surface-bonded poly(ethylene glycol) (PEG) stationary phase. Through a single-step procedure, it concurrently provided column deactivation, stationary-phase coating, and chemical immobilization of the coated film. Sol-gel reactions were carried out within fused-silica capillaries that were filled with properly designed sol solutions containing two sol-gel precursors, two different triethoxysilyl-derivated polyethylene glycols, two sol-gel catalysts, and a deactivation reagent. Hydrolytic polycondensation reactions led to the formation of a sol-gel coating chemically bonded to the inner walls of the capillary. A number of sol-gel coated fused-silica capillary columns were prepared using polyactive PEG derivatives. These columns demonstrated many inherent advantages, the main being the strong anchoring of the coating to the capillary wall resulting from chemical bonding with the silanol groups on the fused-silica capillary inner surface. This chemical bonding yielded strongly immobilized PEG coatings with outstanding thermal stability (up to 320°C). To our knowledge, such a high thermal stability has not been achieved so far on conventionally prepared PEG GC columns. Sol-gel PEG columns provided excellent chromatographic performances: high number of theoretical plates, excellent run-to-run and column-to-column reproducibility, and pronounced selectivity for a wide range of test solutes. Using \( \alpha \)-octadecane as a test solute (\( K = 7.14 \)), an efficiency value of 3200 theoretical plates/m was obtained on a 10 m \( \times 0.25 \) mm i.d. fused-silica capillary column. Five sol-gel PEG columns provided RSD values of 1.09% for column efficiency (solute, \( \alpha \)-octadecane), 1.37% for retention factor (solute, \( \alpha \)-octadecane), and 0.9% for separation factor (for solute pair \( \alpha \) and \( \rho \)-xylene). In five replicate measurements using the same column, RSD values of less than 0.50% for the retention time and 1.36% for retention factor (\( K \)) were obtained.

Poly(ethylene glycol)s (PEGs) have long been one of the most popular polar GC stationary phases.\(^1\) PEG stationary phases are very suitable for the GC analysis of oxygenated compounds and alcohols.\(^2\) Their unique selectivity makes them an excellent choice for separating polar compounds with similar boiling points that are otherwise difficult to separate on silicone-based stationary phases.\(^2\) Despite such advantages, PEG phases are not as widely used in GC practice as the polysiloxanes phases. The deficiencies of conventional column technology\(^3\) with respect to stationary-phase immobilization for polar stationary phases such as PEGs\(^4\) coupled with unfavorable phase characteristics such as low oxidation stability,\(^5\) low thermal stability,\(^5\) phase degradation at low column temperatures,\(^6\) and high minimum working temperature for certain PEGs\(^7\) (e.g., \( \approx 70^\circ \text{C} \) for Carboxypak 20M) appear to be the major shortcomings in using PEGs as stationary phases. Most of these drawbacks can be eliminated by developing effective methods for the immobilization of PEG stationary phases, and many methods have been put forward to accomplish this.\(^8\)-\(^13\) A majority of these methods employ free radical cross-linking reactions for the immobilization of the stationary phases. Such immobilization methods have inherent drawbacks. In many cases, only partial immobilization is achieved.\(^14\) Apart from this, free

\(^{1}\) Chayen, P. J. J. Chromatogr. 1969, 327, 1-22.

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Table 1. Ingredients Used in the Sol Solution

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<th>INGREDIENTS</th>
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</tr>
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<td>Methyltrimethoxysilane (MTMOS)</td>
<td>sol-gel precursor</td>
<td>CH$_3$Si(OCH$_3$)$_3$</td>
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<td>Bis(trimethoxysilyl)benzene (BIS)</td>
<td>sol-gel precursor</td>
<td>O$_3$Si(CH$_3$)$_3$CH$_2$OCH$_3$</td>
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<td>Methoxypropylene glycol-silane</td>
<td>PEG with one sol-gel</td>
<td>H$_2$CO$_2$(CH$_2$O)$_n$(CH$_2$)$_2$N-SiN(CH$_2$)$_2$Si-OC$_3$H$_6$</td>
</tr>
<tr>
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<td>active end</td>
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<td>Poly(ethylene glycol)-bis-silane</td>
<td>PEG with two sol-gel</td>
<td>H$_3$CO$_2$(CH$_2$O)$_n$(CH$_2$)$_2$N-SiN(CH$_2$)$_2$Si-OC$_3$H$_6$</td>
</tr>
<tr>
<td>(PEG 2)</td>
<td>active ends</td>
<td></td>
</tr>
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<td>PEG polymer</td>
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<td>1,1,1,3,3-hexamethyldisilazane</td>
<td>deactivating reagent</td>
<td>H$_3$Si(SiH)$_3$</td>
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<td>(HMDS)</td>
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</tr>
<tr>
<td>Trifluoroacetic acid (TFA)</td>
<td>Catalyst</td>
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<tr>
<td>Ammonium Fluoride</td>
<td>Catalyst</td>
<td>NH$_4$F</td>
</tr>
</tbody>
</table>

Scheme 1. Hydrolysis of the Alkoxy silane-Containing, Sol-Gel-Active Ingredients of the Sol Solution

\[
\begin{align*}
\text{H}_2\text{CO}-\text{Si}-\text{OCH}_3 + 3 \text{H}_2\text{O} & \xrightarrow{\text{Catalyst}} \text{HO}-\text{Si}-\text{OH} + 3 \text{CH}_3\text{OH} \\
\text{methyltrimethoxysilane} & \\
\text{H}_2\text{CO}-\text{Si}-\overset{(\text{CH}_2)}{(\text{CH}_2)}\overset{(\text{CH}_2)}{(\text{CH}_2)}\text{Si}-\text{OCH}_3 & + 6 \text{H}_2\text{O} \xrightarrow{\text{Catalyst}} \text{HO}-\overset{(\text{CH}_2)}{(\text{CH}_2)}\overset{(\text{CH}_2)}{(\text{CH}_2)}\text{Si}-\text{OH} + 6 \text{CH}_3\text{OH} \\
\text{bis(trimethoxysilyl)benzene} & \\
\text{R'}-\text{PEG}-\overset{(\text{Si})}{(\text{OC}_3\text{H}_5)} & + 3 \text{H}_2\text{O} \xrightarrow{\text{Catalyst}} \text{R'}-\text{PEG}-\overset{(\text{Si})}{(\text{OH})} + 3 \text{CH}_3\text{OH} \\
\text{trioxy silane-terminated poly(ethylene glycol)} & \\
\text{where, R'} & \text{a}\text{is a functional group; } A \text{a}\text{is a spacer.} & \\
\text{poly(ethylene glycol) with trioxy silane derivatized ends} &
\end{align*}
\]

radical reactions tend to yield varying degrees of cross-linking in columns prepared by using the same stationary phase, making it difficult to achieve good columns to column reproducibility.

Considering the above-mentioned problems and drawbacks of current GC column technology for PEG stationary phases, the goal of this research was to develop an effective method for the preparation of immobilized PEG columns free from the above-mentioned drawbacks. The new method would provide enhanced thermal stability, efficiency, and chromatographic selectivity. These objectives were successfully accomplished by using sol-
Appendix F (Continued)

Scheme 2. Formation of Sol–Gel PEG Polymer Network Due to Polycondensation of the Silanol-Containing Moieties Formed as a Result of Hydrolysis of the Sol–Gel-Active Ingredients in the Sol Solution

**(Growing Sol–Gel PEG Polymer)**

gel technology to chemically immobilize PEG stationary phases. Sol–gel coating technology described in this paper was specifically designed for PEG stationary phases that were efficiently incorporated into a sol–gel network. During the course of sol–gel reactions, a portion of the created organic–inorganic hybrid sol–gel PEG polymer became chemically bonded to the fused silica capillary inner walls producing a surface-bonded stationary-phase coating, with inherently effective chemical immobilization, outstanding thermal and solvent stability, and excellent gas chromatographic performance.

**EXPERIMENTAL SECTION**

Materials and Chemicals. Fused-silica capillary (250 μm i.d.) was obtained from Polymicro Technologies Inc. (Phoenix, AZ). Polypyrrole microcentrifuge tubes (1.5 mL) and HPLC grade methylene chloride were purchased from Fisher Scientific (Pittsburgh, PA). Methyltrimethoxysilane (MTMOS, 98%), 1,1,1,3,3,3-hexamethyldisilazane (HMDS, 99.9%), trifluoroacetic acid (TFA, 99%), and ammonium fluoride (99%, 9+5%) were purchased from Aldrich (Milwaukee, WI). Bis(trimethoxysilyl)ethyl)benzene was purchased from Celenst Inc. (Hulltown, PA). Methoxypoly(ethylene glycol)–silane (PEG1) and poly(ethylene glycol)–benzaldehyde (PEG2) were purchased from Sherwater Polymers, Inc. (Huntsville, AL).

Equipment. Chromatographic experiments were carried out on a Shimadzu model 17A capillary GC system. A homemade capillary filling/purging device was used for filling the capillary with the coating sol solution under helium pressure. A Scientific Products model S8223 Vertex GC system was used for thorough mixing of various solution ingredients. A Microcentrifuge model APO 5700 centrifuge was used to separate the sol solution from the precipitate, if any. A Barnstead model 40741 Nanopure deionized water system was used to obtain 17.8 MΩ water.

**Hydrothermal Treatment.** Before coating, the fused-silica capillary was hydrothermally treated. For this, first the fused-silica capillary was sequentially rinsed with methylene chloride, methanol, and deionized water followed by a brief helium purge for 5 min. Both ends of the capillary were then sealed using an oxyacetylene torch, and the column was further conditioned in a GC oven by programming the temperature from 40 to 230 °C at a rate of 4 °C min⁻¹ with a final temperature hold time of 120 min. After this thermal treatment, the column was cooled to room temperature and the sealed ends were cut open using an aluminum wafer. Following this, the column was installed in a GC oven and again thermally conditioned under helium purge using the same temperature program as described above. At this point, the capillary was ready to be coated with the sol–gel stationary phase.

**Column Preparation.** In the present work, our previously reported general procedure for preparing open tubular sol–gel columns was carefully modified to meet the specific requirements for the preparation of sol–gel PEG GC columns. The sol solution ingredients used to prepare sol–gel PEG columns are listed in Table 1.

The sol–gel coating solution was prepared as follows: 35 mg of PEG 1 and 15 mg of PEG2 (sol–gel active organic ligands) were dissolved in 600 μL of methylene chloride (solvent) contained

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Appendix F (Continued)

Scheme 3. Chemical Bonding of the Growing Sol-Gel PEG Polymer to the Inner Walls of the Fused-Silica Capillary

in a polypropylene microcentrifuge vial. A Scientific Products model S8223 Vortex shaker aided the dissolution process (5 min). Then 5 μL of MTMOS (precursor), 10 μL of bistrimethoxysilyl-ethylbenzene (precursor), and 5 μL of HMDS (activating reagent) were sequentially added to the microcentrifuge vial and thoroughly mixed for 5 min to obtain a homogeneous solution. After this, 50 μL of 69% TFA (acid catalyst containing 3% water) was added to the solution and thoroughly mixed. After 10 min, a 28 μL volume of NH₄F solution (20 mg/mL in methanol) was introduced into the vial. The volume of the solution was made up to 1000 μL by adding the required amount of methylene chloride, and the mixture was thoroughly vortexed. The resulting solution was centrifuged at 13,000 rpm (15,682 g) for 5 min. The precipitate at the bottom of the vial, if any, was discarded, and the top clear sol solution was used to fill the hydrothermally treated fused-silica capillary using a helium pressure of 50 psi. After a set period of incubation at room temperature (10–20 min), the solution was expelled from the capillary under the same helium pressure and the capillary was subsequently purged with helium at 50 psi for an additional 60 min. This was followed by temperature-programmed heating in a GC oven from 40 to 150 °C at 2 °C min⁻¹, with a hold time of 50 min at 150 °C and then from 150 to 280 °C at 6 °C min⁻¹, holding it at 280 °C for 120 min. Keeping the temperature programming rate at 6 °C min⁻¹, the column was further conditioned in small steps, holding the column for 120 min at each of the following final temperatures: 300, 320, and 340 °C. The column was then rinsed with 2 mL of methylene chloride and conditioned again from 40 to 320 °C at 6 °C min⁻¹. While conditioning, the column was purged with helium at 1 mL min⁻¹.

RESULTS AND DISCUSSION

Chemical Reactions in Sol-Gel PEG Column Technology.

An understanding of the basic chemical reactions that take place inside the capillary column will provide a clear picture of how the stationary phase is chemically immobilized through sol–gel chemistry. The sol–gel process starts with the catalytic hydrolysis (Scheme 1) of the sol–gel precursors and other sol–gel-active agents in the coating solution, followed by polycondensation of the hydrolyzed products into a sol–gel network.25-28 Scheme 1 illustrates the hydrolysis of the sol–gel precursors (MTMOS and BIS) and the two triethoxysilyl-terminated polyethylene glycols (PEG1 and PEG2).

In the sol solution, these hydrolyzed products then undergo polycondensation reactions illustrated in Scheme 2.

It should be noted that the hydrolysis and polycondensation reactions can take place concurrently, and the reaction sequences in Schemes 1 and 2 are being used only for a vivid illustration of chemical reactions involved in the sol–gel process. Also, polycondensation reactions may take place in an order different from that presented in Scheme 2 (e.g., between PEG1 and PEG2, PEG1 and the precursor, PEG2 and the precursor, PEG1 and BIS, or PEG2 and BIS, and so on). The above reactions take place in a random fashion and result in a growing three-dimensional PEG-based sol–gel polymer network. Since both PEG1 and BIS have


Appendix F (Continued)

Scheme 4. Derivatization of the Residual Silanol Groups in the Sol–Gel PEG Coating with HMDS during Postcoating Thermal Treatment Resulting in Column Deactivation

Sol–gel active functional groups at both ends of their molecular structures, they are likely to get incorporated in the polymer network structure. PEG1, on the other hand, having only one sol–gel active end, is likely to form a pendant side chain in the stationary-phase structure as shown in Scheme 3.

Portions of the sol–gel PEG polymer network growing in the vicinity of the fused-silica capillary inner walls get bonded to it due to condensation with the silanol groups residing on the capillary inner surface. After an appropriate length of incubation, the unbonded bulk portion of the sol solution is expelled from the capillary, leaving behind a surface-bonded sol–gel PEG stationary phase film on the inner walls of the fused-silica capillary (Scheme 3). This chemical bonding provides strong immobilization of the stationary phase, which in turn increases its thermal and solvent stability. The thickness of this coating can be varied by manipulating the incubation residence time of the sol solution or by adjusting the concentrations of various ingredients in the sol–gel coating solution.

In the presented sol–gel approach, the PEG1 and PEG2 played dual roles. First, they served as the organic components for the sol–gel organic–inorganic hybrid stationary phase. Second, their chemical bonding to silanol groups resulted in column deactivation. HMDS in the sol solution served not only as a reagent for chemical derivatization of the residual silanol groups (Scheme 1) in the sol–gel columns to ensure added column inertness but also as a precursor for in situ generation of ammonia, which is known to play a significant role in determining the pore and surface characteristics of sol–gel materials.

The use of MTMOS as a precursor aimed at eliminating the problems inherently associated with the widely used tertialkoxysilane precursors. Sol–gel coatings prepared with the use of tertialkoxysilane precursors show a tendency to crack during the thermal-conditioning step, which may serve as a major hindrance to providing a uniform and highly homogeneous surface coating. The use of an alkyl or aryl derivative of a tertialkoxysilane precursor can effectively overcome this problem. This is due to the relatively open structure and greater flexibility of sol–gel polymeric network resulting from the alkyl- or aryl-derivatized precursors. When subjected to thermal conditioning, stationary phases consisting of such sol–gel polymeric networks can efficiently release the capillary thrust generated on the pore walls during evaporation of the solvent residing in the stationary-phase pores.

The purpose of using a second sol–gel precursor (BIS) was to provide chemical incorporation of a phenyl ring into the stationary-phase structure during the sol–gel process. Due to the presence of a phenyl ring in the polymeric backbone, the created stationary phase can be expected to possess added thermal stability since the steric hindrance offered by the phenyl groups...
Appendix F (Continued)

![Image](image.png)

**Figure 2.** Van Deemter plot for a sol-gel coated PEG column. Conditions: 10 m x 0.25 mm i.d., fused silica capillary column, split injection (100:1), 300 °C; helium carrier gas, FID, 350 °C; decanol (k = 6.4) and octadecane (k = 9.3) as test solutes; isothermal runs at 125 °C.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Before rinsing</th>
<th>After rinsing</th>
<th>Change in k due to rinsing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,6-dimethylaniline</td>
<td>0.13</td>
<td>0.06</td>
<td>16.6%</td>
</tr>
<tr>
<td>2,4-dimethylphenol</td>
<td>0.12</td>
<td>0.09</td>
<td>19.6%</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.11</td>
<td>0.09</td>
<td>19.4%</td>
</tr>
<tr>
<td>Hexadecane</td>
<td>0.08</td>
<td>0.07</td>
<td>12.5%</td>
</tr>
<tr>
<td>Hexane</td>
<td>0.07</td>
<td>0.06</td>
<td>14.3%</td>
</tr>
</tbody>
</table>

*Experimental conditions: column, 10 m x 0.25 mm i.d., fused silica capillary column; stationary phase, sol-gel coated PEG carrier gas, helium injection, split (100:1), 350 °C; detector, FID, 350 °C; temperature programming, 15 °C, constant.*

![Image](image.png)

**Figure 1.** Scanning electron microscopic images of a sol-gel PEG coated 250 μm-i.d., fused silica capillary. (A) Cross-sectional view, magnification 5000 x; (B) surface view of the fine structures on the sol-gel PEG coating, magnification, 20000 x.

serves as an impediment to undesirable rearrangement reactions that could lead to stationary-phase degradation through formation of soluble cyclic compounds responsible for column bleeding. However, these phenyl groups could also potentially increase the rigidity of the stationary phase. Chromatographic properties (e.g., separation efficiency, mass transfer properties, etc.) of the stationary phase might get negatively affected, if the structure is too rigid. Therefore, it is important to maintain a delicate balance between structural rigidity of the stationary phase and its chromatographic performance. To avoid the sol–gel stationary phase becoming too rigid, we selected BIS in which a spacer consisting of two methylene groups is attached to the phenyl group on each side, providing a reasonable degree of flexibility to the sol–gel polymer.

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Appendix F (Continued)

![Graph](image)

Figure 3. GC separation of Grob test mixture on a sol–gel coated PEG column. Conditions: column, 10 m x 0.25 mm I.D. fused-silica capillary column; stationary phase, sol–gel PEG; injection, split (100:1, 300 °C); detector, FID 350 °C; Temperature programming, 40 °C at 6 °C min⁻¹. Peaks: (1) n-decane, (2) n-undecane, (3) 1-octanol, (4) 2,3-butanediol, (5) 1-octanol, (6) methyl decanoate, (7) diethylene glycol, (8) methyl undecanoate, (9) methyl decanoate, (10) 2,6-dimethylphenol, (11) 2,6-dimethylphenol, and (12) 2-ethylhexanoic acid.

<table>
<thead>
<tr>
<th>Table 4. Column-to-Column Reproducibility Data for Efficiency (N), Retention Factor (k), and Separation Factor (a) Obtained on Five Sol–Gel PEG Columns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column efficiency</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Column 1</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
</tbody>
</table>

²Conditions for column efficiency (N) and retention factor (k): column, 10 m x 0.25 mm I.D. fused-silica capillary column; stationary phase, sol–gel PEG; injection, split (100:1, 300 °C); detector, FID, 350 °C; temperature programming, 40 °C constant. Solvent for measuring N and k was cyclohexane. Conditions for separation factor (a²): column, 10 m x 0.25 mm I.D. fused-silica capillary column; stationary phase, sol–gel PEG; injection, split (100:1, 300 °C); detector, FID; 350 °C; temperature programming, 40 °C constant. *For solvent pair of p-xylene.

It is known that sol–gel active organic polymers by themselves may act as deactivating agents.²⁴ PEG-1 and PEG-2 not only served as the sol–gel active organic liquids but also took part in the column deactivation through their condensation with silanol groups responsible for undesirable adsorption activity of GC columns. Such a deactivation mechanism was complementary to the deactivation obtained through the use of HMDS.

TEA (containing 5% water) was used as a sol–gel catalyst. In place of the more conventional catalysts such as strong inorganic acids or strong bases, the selection of TFA as a sol–gel catalyst was based on the findings by Sharp²⁵ that carboxylic acids with pKa values less than 4 were able to provide significantly higher reaction rates than strong inorganic acids or organic acids with a pKa value greater than 4. Thus, the use of TFA, with a pKa value of 0.3,²⁶ provided enhanced gelation speed resulting in a decreased column fabrication time.

The surface characteristics of the created sol–gel PEG coatings were studied by scanning electron microscopy (SEM) and are presented in Figure 1.

Figure 1a gives a vivid illustration of the sol–gel stationary-phase film created on the fused-silica capillary inner surface. Here, the SEM image (magnification of 5000x) shows that the created sol–gel PEG coating is highly uniform, having a thickness of ~1.0 μm. The SEM image in Figure 1b (magnification 20000x), illustrates the fine structures of the sol–gel coated surface. As can be seen in Figure 1b, sol–gel PEG coating possesses enhanced surface area due to microroughening created by these

Table 5. Column-to-Column Reproducibility for Retention Factor (k) and Separation Factor (α) on Five Sol–Gel PEG Columns

<table>
<thead>
<tr>
<th>Solvent</th>
<th>T (°C)</th>
<th>k</th>
<th>α</th>
<th>k RSD (%)</th>
<th>α RSD (%)</th>
<th>k α RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>decanol</td>
<td>100</td>
<td>1.31</td>
<td>1.50</td>
<td>1.51</td>
<td>0.65</td>
<td>1.68</td>
</tr>
<tr>
<td>2,5-dimethoxybenzaldehyde</td>
<td>100</td>
<td>1.64</td>
<td>1.94</td>
<td>1.94</td>
<td>0.65</td>
<td>1.68</td>
</tr>
<tr>
<td>phenothiazine</td>
<td>230</td>
<td>1.28</td>
<td>1.58</td>
<td>1.28</td>
<td>0.55</td>
<td>1.12</td>
</tr>
<tr>
<td>anthracene</td>
<td>230</td>
<td>1.46</td>
<td>1.74</td>
<td>1.44</td>
<td>0.55</td>
<td>1.12</td>
</tr>
<tr>
<td>cyclohexane</td>
<td>40</td>
<td>3.60</td>
<td>3.98</td>
<td>3.68</td>
<td>0.32</td>
<td>1.32</td>
</tr>
<tr>
<td>octane</td>
<td>40</td>
<td>4.80</td>
<td>5.42</td>
<td>4.89</td>
<td>0.82</td>
<td>1.68</td>
</tr>
</tbody>
</table>

*Conditions: column, 10 m x 0.25 mm i.d. fused-silica capillary column; stationary phase, sol–gel PEG injection, split (100:1, 300 °C); detector, FID, 350 °C.*

decanol and 0.13 mm for octane. This corresponded to 3200 and 3000 theoretical plates/m for decanol and octane, respectively. The right-hand side of the Van Deemter plot characterizes the mass-transfer process in the column and is represented by the coefficient C in the Van Deemter–Gold equation. This C value can be calculated from the ascending right-hand part of the plot. The slope on this part of the plot is quite small and has a value of ~5.0 x 10⁻⁴ s⁻¹. This value compares favorably with the values commonly obtained for open-tubular columns and indicates the rapid transfer of the solute molecules between the two phases in sol–gel PEG columns. This efficient mass transfer points to the flexibility of the sol–gel surface coating. It should be mentioned that sol–gel column technology does not involve free radical cross-linking reactions, which are normally employed in conventional column technology for immobilizing the stationary phase. Free radical cross-linking reactions often lead to reduced stationary phase flexibility which, in many cases, negatively affects the mass-transfer characteristics of the column. A small C value indicates that the column efficiency will not be greatly affected even if high carrier gas flow rates are used. This makes sol–gel PEG columns well suited for fast analysis.

**Chromatographic Characteristics of Sol–Gel PEG Stationary Phases in GC.** The prepared sol–gel PEG columns showed excellent solvent stability. They were mixed with methyl chloride after sol–gel coating and conditioning steps were completed. The solvent stability was then tested by comparing retention factor (k) values of four different solutes determined in replicate before and after rinsing the column with the solvent (Table 2).

For all polar solutes the average (k) values differed by less than 5% before and after rinsing the column operated under the same experimental conditions. For the nonpolar solute, diocane, this difference was 0.58%, which is significantly higher than those for the polar solutes. This may be explained by the removal of physically incorporated nonpolar materials from the coating during rinsing and by the poor solubility of the nonpolar solute like diocane in PEG. The outstanding solvent stability of sol–gel PEG columns is due to the fact that the sol–gel approach to column technology provides chemical bonding of the stationary phase with the silica surface. Strong chemical immobilization of the stationary phase

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Appendix F (Continued)

to the silica surface is also evident from comparison of pre- and post-injection retention times for Grob’s test mixture solutes belonging to a wide range of chemical classes (Table 3).

Data presented in Table 3 suggest that the solute retention times do not change appreciably (if the manual injections are taken into consideration). Strong chemical immobilization and structural integrity are important attributes of the prepared sol–gel PEG stationary phase that contributed to this excellent retention time reproducibility.

Strong chemical immobilization is also responsible for enhanced thermal stability of the prepared sol–gel PEG columns that can be operated at elevated temperatures up to 320 °C and higher. This operating temperature clearly surpasses the maximum operating temperatures recommended for conventionally coated PEG phases (200–275 °C). Figure 3 represents one such chromatogram illustrating separation of the Grob test mixture on a sol–gel PEG column.

The baseline of the chromatogram showed very low bleeding even at a temperature of 320 °C. To our knowledge, such a high thermal stability has not been achieved so far for PEG columns. Thus, sol–gel column technology provided an effective solution to a difficult problem related to the immobilization of PEG-based stationary phases and offered outstanding thermal stability.

Reproducibility Characteristics of Sol–Gel Coated PEG Columns. An important feature of any newly developed technique is its reproducibility. The reproducibility characteristics greatly influence the reliability and hence the viability of the developed method. In column technology, an important reproducibility criterion is the column-to-column reproducibility. To evaluate this, five sol–gel PEG columns were prepared under identical conditions. Table 4 gives the column efficiency (A), retention factor (k), and separation factor (α) data for selected test solutes and RSD values associated with them.

The RSD values were 1.08% for k, 1.77% for α, and 6.9% for α, respectively. These low RSD values bear testimony to the fact that the developed sol–gel method for the preparation of PEG GC columns is indeed reliable and highly reproducible. Column-to-column repeatability was further examined by injecting isomers such as cis- and trans-4-methylcyclohexanol, phenanthrene and anthracene, and oxyylene and p-xylene on the five sol–gel PEG coated columns. These isomeric solutes were run isothermally at column temperatures appropriate for each pair of solutes. The α value for each solute pair was calculated from the experimentally determined k values of the two solutes in the pair. For the five columns, the calculated RSD values for k and α are presented in Table 5.

The RSD values for retention and separation factors on these five sol–gel PEG columns were less than 1.2 and 1.5%, respectively.
Such low RSD values are indicative of excellent column-to-column reproducibility in terms of both column fabrication procedure and stationary-phase selectivity.

Another important reproducibility parameter is the run-to-run repeatability, which reflects upon the robustness of the stationary-phase coatings to preserve the retention, selectivity, efficiency, and other chromatographic parameters that might be monitored over the runs to gauge the column performance. To evaluate the run-to-run repeatability, Grob mixture was injected in five replicate GC runs. RSD values of less than 0.5% in retention times were obtained for the Grob mixture. These low RSDs can again be attributed to the structural and functional integrity of the sol–gel PEG stationary phases in GC operations.

Deactivation in Sol–Gel Coated PEG GC Columns. Proper deactivation of the polar adsorption sites in the column is a critically important task in GC column technology that greatly influences the separation efficiency and column performance. In general, inadequate column deactivation often results in undesirable effects in chromatographic separations such as peak tailing, reduced peak height, and irreversible solute adsorption, especially for polar analytes. The principle reason for the above undesirable effects is the presence of surface silanol groups, which strongly interact (through hydrogen bonding, dipole–dipole, and dipole–induced dipole interactions) with the polar analytes and ultimately lead to the detrimental effects mentioned above. The deactivation quality of the sol–gel coated PEG columns was evaluated by using free carboxylic acids, amines, and amino alcohols as test solutes. It is well known that separation of free carboxylic acids or aliphatic amines is a challenging task in gas chromatography, more so if they are to be separated on the same column, without adding any stationary-phase modifier. Thus, these two classes of analytes provide a very stringent test of column deactivation. Carboxylic acids are very polar and tend to interact with the residual silanol and other adsorptive sites, resulting in broad and tailing peaks. Similarly, acid–base interactions of the basic analyte with the silanol groups cause them to tail, and in some cases, such analytes may even be irreversibly sorbed by the stationary phase in a poorly deactivated column. Figure 4 represents the gas chromatographic separation of free carboxylic acids, and Figure 5 represents the gas chromatographic separation of aliphatic/aminic amines and amino alcohols (Figure 5A) and aromatic amines (Figure 5B).

Symmetrical peaks for free carboxylic acids, aliphatic and aromatic amines, and amino alcohols obtained on the prepared...
sol–gel PEG column are indicative of high quality of column deactivation. Mention should be made of the fact that no special deactivation steps were employed, nor was any surface/phase modification carried out on the sol–gel PEG columns used to perform these separations. This high quality of deactivation achieved in sol–gel column technology can be attributed to a number of factors inherent in the sol–gel process itself. First, during the sol–gel process, some of the surface silanol groups are derivatized by the growing sol–gel network and others are buried under it. Second, during the coating process, HMDS (an ingredient of the coating solution) becomes physically included in the sol–gel PEG stationary phase and deactivates the column by reacting with the silanol groups mainly during thermal conditioning that follows the column coating process. Third, in the sol–gel approach, deactivation of the column begins with the sol–gel process used to in situ create the stationary phase film. Here the sol–gel–active organic ingredients (PEG 1, PEG 2, BIS) can also take part in the column deactivation. It should be emphasized that, in the sol–gel approach, column deactivation takes place not only on the capillary inner surface but also throughout the entire volume of the stationary-phase film. It is a three-dimensional deactivation process rather than a two-dimensional process inherent in conventional column technology.

In probing the column deactivation quality, it is very important to consider the individual concentration of the analytes in the test mixture. If the analyte concentration is too high, then the adsorptive sites may become saturated with the analytes and one may get good looking peak shapes even for highly polar compounds. Concentration as low as 2 pg/μl of a free carboxylic acid (acetic acid), corresponding to an injected amount of 2 ng, was introduced into a sol–gel coated Carbowax column and perfect peak shape was obtained (Figure 6).

Sol–gel coated PEG columns showed excellent chromatographic performance for a wide range of analytes, evidenced by the following examples of GC separations. Figure 7 represents GC separation of a mixture of aldehydes that often produce broad and trailing peaks on PEG phases. The sharp and symmetrical peaks obtained for aldehydes on the sol–gel PEG columns once again point to the excellent quality of deactivation inherent in the sol–gel column technology (Figure 7).

Figure 8 likewise exemplifies the separation of ketones.

Alcohols are another class of compounds prone to tailing because of their polar interactions with the residual silanol groups. An insufficiently deactivated column will cause peak tailing or reduced peak heights for these polar compounds. As can be seen

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure8.png}
\caption{GC separation of ketones on a sol–gel PEG column. Conditions: column, 10 m × 0.25 mm i.d. fused-silica capillary column; stationary phase, sol–gel PEG; injection, split (100:1); detector, FID at 350 °C. Temperature programming: from 70 °C at 6 °C min⁻¹. Peaks: (1) 2-butanol, (2) 2-pentanol, (3) 3-pentanol, (4) 3-hexanol, (5) 3-heptanol, and (6) 4-heptanol.
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure9.png}
\caption{GC separation of alcohols on a sol–gel PEG column. Conditions: column, 10 m × 0.25 mm i.d. fused-silica capillary column; stationary phase, sol–gel PEG; injection, split (100:1); detector, FID at 350 °C. Temperature programming: from 70 °C at 6 °C min⁻¹. Peaks: (1) 2-butanol, (2) 2-pentanol, (3) 3-pentanol, (4) 3-hexanol, (5) 3-heptanol, and (6) 4-heptanol.
\end{figure}
from Figure 9, sol-gel PEG columns produced good peak shapes for alcohols, minimizing the above-mentioned adverse effects.

Phenols represent an environmentally important class of compounds that are acidic in nature. Figure 10 gives the high efficiency separation of dimethylphenol isomers. Baseline resolution of dimethylphenol isomers demonstrates high selectivity of the newly developed sol-gel PEG stationary phase toward these isomeric polar compounds.

Figure 11 A is the separation of anthracene and phenanthrene, which are baseline resolved on a 10 m long sol-gel PEG column. This indicates the ability of the PEG stationary phases to recognize structural differences in molecules. Figure 11B represents the GC separation of o- and p-xylene. The o- and p-xylene pair was more than baseline resolved with an Rs value of 2.80.

**Polarity Of Sol-Gel Coated PEG Columns.** Polarity of sol-gel coated PEG columns was evaluated by calculating the McReynolds constants.38 The calculated values in Table 6 show that PEG coated columns have an overall polarity value of 1917, as compared to a value of 2353 for silica coated PEG columns.39

This difference in polarity can be attributed to the presence of nonpolar moieties in the chemical structure of the used sol-gel precursors that ultimately become incorporated in the coating structure. A higher polarity could be obtained if the precursors in the sol solution are decreased or precursors containing no nonpolar moieties were used.

**CONCLUSION**

A sol-gel chemistry-based novel approach to prepare high efficiency columns with immobilized poly(ethylene glycol) stationary phases is presented for capillary gas chromatography. The sol-gel approach successfully addresses and eliminates many of the drawbacks associated with conventional column technology for PEG stationary phases. Such shortcomings include difficulties associated with the immobilization of these polar stationary phases, their low thermal stability, and low reproducibility in column preparation and performance. Sol-gel technology facilitates effective chemical bonding between the sol-gel PEG

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**Figure 10.** GC separation of dimethylphenol isomers on a sol-gel PEG column. Conditions: column, 10 m x 0.25 mm i.d., fused-silica capillary column; stationary phase, sol-gel PEG; injection, split (100:1, 500°C); detector, FID, 350°C; Temperature programming: from 65°C at 6°C min⁻¹. Peaks: (1) 2,6-dimethylphenol; (2) 2,5-dimethylphenol; (3) 3,5-dimethylphenol; and (4) 3,4-dimethylphenol.

**Figure 11.** GC separation of isomeric compounds on a sol-gel PEG column. Conditions: column, 10 m x 0.25 mm i.d., fused-silica capillary column; stationary phase, sol-gel PEG; injection, split (100:1, 300°C); detector, FID, 350°C; (A) Temperature, 230°C; Peaks: (1) phenanthrene and (2) anthracene; (B) Temperature, 40°C. Peaks: (1) p-xylene and (2) o-xylene.

**Table 6. McReynolds Constants for Sol-Gel PEG Column**

<table>
<thead>
<tr>
<th>Probe</th>
<th>Benzene</th>
<th>1-Butanol</th>
<th>2-Pentanone</th>
<th>4-Methylacetophenone</th>
<th>Pyridine</th>
</tr>
</thead>
<tbody>
<tr>
<td>l (deg/cm²)</td>
<td>818.20</td>
<td>1500.73</td>
<td>948.34</td>
<td>1188.25</td>
<td>1144.91</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>l for sol-gel PEG</th>
<th>673</th>
<th>590</th>
<th>652</th>
<th>559</th>
</tr>
</thead>
<tbody>
<tr>
<td>l (deg/cm²)</td>
<td>195 (X)</td>
<td>410 (Y)</td>
<td>318 (Z)</td>
<td>486 (S)</td>
</tr>
</tbody>
</table>

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Appendix F (Continued)

stationary phase film and the fused-silica capillary inner surface, leading to excellent stationary-phase immobilization and thermal stability up to 320 °C. Column efficiencies on the order of 3200 theoretical plates/m can be routinely obtained on 250-μm ID, sol–gel PEG capillary columns in GC. Sol–gel PEG stationary phase can be used for efficient separation of difficult-to-chromatograph analytes such as free carboxylic acids and aliphatic amines on the same column. The presented results bear strong evidence that sol–gel PEG columns have the potential to offer a new level of performance in GC separation of polar analytes.

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Appendix G

Sol–Gel Capillary Microextraction

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Sol–gel capillary microextraction (sol–gel CME) is introduced as a viable solventless extraction technique for the preconcentration of trace analytes. To our knowledge, this is the first report on the use of sol–gel-coated capillaries in analytical microextraction. Sol–gel-coated capillaries were employed for the extraction and preconcentration of a wide variety of polar and nonpolar analytes. Two different types of sol–gel coatings were used for extraction: sol–gel poly(dimethylsiloxane) (PDMS) and sol–gel poly(ethylene glycol) (PEG). An in-house-assembled gravity-fed sample dispensing unit was used to perform the extraction. The analysis of the extracted analytes was performed by gas chromatography (GC). The extracted analytes were transferred to the GC column via thermal desorption. For this, the capillary with the extracted analytes was connected to the inlet end of the GC column using a two-way press-fit fused-silica connector housed inside the GC injection port. Desorption of the analytes from the extraction capillary was performed by rapid temperature programming (at 100 °C/min) of the GC injection port. The desorbed analytes were transported down the system by the helium flow and further focused at the inlet end of the GC column maintained at 30 °C. Sol–gel PDMS capillaries were used for the extraction of nonpolar and moderately polar compounds (polycyclic aromatic hydrocarbons, aldehydes, ketones), while sol–gel PEG capillaries were used for the extraction of polar compounds (alcohols, phenols, amines). The technique is characterized by excellent reproducibility. For both polar and nonpolar analytes, the run-to-run and capillary-to-capillary RSD values for GC peak areas remained under 6% and 4%, respectively. The technique also demonstrated excellent extraction sensitivity. Parts per quadrillion level detection limits were achieved by coupling sol–gel CME with GC-FID. The use of thicker sol–gel coatings and longer capillary segments of larger diameter (or capillaries with sol–gel monolithic beds) should lead to further enhancement of the extraction sensitivity.

Developed by Pawliszyn and co-workers, sol–gel microextraction (SPME) has been a major advancement in analytical sample preparation and has provided a solventless pathway to simultaneous sampling, sample preparation, preconcentration, and

sample introduction for instrumental analysis. SPME is based on the principle of equilibrium extraction, where a stationary-phase coating created on the outer surface of a fiber directly extracts analytes from the surrounding medium. Because of the portability of the extraction device, simplicity and speed in operation, and independence of analytical results from the sample volume, SPME is an ideal field sampling technique. In its classical format, SPME uses a fused-silica fiber (typically 100–200 μm in diameter) with a stationary-phase coating on a small end segment (~1 cm) of the fiber. Such a fiber is installed in a specially designed syringe (SPME syringe). The fiber is glued or mechanically mounted into a piece of stainless steel holder positioned concentrically within a needle. During handling and operation, the outer extraction coating is protected from mechanical damage by retracting the fiber into the needle of the SPME syringe. To carry out extraction, the fiber is brought in contact with the analyte by exposing the coated segment of the fiber into the sample. This is accomplished by simply depressing the plunger of the syringe. Analytes in the surrounding medium are extracted by the stationary-phase coating on the external surface of the fiber. Agitation of the sample (e.g., using a stir bar) is often used to assist the extraction process.

Several inherent problems result from the physical construction of the SPME device. The most common practical problems facing SPME users are fiber breakage, mechanical damage of the coating due to scraping, and needle bending. The short length of the coated segment of the fiber translates into low stationary-phase loading available for extraction. This results in low sample capacity of the fiber and imposes limitations on the sensitivity of the technique. Other problems include ghost peaks due to sequestering particles and fiber glue, as well as the memory effect caused by incomplete analyte desorption at the highest allowable temperature. Tentative solutions have been proposed to address some of these practical problems. The use of a metal fiber core provides mechanical strength and enhanced thermal conductivity of the fiber. A septumless injection port has been suggested to avoid sample contamination originating from the septum. Despite all these rapid advancements in SPME, some basic problems still remain to be addressed. These are as follows: (a) enhancing thermal and solvent stability of the coating, (b) improving sample capacity of the fiber, and (c) providing better protection against

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Appendix G (Continued)

mechanical damage of the coating\(^9\) while retaining the technique solventless and portable to achieve integrated sample preparation benefits in a cost-effective way. Coating reproducibility is another important aspect of SPME that deserves improvement. An ideal SPME fiber would also be disposable as opposed to a fixed component of an extraction device. Disposability offers flexibility to the user to extract from both dirty and clean samples.

The coated segment of the fused-silica SPME fiber is susceptible to breakdown since the protective polyimide coating needs to be removed prior to pulling the stationary-phase coating on it. In-tube SPME\(^{10-15}\) overcomes this mechanical instability problem inherent in conventional SPME. In-tube SPME uses a fused-silica capillary with a stationary-phase coating on its inner surface (commonly a piece of GC capillary column) instead of using an externally coated fiber. The potential of an in-tube SPME approach to sample preconcentration was also demonstrated by McComb et al.\(^{16}\) using an INCAT sampling device. These authors utilized a hollow needle containing a short piece of GC capillary column and showed the possibility of effective extraction that can be carried out by the stationary-phase coating on its inner surface.

In recent years, the extraction of analytes by gas chromatography (GC) stationary-phase coatings on the capillary inner surface has received considerable attention. The introduction of in-tube SPME had the primary purpose of coupling SPME to high-performance liquid chromatography (HPLC) for automated applications. The in-tube SPME method uses a flow-through process where a coated capillary is employed for the direct extraction of the analytes from the aqueous sample. The extraction process involves agitation by sample flow in and out of the stationary-phase coating. Successful coupling of in-tube SPME with HPLC as well as HPLC–MS has been achieved for the speciation of organosilicon compounds\(^{17}\) and determination of ranitidine\(^{18}\), \(\beta\) blockers,\(^{19,20}\) carbonate pesticides,\(^{18-21}\) and aromatic compounds.\(^{21}\)

Despite rapid ongoing developments in the areas of in-tube SPME applications, a number of fundamental problems remain to be solved. First, GC capillaries that are used for in-tube SPME typically have thin coatings that significantly limit the sample capacity (and hence sensitivity) of the technique. Conventional static coating techniques\(^{22-24}\) used to prepare stationary-phase coatings in GC columns are designed primarily for creating thin (submicrometer thickness) coatings. So, developing an alternative technique to provide thicker coating thickness suitable for in-tube SPME applications is very important. Second, usually the stationary-phase coatings used in GC capillaries are not chemically bonded to the capillary surface. In conventional approaches, these relatively thin coatings are immobilized on the capillary inner surface through free-radical cross-linking reactions.\(^{25,26}\) Immobilization of thicker coatings (especially the polar ones) is difficult to achieve.\(^{27}\) Third, because of the absence of stationary-phase bonding between the stationary-phase coating and the GC capillary inner wall, the thermal and solvent stabilities of such coatings are typically poor or moderate. When such extraction devices are coupled to GC, reduced thermal stability of thick GC coatings leads to incomplete sample desorption and sample carryover problems.\(^{28-31}\) Low solvent stability of conventionally prepared thick stationary-phase coatings may present a significant obstacle to the hyphenation of in-tube SPME with liquid-phase separation techniques that employ organic or organic–aqueous mobile-phase systems for the desorption of analytes. Solvent stability of the in-tube SPME coatings is, therefore, fundamentally important for further development of the technique.\(^{32}\) Thus, these three problems need to be solved in order to exploit full analytical potential of in-tube SPME.

In this paper, we describe sol–gel open-tubular microextraction technique (sol–gel OTME), the open-tubular version of sol–gel capillary microextraction (CME) that effectively overcomes the above-mentioned shortcomings of conventional coatings used in SPME or in-tube SPME. Sol–gel OTME typically uses a fused-silica capillary internally coated with a sol–gel-based stationary phase. Sol–gel coating technique\(^{22}\) used to prepare the capillaries involves a single-step procedure and allows for the in situ creation of chemically bonded coatings (both thick and thin) that are characterized by high thermal and solvent stabilities. The extraction is carried out by simply connecting the capillary to an in-house-designed, gravity-fed sample dispenser. Extraction capillaries used in sol–gel OTME are disposable, which gives flexibility to the technique. Like SPME, sol–gel OTME remains solventless, portable, inexpensive, and integrated.

**EXPERIMENTAL SECTION**

**Equipment.** Sol–gel CME GC experiments were carried out on a Varian model 3800 capillary GC system equipped with an FID and a Varian model 1090 temperature programmable split/splitless injector. Simple modifications to the split/splitless injector were made such that a two-way press-fit fused-silica connector could be housed inside the liner to provide connection between


Appendix G (Continued)

(THA), ketones (cyclohexanone, hexanophene, heptanophene, decanophene, anthracinone), aldehydes (benzaldehyde, n-octaldehyde, tolualdehyde, n-decylaldehyde, undecyl aldehyde), polyyclic aromatic hydrocarbons (PAHs) (acenaphthylene, fluorene, phenanthrene, fluoranthene), and phenols (2,6-dimethylphenol, 2,5-dimethylphenol, 2,3-dimethylphenol, 3,4-dimethylphenol) were purchased from Aldrich (Milwaukee, WI). Hydroxyl-terminated poly(dimethylsiloxane) (PDMS) and methyltrimethoxysilane (MTMS) were purchased from United Chemical Technologies, Inc. (Bristol, PA). Trimethoxysilane-derivatized poly(ethylene glycols) (M-3E5000 and M-3E3400) were obtained from Shertime Polymers (Huntsville, AL).

Preparation of Aqueous Standard Solutions for Capillary Microextraction. Stock solutions of PAHs (naphthalene, acenaphthylene containing 20% acenaphthene, fluorene, phenanthrene, and fluoranthene) and ketones (4-phenylcyclohexene, anthracinone) were prepared by dissolving 10 mg of each compound in 10 ml of THF in a 10 ml volumetric flask at room temperature. Preparation of 100 and 1 ppb PAH solutions was accomplished by further dilution of this stock solution with deionized water. THF was also used as the initial organic solvent to prepare stock solutions of ketones (valerophene, hexanophene, heptanophene, decanophene) or aldehydes (benzaldehyde, n-octaldehyde, tolualdehyde, n-decylaldehyde, undecyl aldehyde). Stock solutions of dimethylphenol (DMP) isomers (2,6-dimethylphenol, 2,5-dimethylphenol, 2,3-dimethylphenol, 3,4-dimethylphenol), alcohols, and amines were prepared in an analogous way using methanol as the initial organic solvent.

Prior to extraction, all glassware was deactivated. The glassware was cleaned using Sparkleen detergent, rinsed with generous amounts of deionized water, and dried at 150 °C for 2 h. The inner surface of the dried glassware was then treated with a 2% v/v solution of HMDS in methylene chloride, followed by placing of the glassware in an oven at 250 °C overnight. The glassware were then rinsed sequentially with methylene chloride and methanol and further dried in the oven at 100 °C for 1 h. Before use, they were rinsed with generous amounts of deionized water and dried at room temperature in a flow of helium.

Preparation of Sol–Gel-Coated Capillaries. Sol–gel PDMS and poly(ethylene glycol) (sol–gel-PEG) extraction capillaries as well as the sol–gel open-tubular GC columns were prepared according to procedures described elsewhere. Briefly, a previously cleaned and hydrothermally treated fused silica capillary was filled with a specially designed sol solution using a helium pressure operated filling/purging device. The sol solution was prepared by dissolving appropriate amounts of a sol–gel precursor (e.g., methyltrimethoxysilane, tetramethoxysilane, etc.), a sol–gel active organic polymer (e.g., hydroxyl-terminated PDMS, trimethoxysil-terminated PEG, etc.), a surface deactivation reagent (e.g., HMDS, poly(dimethylsiloxane), etc.) and a sol–gel catalyst (e.g., triflic acid) in a suitable solvent system. After filling, the sol solution was allowed to stay inside the capillary for 20–30 min. During this residence time, an organic–inorganic hybrid sol–gel network evolves in the sol solution within the confined environment of the fused-silica capillary, and a thin layer of the evolving sol–gel stationary phase gets chemically bonded

Figure 1. Schematic of a gravity-fed sample dispensing unit for capillary microextraction.
to the capillary walls as a result of condensation reaction with the silanol groups on the capillary inner surface. After the residence time, the residual sol solution was expelled from the capillary under helium pressure. The sol–gel coated capillary was further purged with helium for 1 h and conditioned in a GC oven using temperature programming from 40 °C to 1 °C/min. The capillary was held at the final temperature (50 °C) for sol–gel PDMS and 300 °C for sol–gel PEG) for 5 h under helium purge. The final conditioning temperatures used for the two types of sol–gel coatings were determined by their thermal stabilities. Before being used for extraction, the capillary was sequentially rinsed with methylene chloride and methanol followed by drying in a helium stream under temperature-programmed conditions as described above except for holding the capillary at the final temperature for only 30 min.

Gravity-Fed Sample Dispenser for Capillary Microextraction. The gravity-fed sample dispenser for capillary microextraction (Figure 1) was made by in-house modification of a Chromosax AQ column (Kromarx Glass Co.) consisting of a thick-walled glass cylinder coaxially placed in an acrylic jacket. For this, the bottom screw caps, jacket sealing rings, vinyl O-rings, nylon bed support, and acrylic jacket were removed from the Chromosax AQ column. The thick-walled glass column was uncapped and its inner surface was deactivated by treating the inner surface with a 2% v/v solution of HMDS in methylene chloride followed by heating at 250 °C for 1 h. The column was then cooled to ambient temperature, thoroughly rinsed with liberal amounts of deionized water, and dried in a helium flow. The entire Chromosax AQ column was subsequently reassembled.

Thermal Desorption of Extracted Analytes in the GC Injection Port. To facilitate thermal desorption of the extracted analytes from the sol–gel microextraction capillary for their subsequent introduction into the GC capillary column, the Varian model 1079 split/splitless injector was slightly modified. For this, the quartz wool was removed from the glass insert to accommodate a two-way fused-silica connector within the insert. With the glass insert (now without the quartz wool) in place, the injector end of GC capillary column was press-fit into the lower end of the deactivated two-way fused-silica connector and secured inside the glass insert. After performing capillary microextraction, the extraction capillary was connected to the system in the following way. The capillary column not at the bottom of the injector was loosened and the column was slid up. The extraction capillary was passed through the septum support and press-fit into the fused-silica two-way butt connector. The column was then pulled down until the extraction capillary disappeared below the septum support and remained inside the glass insert. The septum was replaced, and the injector nut and the capillary column nut were tightened down.

Sol–Gel Capillary Microextraction-GC Analysis. To perform capillary microextraction, a previously conditioned sol–gel extraction capillary was vertically connected to the bottom end of the empty sample dispenser (Figure 1). The aqueous sample (25 μl) was placed in the dispenser and allowed to flow through the extraction capillary under gravity for 30 min for equilibration to be established. After this, the microextraction capillary was purged with helium gas at 25 kPa for 1 min and connected to the top end of the two-way press-fit fused-silica connector (housed inside the GC injection port liner) with the GC column inlet connected to its bottom end.

The extracted analytes were then thermally desorbed from the capillary by rapid temperature programming of the injector (at 100 °C/min starting from 30 °C). The nature of the coating used in the capillary determined the final temperature of the ramp (330 °C for sol–gel PDMS and 280 °C for sol–gel PEG-coated capillaries). The desorption was performed over a 5-min period in the splitless mode whereby the released analytes were swept over by the carrier gas into the GC column held at 30 °C to facilitate effective solute focusing at the column inlet. The split went remained closed throughout the entire course of the chromatographic run. Analyte detection was performed using a flame ionization detector (FID) maintained at 350 °C.

RESULTS AND DISCUSSION

Sol–gel technology provides an elegant synthetic pathway to advanced materials"30-33 with a wide range of applications. In the context of analytical microextraction, it allows for the in situ creation of hybrid organic–inorganic stationary phases within separation columns in the form of coatings, monolithic beds, and stationary-phase particles.24 Excellent chromatographic and electromigration separations have been demonstrated using separation columns with sol–gel stationary phases,10-34-35,46 We introduced sol–gel coatings for gas chromatography35 and solid-phase microextraction.36-38
Appendix G (Continued)

demonstrated significant thermal and solvent stability advantages inherent in sol–gel-coated GC columns\(^2\) and SPME fibers.\(^8\) Since then, several other groups have gotten involved in sol–gel research for solid-phase microextraction\(^15,17\) and solid-phase extraction.\(^21\) In this paper, we demonstrate the possibility of using sol–gel-coated capillaries as an effective means of solventless microextraction and call such a microextraction technique sol–gel open-tubular microextraction. Sol–gel OTME is synonymous with in-tube solid-phase microextraction (in-tube SPME) on sol–gel-coated capillaries. It should be possible to materialize a capillary microextraction technique using a sol–gel monolithic bol–sol–gel monolithic microextraction (sol–gel MMP). Both sol–gel OTME and sol–gel MMP can be combined under a general term – sol–gel capillary microextraction (sol–gel CME). In our view, the new terminology – capillary microextraction – provides a better reflection of the technique compared with the conventionally used “in-tube solid-phase microextraction” since the technique it relates to is not necessarily limited to the use of only “solids phases” as the extraction media. In fact, liquid stationary-phase coatings are commonly used in in-tube SPME as well as conventional SPME.

Sol–gel technology allows for the creation of coatings on the inner surface of open-tubular GC, CE, and SEC columns\(^5,13,14,31\) as well as on the outer surface of substrates of different shapes and geometry (e.g., SPME fibers\(^31,40,75\)). It is applicable to the creation of silica-based\(^31,35\) and transition metal-based\(^36,37\) materials. In the context of capillary separation and sample preconcentration techniques, an important attribute of sol–gel coating technology is that it provides surface coatings that get automatically bonded to the substrate surfaces containing sol–gel-active functional groups (e.g., silanol groups). Mention should be made here that sol–gel technology effectively achieves stationary-phase coating, its immobilization, and deactivation in one single step\(^6\) instead of multiple time-consuming steps involved in conventional coating technology.

The use of the stationary-phase coating on the inner surface of a fused silica capillary eliminates the coating scraping problem inherent in fiber-based SPME and significantly reduces the possibility of sample contamination. Furthermore, the protective polyimide coating on the outer surface of the fused silica extension capillary remains intact and adds flexibility to the extraction device as compared with traditional SPME fibers.

Sol–gel PDMS and PEG-coated capillaries were used in conjunction with a gravity-fed sample dispenser (Figure 1) to achieve our objective of developing a simple and reproducible method for the extraction of analytes from aqueous media. Our aim was to make a contribution to the further development of SPME technology by using sol–gel extraction media whose advanced material properties would help to overcome some basic problems inherent in either fiber-based SPME or in-tube SPME with conventional coatings. Sol–gel OTME typically uses a short length of fused silica capillary coated internally with sol–gel stationary phase, which is chemically bonded to the substrate. Because of this chemical bonding, sol–gel coatings have significant temperature- and solvent-stability advantages over physically held conventional coatings. It should be mentioned that Supelco recommends that commercial PDMS fibers should not be exposed to nonpolar organic solvents such as hexane.\(^7\)

Figure 2 represents scanning electron microscopy (SEM) illustrating the internal structures of two 250 µm id. fused silica capillaries with sol–gel PDMS (Figure 2A) and sol–gel PEG (Figure 2B) coatings on the inner surface. As can be seen from these SEM images, the coatings in these microextraction capillaries are remarkably uniform in thickness. The thickness of the sol–gel PDMS coating was estimated at ~0.6 µm, while that for the sol–gel PEG coating at ~0.4 µm.

Figure 3 and Table 1 present experimental data that illustrate different aspects of open-tubular microextraction of PAHs performed on an aqueous sample at ppb and sub-ppb level concentrations of the analytes using a sol–gel PDMS-coated capillary. The repeatability data presented in Table 1 show that sol–gel OTME GC provides excellent run-to-run repeatability in solute peak areas (less than 5%) and retention time (less than 0.2%). The data on the detection limits demonstrate high sensitivity of capillary microextraction. For example, by hyphenating sol–gel OTME with GC–FD, a detection limit of 300 parts per million (ppm) was achieved for naphthalene extracted on a 250 µm id. sol–gel PDMS-coated capillary with a relatively thin coating (0.6 µm). Mention should be made here that sol–gel coating technology can easily produce stable thick coatings\(^30,31\) (~1 µm). For example, Zeng et al.\(^7\) recently reported SPME on sol–gel-coated fibers with a coating thickness of 76 µm. The use of microextraction capillaries with thick sol–gel coatings should lead to higher sensitivity of capillary microextraction. It can be expected that the use of capillaries with larger inner diameters and thicker sol–gel coatings should lead to further enhancement of this extraction sensitivity.

Figure 4 represents a gas chromatogram of several free aldehydes extracted from an aqueous medium by OTME using a sol–gel PDMS-coated capillary. Analytical determination of aldehydes is often performed through derivatization into less polar or easy to detect forms.\(^8\) In this work, aldehydes were extracted and analyzed without derivatization. This became possible thanks to the outstanding material properties of sol–gel PDMS coating used both in the microextraction capillary and in the GC separation column. The organic–inorganic nature of the sol–gel PDMS coating provides sorption sites for both the polar and nonpolar. The high quality of column deactivation achieved through sol–gel column technology\(^9\) allows for the GC analysis of aldehydes without derivatization. From an analytical standpoint,\(^7\) USEPA Catalog 2002: Chromatography Protocols for Analytical and Purifica-
Appendix G (Continued)

Table 1. Peak Area and Retention Time Repeatability Data for ppb and Sub-ppb Level Concentrations of PAHs, Aldehydes, and Ketones Obtained in Five Replicate Measurements by OTME-GC Using Sol–gel PDMS Coatings^a

<table>
<thead>
<tr>
<th>Chemical Class</th>
<th>Name</th>
<th>Peak Area Repeatability ((a = 5))</th>
<th>Eth Repeatability ((a = 5))</th>
<th>Detection Limits (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAHs</td>
<td>naphthalene</td>
<td>253 414.2  2.7  16.176  0.221  0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>acenaphthene 80%</td>
<td>73 189.5  2.5  19.710  0.168  0.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>acenaphthene 20%</td>
<td>39 683.4  2.8  20.112  0.181  0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>fluorene</td>
<td>132 658.8  3.3  23.671  0.205  0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>phenanthrene</td>
<td>92 212.3  3.6  23.376  0.148  0.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>anthracene</td>
<td>128 405.4  4.0  26.029  0.167  0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldehydes</td>
<td>benzaldehyde</td>
<td>30 013.0  6.0  16.757  1.904  103.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>cinnamaldehyde</td>
<td>174 300.5  4.9  17.770  3.030  104.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>valeraldehyde</td>
<td>60 002.1  5.2  18.806  3.010  62.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>isovaleraldehyde</td>
<td>138 555.7  4.7  19.330  2.250  28.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketones</td>
<td>valeric acid</td>
<td>31 364.5  4.8  19.146  0.993  215.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>hexadecane</td>
<td>65 197.5  4.3  20.624  0.077  109.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>heptadecane</td>
<td>71 330.6  4.5  21.663  0.006  162.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4′-phenylcetophenone</td>
<td>43 638.0  5.6  24.189  0.035  117.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>decanoic acid</td>
<td>100 165.0  4.2  24.561  0.176  55.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>undecanoic acid</td>
<td>201 529.8  3.8  25.239  0.133  32.67</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a Experimental conditions for capillary microextraction and GC analysis are same as in Figures 3 (PAHs), 4 (aldehydes), and 5 (ketones).

^b Detection limits were calculated for a signal-to-noise ratio (S/N) of 3 using the data presented in Figures 3 (PAHs), 4 (aldehydes), and 5 (ketones).

The possibility of extraction and gas chromatographic analysis of underivatized aldehydes by OTME-GC is important and should provide simplicity, speed, sensitivity, and accuracy in aldehyde analysis.

Figure 5 represents a gas chromatogram illustrating OTME-GC analysis of several ketones extracted from an aqueous sample using a sol–gel PDMS coated capillary. Like aldehydes, ketones are also often derivatized for analysis. Using a sol–gel-coated extraction capillary and separation column, no derivatization step was necessary either for extraction or for GC separation. Two important features can be observed in this chromatogram. First, the peaks are sharp and symmetrical, which is indicative of effective focusing of the analytes at the column inlet after their desorption as well as excellent performance of the sol–gel PDMS column used for GC separation. Second, although all analyte concentrations in the aqueous sample were practically the same (100 ppb), the analyte peak height increased with the increase of the molecular weight of the ketone. This might be the consequence of two distinctive phenomena: (1) higher loss of the more volatile ketones during the postextraction purging step of the microextraction capillary and (2) displacement of the lower molecular weight ketones by the ones with higher molecular weights. However, considering the fact that the analyte concentrations were sufficiently low (100 ppb), and that sol–gel coatings are characterized by enhanced surface area, it is not likely that the coating was overloaded at this concentration level. So, it is...
Appendix G (Continued)

Figure 3. Capillary microextraction analysis of PAHs at 1 ppb level concentration (except for acenaphthene (200 ppb) and acenaphthylene (800 ppb)) using a sol–gel PDMS-coated capillary. Extraction conditions: 3.5 cm × 0.25 mm i.d. micro-extraction capillary, extraction time 30 min (gravity feed at room temperature). Other conditions: 10 cm × 250 μm i.d. sol–gel PDMS column; splitless injection (splitless desorption); injector temperature 300 °C at a rate of 15 °C/min; column temperature programmed from 30 to 250 °C at a rate of 15 °C/min; helium carrier gas: FID 350 °C. Peaks: (1) naphthalene, (2) acenaphthene, (3) acenaphthylene, (4) fluorene, (5) phenanthrene, and (6) fluoranthene.

more like that the peak size discrimination of the ketones was caused primarily by the first factor.

The sol–gel OTME-GC for aldehydes and ketones described herein provides a number of important advantages over sample preparation techniques coupled to HPLC. First, the fact that no derivatization is needed makes the procedure faster, simpler, and more accurate. Second, since the flame ionization detector used for GC analysis inherently possesses several order of magnitude higher sensitivity compared with the UV detector commonly used with HPLC analysis, the described procedure also provides sensitivity advantage. The OTME-GC analysis of aldehydes and ketones is also characterized by low run-to-run relative standard deviation (RSD) values (Table 1). For five replicate measurements, RSD values of about 6% and 4% were obtained for solute peak area and retention time, respectively. The only exception was benzaldehyde that had a retention time RSD value of 1.9%, which is significantly higher than the RSD values for the rest of the aldehydes and ketones studied. At this time, we do not have a good explanation for this anomalous behavior of benzaldehyde.

Figure 6 presents OTME-GC analysis of phenolic compounds at 10 ppb level concentrations using a sol–gel PEG-coated microextraction capillary. A sol–gel PEG-coated capillary column was used for their GC analysis. The analysis of phenols is important from an environmental point of view since some phenols are registered in the U.S. EPA's priority pollutant list. The extraction and GC analysis were done without derivatization, although analysis of these polar compounds often requires derivatization. Under the experimental conditions used, the detection limit for this analysis was ~0.1 ppb. The sharp symmetrical peaks and the low detection limits obtained are indicative of the high extraction efficiency of the sol–gel PEG-coated microextraction capillary and excellent analytical performance of the sol–gel PEG column used. Conventional coatings for the analysis of phenols often show carryover problems because of the strong interaction of polar analytes with the coatings. Effective release of the extracted polar analytes from the coatings requires application of high desorption temperature. However, the relatively low thermal stability of conventionally prepared thick coatings does not allow for the application of high temperatures during the analyte desorption step of the analysis, resulting in only partial

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Appendix G (Continued)

Figure 5. Capillary microextraction analysis of ketones using a sol-gel PDMS-coated capillary at 160 ppb analyte concentration. Extraction conditions are the same as in the Figure 3 caption. Other conditions: 10 m x 250 μm i.d. sol-gel PDMS column; splitless injection (splitless desorption); injection temperature 40–300 °C at a rate of 10 °C/min; column temperature programmed from 30 (hold for 5 min) to 250 °C at a rate of 15 °C/min; helium carrier gas; FID 350 °C; extraction time, 30 min (gravity feed at room temperature). Peaks: (1) valeraldehyde, (2) hexanal, (3) heptanal, (4) 4-phenylacetophenone, (5) decanal, and (6) anthracene.

Figure 6. Capillary microextraction analysis of dimethylphenol isomers using a sol-gel PEG-coated capillary at 10 ppb analyte concentration. Extraction conditions are the same as in the Figure 3 caption. Other conditions: 10 m x 250 μm i.d. sol-gel PEG column; splitless injection (splitless desorption); injection temperature 40–300 °C at a rate of 10 °C/min; column temperature programmed from 30 (hold for 5 min) to 250 °C at a rate of 15 °C/min; helium carrier gas; FID 350 °C; extraction time, 30 min (gravity feed at room temperature). Peaks: (1) 2,6-xylene, (2) 2,4-xylene, (3) 2,3-xylene, (4) 2,5-xylene, (5) 1,3-xylene, and (6) 1,4-xylene.

release of the extracted analytes. This incomplete desorption of the extracted analytes is responsible for the carryover problem. The sol-gel PEG-coated extraction capillary showed consistent performance at a desorption temperature of 280 °C. No carryover problems were observed for polar or nonpolar analytes on sol-gel PEG or sol-gel PDMS-coated microextraction capillaries.

Figure 7A illustrates the extraction kinetics of fluorene (a nonpolar analyte) and decanoic acid (a moderately polar analyte) on a sol-gel PDMS-coated microextraction capillary. Extraction kinetics of 2,3-dimethylphenol on a sol-gel PEG-coated capillary is illustrated in Figure 7B. The extractions were carried out using aqueous samples containing 1 ppm concentration of each analyte. As can be seen from Figure 7A, the extraction equilibrium for fluorene was practically reached after 15 min of extraction while for decanoic acid it required ~35 min of extraction to reach the plateau on the extraction curve. Such differences in the extraction behavior of the two analytes can be explained on the basis of the differences in their hydrophobicity. The highly nonpolar nature of fluorene makes it more susceptible to hydrophobic interaction and facilitates its extraction by the nonpolar PDMS moieties on the sol-gel coating. This is evident from the steeply rising beginning part of the fluorene extraction curve in Figure 7A. The higher polarity of the ketone makes it more hydrophilic, which leads to a slower extraction process as is evidenced by the more gradually rising nature of the extraction curve for the ketone. For the sol-gel PEG-coated capillary (Figure 7B), the extraction equilibrium for 2,3-dimethylphenol was reached within 30 min.

Highly polar compounds such as alcohols, amines, and phenols have higher affinity for water, and conventional nonpolar phases (e.g., PDMS) are usually not very efficient for their extraction from an aqueous phase. Polar coatings are normally used for the extraction of these highly polar analytes. However, creation of thick coatings of polar stationary phases and their immobilization on a substrate are associated with technical difficulties. Previously, we showed that these polar compounds can be satisfactorily extracted and analyzed using sol-gel PDMS-coated fibers. This becomes possible thanks to the organic−inorganic hybrid nature of the sol−gel PDMS coatings characterized by the presence of both polar and nonpolar sorption sites. Sol−gel coating technology allows for the creation of both polar and nonpolar PDMS coatings with equal ease and versatility. In the present work, we demonstrate the possibility of efficient extraction of these polar analytes from an aqueous environment using open-tubular capillary microextraction on sol−gel poly(ethylene glycol) (sol−gel PEG) coatings.
Appendix G (Continued)

Table 2. Peak Area and Retention Time Repeatability Data for ppb Level Concentrations of Phenols, Alcohols, and Amines Obtained in Five Replicate Measurements by OTME-GC Using Sol−Gel PEG Coatings

<table>
<thead>
<tr>
<th>Chemical Class</th>
<th>Name</th>
<th>Peak Area Repeatability</th>
<th>Retention Time Repeatability</th>
<th>Detection Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4-dimethylphenol</td>
<td>39.360.7 ± 1.6</td>
<td>19.445 ± 0.071</td>
<td>16.06ppb</td>
<td></td>
</tr>
<tr>
<td>2,5-dimethylphenol</td>
<td>86.727.4 ± 1.5</td>
<td>20.016 ± 0.007</td>
<td>7.085ppb</td>
<td></td>
</tr>
<tr>
<td>2,3-dimethylphenol</td>
<td>110.450.4 ± 1.0</td>
<td>20.326 ± 0.110</td>
<td>6.901ppb</td>
<td></td>
</tr>
<tr>
<td>3,4-dimethylphenol</td>
<td>70.653.3 ± 2.0</td>
<td>20.717 ± 0.119</td>
<td>9.421ppb</td>
<td></td>
</tr>
<tr>
<td>Alcohols and Amines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decyl(2-ethylhexyloxylamine</td>
<td>137.780.0 ± 3.0</td>
<td>14.082 ± 0.171</td>
<td>4.988ppb</td>
<td></td>
</tr>
<tr>
<td>Myristyl (C14) alcohol</td>
<td>133.076.0 ± 4.2</td>
<td>16.524 ± 0.152</td>
<td>1.992ppb</td>
<td></td>
</tr>
<tr>
<td>Arachidyl (C20) alcohol</td>
<td>138.103.6 ± 3.8</td>
<td>17.728 ± 0.153</td>
<td>2.318ppb</td>
<td></td>
</tr>
<tr>
<td>Benzylamine</td>
<td>64.498.7 ± 4.0</td>
<td>18.768 ± 0.161</td>
<td>5.976ppb</td>
<td></td>
</tr>
<tr>
<td>Stearyl (C18) alcohol</td>
<td>64.410.0 ± 3.0</td>
<td>19.483 ± 0.147</td>
<td>3.412ppb</td>
<td></td>
</tr>
<tr>
<td>Arachidyl (C20) alcohol</td>
<td>107.330.0 ± 4.0</td>
<td>21.070 ± 0.133</td>
<td>4.182ppb</td>
<td></td>
</tr>
</tbody>
</table>

a Experimental conditions for capillary microextraction and GC analysis are the same as in Figures 6 (phenols) and 8 (alcohols and amines). b Detection limits were calculated for a signal-to-noise ratio (S/N) of 3 using the data presented in Figures 6 (phenols) and 8 (alcohols and amines).

Figure 7. (A) Illustration of the extraction kinetics of fluorene (■) and decachlorothane (▲) obtained on a 3.5 cm x 250 μm i.d. sol-gel PEG-coated microextraction capillary using 1 ppm aqueous solutions. Thermal desorption and GC conditions are the same as in the captions for Figures 3 and 5, respectively. (B) Illustration of the extraction kinetics of 2,3-dimethylphenol obtained on a 3.5 cm x 250 μm i.d. sol-gel PEG-coated microextraction capillary using 1 ppm aqueous solution. Thermal desorption and GC conditions are the same as in the Figure 6 caption.

Figure 8 illustrates a gas chromatogram of a mixture of alcohols and amines (10 ppb each) that were extracted from an aqueous sample using a sol−gel PEG-coated microextraction capillary. No derivatization was needed either for extraction or for GC analysis of these highly polar compounds. Excellent peak shapes, detection sensitivity, and extraction efficiency are evident from the chromatographic data presented in Figure 8 and Table 2. The run-to-run repeatability data for the phenols, alcohols, and amines, presented in Table 2 in terms of peak area and retention time RSD values, are also remarkable. For these highly polar analytes, the peak area and retention time RSD values were less than 4.5% and 0.2%, respectively.

The capillary-to-capillary reproducibility in open-tubular microextraction was evaluated for the two types of sol−gel coatings.
Appendix G (Continued)

used in this work—sol–gel PDMS and sol–gel PEG coatings. For this, three identical segments of each type of sol–gel-coated capillary were used. Fluorene was used as the test solute for the sol–gel PDMS-coated capillary while decacone served the same purpose for the sol–gel PEG-coated microextraction capillary. A total of six extractions (30 min each) were carried out on each capillary using 1 ppm aqueous solutions containing the respective test solute. The RSDs of the mean GC peak area for the two test solutes on sol–gel PDMS and sol–gel PEG capillaries were 3.0% and 3.0%, respectively. These low RSD values are indicative of excellent capillary-to-capillary reproducibility in sol–gel open tubular microextraction.

In the present work, OTME was performed using 3.5 cm long sol–gel-coated capillary segments. The length of the extraction capillary used was limited by the linear dimensions of the glass insert in the injection port of the used GC (Varian 3800D) and the length of the two-way pressfit connector. In this format, the entire length of the extraction capillary was contained inside the GC injection port. However, it should be possible to use longer sol–gel-coated capillary segments to enhance the extraction sensitivity. Currently, we are exploring this possibility.

CONCLUSION

For the first time, sol–gel-coated capillaries were used for solventless microextraction and sample preconcentration. The newly developed technique was termed sol–gel open-tubular microextraction. Two types of sol–gel coatings (sol–gel PDMS, sol–gel PEG) were effectively used for the extraction of analytes belonging to various chemical classes. Parts per trillion (ppt) and parts per quadrillion level detection sensitivities were achieved for polar and nonpolar analytes. Further sensitivity enhancement should be possible through the use of thicker sol–gel coatings in conjunction with longer extraction capillaries or capillaries of larger inner diameters. The sol–gel-coated capillaries are characterized by enhanced thermal and solvent stabilities (a prerequisite for efficient analyte desorption), making them very suitable for coupling with both GC and HPLC. Sol–gel capillary microextraction showed remarkable run-to-run and capillary-to-capillary repeatability and produced peak area RSD values of less than 6% and 4%, respectively.

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Appendix H

Synthesis of benzyl-terminated dendrons for use in high-resolution capillary gas chromatography

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Abstract—The synthesis, silane functionalization, and facile silica attachment of dendritic monomers containing terminal benzyl groups are presented. For the first time, a simple one-step procedure is described that leads to the in situ creation of a surface-bonded sol-gel dendritic stationary phase on the inner walls of a fused silica capillary; such phases showed unique selectivities in high-resolution capillary gas chromatography. © 2001 Elsevier Science Ltd. All rights reserved.

In the past decade, highly functionalized branched materials have found use in numerous applications, such as host-guest chemistry, carbohydrate chemistry, metallo-dendrimers, catalysis, and surface chemistry. These various applications were made possible due to either internal or external modifications of dendrimers, which possess a general spherical shape, and thus are capable of intermolecular interactions with the immediate environment as well as an inner regime capable of utilitarian supramolecular characteristics.

We are not aware of any application of functionalized dendrons as a stationary phase in capillary gas chromatography, although their applications as mobile phase additives in liquid-phase separations have been reported. Herein, we describe the synthesis, extended silane functionalization, and silica attachment procedure for highly branched building blocks containing benzyl ether terminal groups.

Access to the desire dendrons 3, 8, and 13 (Fig. 1) was facilitated by the use of the previously reported amine and isocyanate monomers 1 and 2, respectively. Thus, the preparation (Scheme 1) of the nitrobenzyl ether 5 and its larger analog 10 was effect by reaction of 4-amino-4-[3-(benzyloxy)propyl]-1,7-dibenzylxyl-heptane (1) with the triacid 4 and nonacid 9, respectively, using standard DCC coupling conditions. Formation of the nitrobenzyl ethers 5 and 10 was confirmed (1H NMR) by the observation of an upfield shift of the carboxyl carbon resonance (5: 173.5 ppm and 174.9 ppm to 169.9 ppm and 171.9 ppm, respectively). The acetate 6 was obtained by reacting 5 with the sodium acetate anion (1:6). Reduction of the nitro groups with NaBH₄ in a MeOH-CH₂Cl₂ mixture at 50°C afforded the corresponding amines 6 and 11, whose structures were identified by the traditional chemical shift (¹H NMR) of the signal for the C=O from 9.49 ppm to 7.29 ppm and 9.81 ppm to 7.32 ppm, respectively, confirming the desired C=O→NO₂ to C=O→NH₂ transformation.

Treatment of amines 6 and 11 with 0.4 equiv. of triphosgene in the presence of Et₃N in CH₂Cl₂ gave the corresponding isocyanates 7 and 12. Their structures were characterized (¹H NMR) by the absorption of chemical shifts from 52.6 and 49.3 ppm to 61.9 and 60.3 ppm, respectively, corresponding to the C=O→NH₂ to C=O→NCO conversion, as well as the appearance of a new peak at ca. 122 ppm for the NCO group. Furthermore, the IR spectrum for each showed the typical isocyanate peak at 2225 and 2248 cm⁻¹ and the ESI-MS and MALDI-TOF further confirmed their assignment by a peak at m/z 1646.2 [M⁺] (7: ca. m/z 1646.2 [M⁺]) and m/z 5080.2 [M⁺] (12: ca. m/z 5080.8 [M⁺]) respectively.

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Dendritic silane reagents 3, 8, and 13 were then synthesized by treatment of these monomers possessing the focal isocyanate moiety and terminal benzyloxy groups with 3-(triethoxysilyl)propylamine in dry CH₂Cl₂ at 25°C for 1 h. Their preparation was confirmed by ¹³C NMR in all cases by the presence of a new urea (NHCONH) carbon peak at ca. 157 ppm, as well as (IR) new absorbance peaks at 1100 and 1650 cm⁻¹ for the silane group (Si-O) and urea carbonyl group; the absorbance peak (2250 cm⁻¹) indicative of the -NCO moiety was absent.

Silica attachment of the sol-gel active dendritic reagents (3, 8, or 13) in the presence of added methyltrimethoxysilane (MTMS), and catalyzed by TFA proceeded smoothly via hydrolysis and subsequent polycondensation to form an organic-inorganic hybrid polymer network with the dendron as the organic constituent. Conducting the reaction within a clean fused silica capillary column provided a simple means for effective column deactivation as well as stationary film immobilization.²⁴

Fig. 2 presents three gas chromatograms obtained on three different sol-gel columns with the first (3) (A), second (8) (B), and third generation (13) (C) benzyl-terminated dendritic appendages were incorporated into stationary phases. A Grob test mixture,³⁵ containing compounds from different chemical classes, was used as the sample probe. As can be seen in Fig. 2, the sol-gel dendrimer stationary phase can provide well-behaved chromatographic peaks for both polar and non-polar components. The elution pattern for individual components of this mixture is very different from that
Appendix H (Continued)

Scheme 1. Synthesis of second and third generation building blocks: (a, d) DCC, 1-HOBT, 25°C, dry DMF, 48 h; (b, e) NaBH₄, NiB, 50°C, MeOH, 1 h; (c) Et₃N, triphosgene, 25°C, CH₂Cl₂, 1.5 h; (f) Et₃N, triphosgene, 55°C, CH₂Cl₂, 1.5 h.

obtained on conventional columns. For example, unlike conventional PDMS or related capillary GC columns that elute 2,3-butanediol as the first peak under standard operating conditions, on benzyl-terminated sol-gel dendrimerized columns, the first eluted component is n-decane. In this respect, the sol-gel dendrimerized column behaved more like a polar column (e.g. PEG type) characterized by early elution of n-alkanes. A closer look at the chromatograms in Fig. 2 reveals that compared with conventional PDMS column, the functionalized sol-gel column provided a different elution order for all peaks (except for methyl dodecanoate, which elutes last in both cases). This selectivity of the sol-gel functionalized stationary phase in GC may be attributed to the specific molecular architecture of these modified stationary phases. A marked difference in chromatographic selectivity is observed between different generations of these stationary phases, coating different generations of dendritic moieties. For example, an efficiency value of 3,200 theoretical plates/m was obtained on a 10 mm x 0.25 mm i.d. column coated with the third generation dendrimerized sol-gel stationary phase. This corresponded to a minimum plate height of 0.31 mm and an optimum flow rate of 25 cm/s. Efficiency values on the order of only 1,000 plates/m were obtained on the columns coated with the related first and second generation stationary phases.

Based on this preliminary study, in which the dendrimerized reagent possessed simply benzyl ether termini and area connectivity, it can be concluded that the dendritic architecture provides unique selectivity in capillary GC separations, and adds chromatographic and electro-migration separations to potential utilitarian purposes for dendrons. In view of the facile ability to create diverse useful canopies that can enshrine different specific functionality to achieve an appropriate molecular interaction and response event(s), installation of these complimentary supramolecular relationships for chromatographic interactions can lead to enhanced...
Appendix H (Continued)

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References


selectivity in separations. The use of sol-gel chemistry to chemically bind these molecules to the capillary inner surface in the form of a stable organic-inorganic hybrid stationary phase coating opens new possibilities for enhanced performance and stability in differential migration separation techniques.
ABOUT THE AUTHOR

Abuzar Kabir was born in Jessore, a small city in Bangladesh where he received his elementary, secondary and higher secondary education. In 1984, he moved to Dhaka, the country’s capital, to pursue higher studies. He was admitted into the University of Dhaka where he received his B.Sc. (Honors) and M.Sc. degrees in Applied Chemistry and Chemical Technology in 1990 and 1991, respectively. In 1993, he started his professional career in the Department of Applied Chemistry & Chemical Technology, University of Dhaka, Bangladesh as a lecturer and was promoted to assistant professor in 1996. He came to the United States in 1999 and joined the Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, USA as an M.S. student. On the first day of the new millennium he came to Tampa to join the Department of Chemistry, University of South Florida (USF) where he continued his higher education as a Ph.D. student. He joined Dr. Abdul Malik’s research group and started his investigation on developing novel sol-gel organic-inorganic hybrid material systems for chromatographic separation and analytical sample preparation. His research at USF has resulted in 8 publications in international journals and 3 US patent applications.