Development of Novel Biocompatible Hydrogel Coatings for Implantable Glucose Sensors

by

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Date of Approval: November 19, 2008

Keywords: poly(2-hydroxyethyl methacrylate), 2,3-dihydroxypropyl methacrylate, N-vinyl pyrrolidone, freezing water, nonfreezing water, drug delivery

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ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my dissertation advisor, Dr. Julie Harmon, for her guidance, support, and encouragement in completing my Ph.D. research project. Thank you for giving me the freedom to pursue this project and for developing my research career as a ‘scientist’. I will be forever grateful to you for your generosity, kindness, and wisdom over the years. I extend my appreciation to the members of my Ph.D. dissertation committee, Dr. Edward Turos, Dr. Romann Manetsch, Dr. Xiao (Sheryl) Li, Dr. Francis Moussy, Dr. Kirpal S. Bisht, who encouraged and leaded me to the right directions during my Ph.D. study. I would also like to thank Dr. David Rabson, who served as the external committee chairperson for my dissertation defense.

I acknowledge Mr. Jay Bieber for his assistance with SEM analysis, Benz Research & Development (Sarasota, FL) for the generous supply of ultra high purity monomers, all of my fellows in Polymer Materials lab and my Chinese friends in the USF for their support and encouragement.

Last but certainly not least, I especially thank my parents and parents-in-law who have always supported and encouraged me with unconditional charity and their prayer. I am forever grateful to my husband Yangyang Zhang. He is always there with devotion, patience, love, and constant cheers. I am also grateful to my sweet little daughter, Yuxin Zhang, who is a blessing and a treasure of my heart.
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(0.01MKH₂PO₄)

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<tr>
<td>NIH</td>
<td>National Institute of Health</td>
</tr>
<tr>
<td>PI</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
</tr>
<tr>
<td>Ag/AgCl</td>
<td>Silver/silver chloride</td>
</tr>
<tr>
<td>GOx</td>
<td>Glucose oxidase</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
</tr>
<tr>
<td>PEG</td>
<td>poly(ethylene glycol)</td>
</tr>
<tr>
<td>HEMA</td>
<td>hydroxyethylmethacrylate</td>
</tr>
<tr>
<td>DLC</td>
<td>diamond-like carbon</td>
</tr>
<tr>
<td>PU</td>
<td>Polyurethane</td>
</tr>
<tr>
<td>EPU</td>
<td>Epoxy polyurethane</td>
</tr>
<tr>
<td>PC</td>
<td>phosphorylcholine</td>
</tr>
<tr>
<td>MPC</td>
<td>2-methacryloyloxyethyl phosphorylcholine</td>
</tr>
<tr>
<td>DX</td>
<td>Dexamethasone</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VP</td>
<td>N-vinyl pyrididone</td>
</tr>
<tr>
<td>DHPMA</td>
<td>2,3-dihydroxypropyl methacrylate</td>
</tr>
<tr>
<td>EGDMA</td>
<td>ethylene glycol dimethacrylate</td>
</tr>
<tr>
<td>IPN</td>
<td>interpenetrating polymer network</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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<tr>
<td>UV</td>
<td>Ultra-violet</td>
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<tr>
<td>EWC</td>
<td>Equilibrium water content</td>
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<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<tr>
<td>PVA</td>
<td>poly(vinyl alcohol)</td>
</tr>
<tr>
<td>PAAm</td>
<td>poly(acrylamide)</td>
</tr>
<tr>
<td>SD</td>
<td>sorption degree</td>
</tr>
<tr>
<td>S</td>
<td>sensitivity</td>
</tr>
<tr>
<td>$T_{90%}$</td>
<td>response time</td>
</tr>
<tr>
<td>$W_f%$</td>
<td>freezing water content</td>
</tr>
<tr>
<td>$W_{nf}%$</td>
<td>Non-freezing water content</td>
</tr>
<tr>
<td>HG</td>
<td>hydrogel</td>
</tr>
<tr>
<td>$T_g$</td>
<td>Glass transition temperature</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>EWF</td>
<td>equilibrium water fraction</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffer saline</td>
</tr>
<tr>
<td>I.V.</td>
<td>Intravascular</td>
</tr>
<tr>
<td>IACUC</td>
<td>Institutional Animal Care and Use Committee</td>
</tr>
<tr>
<td>$T_c$</td>
<td>Crystallization temperature</td>
</tr>
<tr>
<td>$T_m$</td>
<td>Melting temperature</td>
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<tr>
<td>EWF</td>
<td>Equilibrium water fraction</td>
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<td>FWF</td>
<td>Freezing water fraction</td>
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<td>NFWF</td>
<td>Non-freezing water fraction</td>
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Development of Novel Biocompatible Hydrogel Coatings
for Implantable Glucose Sensors

Chunyan Wang

ABSTRACT

Due to sensor -tissue interactions, currently none of the commercially available glucose sensors are capable of continuous, reliable monitoring of glucose levels during long-term implantation. In order to improve the lifetime of implanted glucose sensors, two series of biocompatible novel hydrogel coatings were designed, synthesized and the physical properties were measured.

Different hydrogels with various 2,3-dihydroxypropyl methacrylate (DHPMA) compositions were coated onto glucose sensors. Results show that the higher freezable water content, swelling rate and uniform porosity that resulted from high DHPMA content increased the sensitivity and shortened the response time of glucose sensors. The linear range of a glucose sensor coated only with hydrogel is short, however, the range can be improved by coating the epoxy- polyurethane (PU) with a layer of hydrogel. Since the hydrogel minimizes the fibrosis and inflammation, it shows promise for use in implantable glucose sensors. However, the in vivo experiment shows only 25% of sensors still worked after 4 weeks. In order to overcome problems present in the first series of experiments, another series of novel hydrogels with various N-vinyl pyrrolidone (VP) contents was developed. This study has provided a feasible approach to design and select the properties
of the copolymer for coating implantable biosensors. The in vivo experiments demonstrate that a hydrogel coating significantly improved the performance of implanted glucose sensors.

In order to suppress the acute inflammation caused by the surgery, dexamethasone-21 phosphate disodium salt (DX-21) was incorporated to a series of poly (HEMA-DHPMA-VP) hydrogels to investigate the drug delivery in vitro. All hydrogels showed a high initial release, followed by slow, long term release during the next hours to days. This release pattern is believed to be optimum for implanted glucose sensors suppressing the acute and chronic inflammation.

Water structures in hydrogels swollen in different media water, PBS and DX-21 solution were also investigated. 1HEMA:1DHPMA copolymer and VP-HEMA-DHPMA copolymers imbibed higher freezable water fractions in DX-21 solution. The ratio of transporting water mass to DX-21 mass is 9.6 which is independent of the hydrogel composition.
CHAPTER 1

INTRODUCTION

The aim of this project is to design, synthesize and characterize novel biocompatible hydrogels for the coatings of implantable glucose sensors and drug delivery system to enhance implanted glucose sensors’ function and lifetime in vivo.

This project has been funded by the National Institute of Health (NIH). The research has been conducted under the supervision of co-PI, Dr. Julie Harmon of the Department of Chemistry (USF).

1.1. Diabetes

Diabetes is a chronic disease which involves regulatory problems with the hormone insulin. Insulin is released from the pancreas to regulate the amount of glucose in the bloodstream. When the pancreas doesn’t produce the correct level insulin, or the body does not respond appropriately to insulin, the symptoms of diabetes occur. According to American Diabetes Association (ADA), there are 23.6 million children and adults in the United States, or 7.8% of the population, who have diabetes. (Diabetes, Oct.20, 2008)

There are two types of diabetes. Type 1 diabetes occurs when the insulin-producing cells of the pancreas (called beta cells) are destroyed by the
immune system. People with type 1 diabetes don’t produce insulin and must use insulin injections to control their blood glucose level. It is estimated that 5-10% of Americans who are diagnosed with diabetes have type 1 diabetes. Unlike people with type 1 diabetes, people with type 2 diabetes produce insulin. However, the insulin their pancreas secretes is either not enough or the body is unable to recognize insulin and use it properly. When there isn't enough insulin or the insulin is not used as it should be, glucose can't get into the body's cells. About 90 to 95% people with diabetes have type 2 diabetes. (Diabetes, Oct.21, 2008)

Long-term high glucose levels can cause a number of long-term, sometimes life-threatening complications including heart disease and stroke, high blood pressure, blindness, kidney disease, nervous system disease, dental disease; therefore it is crucial to control and monitor the blood glucose level.

1.2. Implantable Glucose Sensors

In order to regulate the blood glucose concentration, it is important for diabetics to have the ability to monitor blood glucose concentration. Diabetes patients are used to self-monitoring blood glucose levels by finger pricking to obtain blood samples. This kind of monitoring is discontinuous since it depends on how frequently the blood glucose detection is performed. It is very difficult to record frequent enough determinations every day because the patients are unwilling to withstand the pain associated with frequent finger pricking. Currently, continuous glucose monitoring systems, glucose sensors, are being developed as an alternative to
the present method. Glucose sensors can continuously detect changes of blood glucose levels, and therefore provide information on how to optimize insulin therapy and to control the patients’ metabolism.

Since the first enzyme electrode was constructed for the measurement of blood sugar in the 1960s (Clark et al. 1962), considerable amount of research has been devoted to the development of glucose sensors. Many kinds of glucose sensors have been studied including non-invasive glucose sensors such as optical glucose sensors or sensors designed to detect the glucose concentration in tears (Baca, 2006) or urine (Shieh, 1997), mini-invasive glucose sensors which can be implanted in the subcutaneous tissue, vascular bed (Armour et al. 1990; Frost and Meyerhoff, 2002) or transferring interstitial fluid outside the body and detecting by micro dialysis device (Steinkuhl et al. 1996). A non-invasive glucose sensor is the most optimum method for patients and because it overcomes the biocompatibility problems. However the precision of this method needs to be improved for clinical applications. Micro dialysis needs recalibration at least once daily and has the time lag between 5 and 45 min caused by the length and diameter of the tube and the rate of the pumping. Due to the risk of inserting the glucose sensor in the intravascular compartment for a long period, most studies have focused on the development of needle-type glucose sensors for subcutaneous glucose monitoring (Chen et al. 1992; Matthews et al. 1988).
Figure 1.1 Schematic diagram of the sensor. Figure adapted from (Moussy et al. 1993)
Figure 1.1 shows the typical needle-type implantable glucose sensor which is a two electrode system including a central platinum (Pt) anode as working electrode and a silver/ silver chloride (Ag/AgCl) reference electrode surrounding around Pt anode. Glucose oxidase (GOx) is immobilized between the coiled Pt electrode. The entire sensor is coated with a Nafion membrane to protect other components from biological degradation. Once glucose passes through Nafion membrane, in the presence of oxygen, it is oxidized by GOx and produces hydrogen peroxide (H₂O₂). Hydrogen peroxide is oxidized by a polarization voltage of about +700 mV at Pt electrode surface, thus producing an electric current that is monitored (Abel and von Woedtke, 2002). The chemical reactions are:

\[
\text{Glucose} + \text{O}_2 \xrightarrow{\text{GOx}} \text{Gluconic Acid} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 \xrightarrow{700\text{mV}} 2\text{H}^+ + \text{O}_2 + 2\text{e}^-
\]

In vitro and animal experiments initially showed encouraging results. However the long-term stability of the signal is insufficient due to the low biocompatibility of the electrode surface.

1.3. Biocompatibility of Implanted Glucose Sensors

The implanted glucose sensors are not reliable if they progressively lose function after several days in vivo. It is believed this is caused by the events that affect the sensor itself (e.g. degradation, fouling), as well as by changes in the tissue surrounding the sensor, caused by implantation (Moussy, 2000).
When glucose sensors are implanted within the body membrane, biofouling processes start immediately. Low molecular weight solutes, such as ions, small organic molecules, and high molecular weight composites, such as proteins and enzymes, will deposit on the surface of the sensors. Surface deposition of the low molecular weight solutes on the metallic electrodes, such as Pt, can cause degradation or corrosion of the metallic component. These problems can be avoided by coating electrodes with polymers such as polyurethane, cellulose and cellulose acetate, and Nafion. The initial step of protein adsorption could result in an unstable protein layer, which may alter or totally block the substrates’ transportation, and therefore diminishing sensor’s in vivo performance. Once a protein layer has formed, cellular interactions occur with protein coated sensors shown in Figure 1.2 (Fraser, 1997). These interactions lead to adhesion and activation of cells which characterize the inflammatory response. In such a case the diffusion distance of the glucose and oxygen is greatly increased, and the true glucose concentration surrounding the implantation site is different from that in non-inflamed subcutaneous tissue (Rebrin et al. 1992). Pickup et al. (Pickup et al. 1993) reported protein and cellular accumulation on the tips of the non-functioned glucose sensors after only five hours of implantation. Figure 1.3 shows scanning electron microscopy (SEM) Photographs of Tips of Glucose Sensors. The functioning sensor shows minimal biofouling, but the non-functioning sensor shows significant protein and cellular accumulation.
When substances from the body are able to penetrate the outer membranes and alter the metal electrode surface, electrode fouling, sometimes referred to as electrode passivation, occurs and causes a decrease sensor signal (Reddy and Vadgama, 1997; Linke et al. 1999).

In the case of fibrosis, the smooth solid sensors become surrounded by the fibrous capsule. The fibrous capsule surrounding the sensor presents a structurally distinct barrier of collagen fibrils, which might have an effect on the substrate mass transport, resulting in a delay in sensor response and an underestimation of analytes (Frazer, 1997). Figure 1.4 shows dense fibrous capsule tissue surrounding a glucose sensor tip after 10 days of implantation in subcutis (Ertefai and Gough, 1989).

Figure 1.2 The initial protein adsorption is followed by cellular adhesion onto the protein-coated surface of the device. Figure adapted from (Voskerician and Aderson, 2006)
Figure 1.3 SEM photographs of tips of glucose sensors. (A) Control sensor not implanted; (B) Functioning sensor showing minimal biofouling; (C) Non-functioning sensor showing significant protein and Cellular accumulation. Figure adapted from (Pickup et al. 1993)
Figure 1.4 Light micrograph image of glucose sensor tip after 10 Days of implantation in subcutis. Figure adapted from (Ertefai and Gough, 1989).
1.4. Strategies to Improve Sensor Biocompatibility

It can be seen that modification of the interface between sensor and tissue to control inflammation and fibrosis will enhance a glucose sensor’s biocompatibility, function and lifespan. Thus strategies to improve sensor biocompatibility include modifications to reduce protein adsorption and local drug delivery of tissue response modifiers.

Modifications to Reduce Protein Adsorption

Protein adsorption is controlled by the characteristics of the material including topography, charge density, distribution and mobility, surface groups (chain length, hydrophobicity, and hydrophilicity), structural ordering (soft to hard segment ratio and distribution), and the extent of hydration (Wisniewski et al., 2000), therefore the simple strategy to improve sensor biocompatibility is to reduce protein adsorption by surface modification. One approach to modify the surface of glucose sensors is to incorporate poly(ethylene glycol) (PEG) into a poly(hydroxyethylmethacrylate). The PEG chains tend to line up parallel to each other and perpendicular to the surface to present a water rich phase that resists penetration by many proteins (Claesson, 1993). Compared to the same membranes without PEG, PEG in the outer membrane induced less fibrous encapsulation after subcutaneous implantation in rats. But there is no report about improvement in sensor performance (Quinn et al. 1995; Quinn et al. 1997).

The diamond-like carbon (DLC), so-called “inert” materials are also used to coat on glucose sensors to enhance hemocompatibility as determined by both
sensitivity change and surface deposition of blood components examined by SEM. (Higson and Vadgama, 1995).

Another strategy to reduce protein adsorption is to develop biomimetic coatings containing phosphorylcholine (PC) groups. It has been demonstrated that PC coated glucose sensor could diminish protein adsorption (Yang et al. 2000). One example of making biomimic membrane is to self-assemble phospholipids on the polymer surface containing a phosphorylcholine group, 2-methacryloyloxyethyl phosphorylcholine (MPC) and hydrophobic alkyl group. The surface shows an excellent resistance for both protein adsorption and blood cell adhesion. The relative output current of the sensor covered with this membrane was maintained as the initial level even after 14 days of subcutaneous implantation in a rat (Ishihara, 2000).

Other methods to reduce protein adsorption include the use of alginate/polylysine gel (Shichiri et al. 1988), PHEMA/polyurethane (PU)(Shaw et al. 1991), NafionTM (perfluorosulphonic acid) membrane(Wilkins et al. 1995; Moussy et al. 1994), crosslinked albumin(Armour et al. 1990), cellulose(Kerner et al. 1993).

Local Drug Delivery Strategies

Site specific, controlled delivery of tissue response modifiers can be used alone or combine with above surface modifications to help control the tissue reaction. The anti-inflammatory drug dexamethasone (DX) contained in microspheres is incorporated into a hydrogel and coated onto glucose sensors. The in vivo experiment results show that eluting dexamethasone successfully controled negative
tissue reactions at the sensor-tissue interface by reducing the level of inflammation-mediation cells to those observed in normal tissue (Patil et al. 2004; Norton et al. 2005).

Controlled nitric oxide (NO) or vascular endothelial growth factor (VEGF) delivery also can suppress the tissue reactions and improve biosensor performance (Norton et al. 2005; Shin and Schoenfisch, 2006).

1.5. Hydrogel

Hydrogels are three-dimensional cross-linked polymer networks which are designed to swell but not dissolve in water. They can be chemically cross-linked by a covalent bond with multi-functional cross-linkers or physically cross-linked by physical cross-links from entanglements, association bonds such as hydrogen-bond, Van der Waals forces, hydrophobic forces or crystalline forces (Peppas, 1986, V1).

Due to high water absorption, low interfacial tension, high permeability to small molecules, and the soft and rubbery nature, hydrogels are well suited for biomaterials. Therefore, the biomedical applications of hydrogels, such as sutures, contact lenses, and drug delivery vehicles are of most interest. Table 1.1 lists some biomedical applications of hydrogels summarized from Peppas’Book (Peppas, 1986).
Table 1.1 Some biomedical applications of hydrogels

<table>
<thead>
<tr>
<th>Biomedical applications</th>
<th>Example Materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood compatible applications</td>
<td>Poly(vinyl alcohol)(PVA), Polyacrylamide(PAAm), Poly(N-vinylpyrrolidone)(PVP), Poly(hydroxyl-methacrylate)(PHEMA), poly(ethylene, oxide)(PEO), Poly(ethylene glycol)(PEG), cellulose</td>
</tr>
<tr>
<td>Contact lenses</td>
<td>PHEMA, P(VP-co-HEMA), acrylamide and acrylonitrile based hydrogels, P(HEMA-co-MMA)</td>
</tr>
<tr>
<td>Artificial tendons</td>
<td>Fiber (polyethylene terephthalate) reinforced hydrogel</td>
</tr>
<tr>
<td>Controlled release devices</td>
<td>PHEMA, P(HEMA-methyl acrylate) copolymers, P(HEMA-MMA) copolymers, P(HEMA-polymethacrylic acid) copolymer, PVA, PVP,</td>
</tr>
<tr>
<td>Membranes for biomacromolecular separations for plasmapheresis, artificial kidneys, artificial liver</td>
<td>P(glycerolmethacrylate-co-MMA), P(Hydroxypropyl methacrylate-MMA), pure PHEMA, PVA, PVP</td>
</tr>
<tr>
<td>Sutures</td>
<td>PHEMA</td>
</tr>
<tr>
<td>Artificial skins</td>
<td>PVA-formaldehyde foam, PHEMA</td>
</tr>
</tbody>
</table>
Hydrogels commonly are classified into neutral hydrogels, ionic hydrogels and swollen interpenetrating polymeric networks (IPN). Neutral hydrogels are based on neutral monomers, while ionic hydrogels are based on the ionic monomers such as acryl acid, methacrylic acid. An interpenetrating polymer network (IPN) is a combination of two polymers one of which must be synthesized, or crosslinked in the immediate presence of the other. For an interpenetrating polymeric network (IPN) hydrogel system, there is no chemical bonding between two polymer networks, but there are physical entanglements (Liu et al. 2003). Covalent crosslinked gels do not dissolve in organic solvents even upon the addition of heat; whereas non-covalent crosslinked gel will eventually dissolve in solvents or melt upon the addition of heat (LaPorte, 1997). Thus physically crosslinked gels are not stable in heat and solvent environment. The properties of synthetic hydrogels can be controlled by chanaging the ratio of hydrophilic to hydrophobic monomers. Neutral hydrogels are more biocompatible than the ionic hydrogels since more proteins will absorb on the surface of ionic materials (Peppas, 1986), and crosslinked neutral hydrogels are more stable than the interpenetrating hydrogels. In this project the neutral hydrogels are polymerized for the sensor coatings because the nonionic hydrogels exhibit minimal protein build-up and excellent pH stability (Gates and Harmon, 2001).

Some common synthetic hydrogels being used as biomaterials include PHEMA, poly(2,3-dihydroxypropyl methacrylate) (PDHPMA), PAAm, PVP, and PVA.
Poly(hydroxyalkyl methacrylates)(PHEMA) Hydrogels

PHEMA is the most studied hydrophilic polymer in the biomedical industry. Since Wichterle and Lim polymerized HEMA hydrogels in 1960 (Wichterle and Lim, 1960) for contact lenses, PHEMA hydrogels have received a lot of interest.

The biocompatibility of PHEMA hydrogels is primarily due to high water content absorbed into the hydrogel network. Initiators, reaction by-products, residual monomer, and impurities can be extracted with successive aqueous washings, so the likelihood of toxic or local immune reactions decreases in biomedical applications. When used as an implant, the rubbery nature of the hydrated hydrogel reduces mechanical irritation of surrounding tissue. The low interfacial tension between the hydrogel surface and aqueous solution minimizes protein adsorption and possibly cell adhesion (Ratner and Hoffman, 1976).

Cross-linker impurities are present in all methacrylate monomers, so it is usual to obtain crosslinked gel instead of linear PHEMA. PHEMA hydrogels can be prepared by bulk polymerization or solution polymerization using heat initiators or photoinitiators. The most popular method for preparing PHEMA hydrogels is by solution polymerization of the monomer, HEMA, in the presence of a cross-linking agent. Different solvents can be used for solution polymerization such as dimethyl sulfoxide(DMSO), propanol, glycerol, ethylene glycol, cyclohexanol, toluene, and dimethyl formamide. Water is commonly used as a solvent in the preparation of PHEMA hydrogels. Below a critical solvent concentration (40 to 60% water by
weight), the gel thus formed is homogeneous, nonporous, and optically transparent. When the solvent concentration is above this critical value, the resulting gel is heterogeneous and has a macro-porous structure, and is optically opaque (Laporte, 1997).

The water content of PHEMA hydrogels is usually relatively low(<45 wt%), which results in the limited applications of these hydrogels (Lee and Lin, 2003). Furthermore, PHEMA does not have the stringent mechanical properties needed for many biomedical applications. It was found that mechanical properties could be improved by crosslinking with ethylene glycol dimethacrylate(EGDMA). Water content can also be increased by copolymerizing with other monomers such as DHPM or vinyl monomer, VP (Laporte, 1997). (Table 1.2)

**Poly(N-Vinyl-2-pyrolidone)(PVP) Hydrogels**

The backbone and ring structure of PVP provide hydrophobicity while the highly polar amide group provides hydrophilicity similar to that of a protein, but is physiologically inactive. The size of VP repeating structure along the main carbon chain produces free volume in the material, resulting in flexibility and water solubility. PVP was first used as a blood plasma substitute and extender during World War II (Robinson et al. 1990) VP can also be copolymerized with other materials to modify properties for various medical applications (Laporte, 1997). PVP is a major component of some contact lenses and has been proposed as a material that can enhance blood compatibility (Francois et al. 1996).
PVP hydrogels have also been used as cell culture substrates. Interpenetrating polymer networks (IPNs) composed of PVP and gelatine have been studied as non-fouling materials for devices in contact with blood (Lopes and Felisberti, 2003). It has been shown that 70% PVP exhibits optimum swelling degree and compression strength. In biocompatibility tests, 70% PVP hydrogels showed no deleterious effect to the cells but were not good as gelatin for cell growth. As it turns out, 50% PVP retains the best properties of both polymers with higher water swelling degree, compression strength and biocompatibility. Risbud et al. (Risbud et al. 2000) reported a growth promoting effect in a PVP-co-chitosan (a linear polysaccharide) hydrogel as compared to chitosan gels alone. Janson et al. studied porous VP-BMA (n-butyl methacrylate) copolymers as scaffolds for bone tissue engineering. In their study they reported that 50:50 (VP: BMA) is less cytotoxic and more biocompatible than 70:30 counterpart in vivo (Janson et al. 2005). From these research results, it can be concluded that VP can enhance the water content, mechanical properties and porous structures of hydrogels at the same time, behavior unusual for a hydrogel. High VP percent hydrogels show less biocompatibility, but 50% of PVP hydrogels are biocompatible. Furthermore, PVP can be coated on the polyurethane surface and firmly attach to the surface (Francois et al. 1996). This property is essential for this project.
<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Structure</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-hydroxyethyl methacrylate (HEMA)</td>
<td><img src="image1" alt="Structure" /></td>
<td>130.14</td>
</tr>
<tr>
<td>2,3-dihydroxypropyl methacrylate (DHPMA)</td>
<td><img src="image2" alt="Structure" /></td>
<td>160.17</td>
</tr>
<tr>
<td>N-Vinyl-2-Pyrrolidinone (V P)</td>
<td><img src="image3" alt="Structure" /></td>
<td>111.14</td>
</tr>
<tr>
<td>Ethylene dimethacrylate (EGDMA)</td>
<td><img src="image4" alt="Structure" /></td>
<td>198.22</td>
</tr>
<tr>
<td>1-phenyl-2-hydroxy-2-methylpropan-1-one (Benacure 1173)</td>
<td><img src="image5" alt="Structure" /></td>
<td>164.00</td>
</tr>
</tbody>
</table>
Poly (2,3-dihydroxypropyl methacrylate) (PDHPMA)) Hydrogels

In comparison with PHEMA, PDHPMA is more hydrophilic and even soluble in water since it contains two hydroxyl groups in each of its repeat units (Hou et al. 2002). DHPMA is often copolymerized with HEMA or VP to make hydrogels for soft contact lenses (Wang et al. US patent). Clinical experiments show that hydrogels used as contact lenses will dehydrate especially at high water contents (McConville and Pope, 2001). Gates found that DHPMA exhibits decreased water loss rate (Gates et al. 2003).

Photopolymerization of Hydrogel by Ultraviolet light (UV)

Polymerization of hydrogels can be initiated by γ radiation, heat or Ultraviolet (UV) radiation with initiators or they can self-initiate. UV radiation is frequently used to induce cross-linking of polymers, because it requires less time, less energy, and generates little heat during polymerization (Laporte, 1997). In addition, heat sensitive substrates such as plastics, printed circuit boards, paper, and wood can be coated and cured by UV (Nichols et al. 2001; Koleske, 2002).

There are three main components in UV curing system: monomers or oligomers, photo-initiators and additives. First, photo-initiators absorb high intensity UV light and are raised to an excited state. From their radiation-excited state, the photo-initiators photolyze or degrade directly or indirectly into free radicals. These free radicals initiate the very rapid polymerization and crosslinking of monomers (Lee et al. 2006). Steps of UV polymerization are listed in Figure 1.5.
According to the process for forming the initiating radicals, photo-initiators can be divided into two classes. Norrish Type I photo-initiators (cleavage) photolyze through a homolytic fragmentation mechanism or through a-cleavage, and thereby directly form free radicals capable of initiating polymerization. (Figure 1.6)

Norrish Type II photo-initiators (H-abstraction) are activated with radiation and form free radicals by hydrogen abstraction or electron extraction from a second compound (coinitiator) that becomes the actual initiating free radical. In addition, the Norrish Type II reactions are intra-molecular, so Type II photo-initiators are more easily affected by the environment such as oxygen and viscosity (Lee et al. 2006).

The majority of Type I photo-initiators are aromatic carbonyl compounds which can carry out fragmentation by α-cleavage or β-cleavage. The most important fragmentation in photo-initiator molecules is the α-cleavage of the carbon-carbon bond between the carbonyl group and the alkyl residue in alkyl aryl ketones (Figure 1.7). 1-phenyl-2-hydroxy-2-methyl-propan-1-one (Benacure 1173) is a very popular photo-initiator (structure shown in Table 1) because it is highly reactive, thermally stable, does not yellow and can be used in water based formulations (Crivello and Dietliker, 1998).
Figure 1.5 Various steps in a photoinitiated polymerization
Type I Photoinitiator: unimolecular fragmentation

\[ \text{PI} \xrightarrow{hv} \text{PI}^* \rightarrow \text{unimolecular reaction (fragmentation)} \rightarrow R_1^- + R_2^- \]

Type II photoinitiator: bimolecular fragmentation

\[ \text{PI} \xrightarrow{hv} \text{PI}^* + \text{COI} \rightarrow \text{biomolecular reaction} \rightarrow R_1^- + R_2^- \]

Figure 1.6 Types of photoinitiator. (Crivello and Dietliker, 1998)
Previous investigations into the biocompatibility of PHEMA and PDHPMA as homo- and copolymers by UV polymerization showed that hydrogels containing 80% DHPMA and 20% HEMA were found to induce minimal to no fibrosis when implanted subcutaneously in rats (Mohomed et al. 2006). Unfortunately, as it sorbed large amounts of water, the mechanical stability of the high content DHPMA copolymers (80% DHPMA:20% HEMA) and the PDHPMA homopolymer decreased, and the samples were easily fragmented. The biocompatible hydrogels were also coated onto the polyurethane (PU)/epoxy coated metal sensor by dip-coating, or in situ polymerization. It was noted that the PHEMA coating easily delaminated from the PU/epoxy coating once swollen in water (Mohomed, 2006).

To improve the lifespan of glucose sensors, the objectives of hydrogel coatings are:
1) the coating must allow glucose, oxygen and hydrogen peroxide to diffuse freely, while causing minimal fibrosis and inflammation.

2) it should be easily coated onto the surface of implantable glucose sensors

3) it should attach firmly and permanently to the glucose sensors and strong enough not to break during the implantation period.

4) it must not deter the performance of the implantable glucose sensors.

5) it should deliver anti-inflammatory drug and release drug to suppress both acute and chronic inflammatory.

According to previous work by Mohomed, the formulations of hydrogel have based on HEMA and DHPMA copolymer for its excellent biocompatibility, but well modified with another monomer to improve the coating properties.
CHAPTER 2

SYNTHESIS AND PERFORMANCE OF NOVEL HYDROGELS COATING
FOR IMPLANTABLE GLUCOSE SENSORS

2.1. Introduction

State of the art implantable glucose sensors do not work reliably and have a short life after implantation (Moussy, 2002). This in vivo loss of function is caused by tissue reactions surrounding the sensor such as fibrosis and inflammation (Mang et al. 2005). In order to improve the lifetime of implantable glucose sensors, the biocompatibility of the coatings must be monitored (Gerritsen et al. 2000). Many biocompatible coatings have been prepared for sensors such as hydrogels (Praveen et al. 2003; Galeska et al. 2001; JP 60171140; USP 5786439), silica-based hybrid materials (Kros et al. 2001), sol-gel coatings (Gerritsen et al. 2000), diamond-like carbon films and diamond-like carbon-coated anodized aluminum oxide nanoporous membranes (Narayan et al. 2007). All of these coatings showed some potential for use in sensors, but formulations need to be optimized at this time.

The outer interfacial coatings of implantable glucose sensors must be stable in biological fluids, ensure the transport of glucose to the sensors, resist the deposition of the proteins and other interferents and minimize fibrous encapsulation. Three-dimensional cross-linked polymer networks known as hydrogels have been shown to
meet these requirements and have the added benefit of swelling in water without dissolving (Praveen et al. 2003; Kros et al. 2001; Gerritsen et al. 2000).

Poly(hydroxyethyl methacrylate) (PHEMA) hydrogels were first polymerized and studied for biological use by Wichterle and Lim in the early 1960s (Wichterle and Lim, 1960; Wichterle and Lim, 1961). Since they are biocompatible, PHEMA hydrogels have been used in biomedical and pharmaceutical applications such as contact lenses, implants and drug delivery systems. PHEMA was first used in glucose sensors by John Christopher, et al in 1990 (Pickup and Claremont, 1990). Sensors coated with PHEMA were stable for 24hrs in vitro at 37°C. However, much longer life times are required. PHEMA hydrogels are clear, exhibit smooth surfaces and are biocompatible (Rozakis et al. 2005). Drawbacks of using PHEMA gels are that their mechanical properties are poor and equilibrium water contents are relatively low, <45 wt% (Lee and Lin, 2003); water uptakes of 200 wt% are needed for optimum operation (Van Antwerp et al. 1998). Mechanical properties can be increased by preparing PHEMA hydrogels with ethylene glycol dimethacrylate cross-linker (EGDMA). Equilibrium water contents can be increased by preparing PHEMA hydrogels with more hydrophilic monomers such as 2, 3-dihydroxypropyl methacrylate (DHPMA) (Yasuda et al. 1966; Macret and Hild, 1982) or N-vinyl pyrolidinone (VP) (Laporte, 1997). According to previous investigations of hydrogel (80%DHPMA:20%HEMA) coating for implantable glucose sensors, it was found that the biocompatibility and equilibrium water content are improved (Mohomed. 2006).
Unfortunately, as it sorbed large amount of water the mechanical stability of the high content DHPMA copolymers (80% DHPMA: 20% HEMA) decreased, and the samples were easily fragmented. The biocompatible hydrogels were also coated onto the polyurethane (PU)/epoxy coated metal sensor by dip-coating, or in situ polymerization. It was noted that hydrogel coatings easily delaminated from the PU/epoxy coating once swollen in water (Mohomed, 2006).

Since PVP was first used as a blood plasma substitute and extender during World War II (Robinson et al. 1990), VP can be copolymerized with other materials to modify properties for various medical applications (Laporte, 1997). VP can enhance the water content, mechanical properties and porous structures of hydrogels at the same time, behavior unusual for a hydrogel. High VP percent hydrogels show less biocompatibility, but 50% of PVP hydrogels are biocompatible (Lopes and Felisberti, 2003; Risbud et al. 2000). Furthermore, PVP can be coated on the polyurethane surface and firmly attach to the surface (Francois et al. 1996). This property is essential for this project.

The goal of this study was to develop a novel hydrogel copolymer with HEMA, DHPMA, VP and EGDMA which was facile to coat on the glucose sensors, did not deter the detection of glucose and exhibits improved biocompatibility. In order to optimize the compositions of hydrogels swelling, SEM and water structure of hydrogels were characterized. Different formulations hydrogels were coated on the implantable glucose sensors by UV curing. Glucose sensors used for this study were
based on the long term excess enzyme loading coil-type implantable amperometric sensors (Yu et al. 2005; Yu et al. 2006). *In vitro* and *in vivo* evaluations of the hydrogel coated glucose sensors with and without PU were also undertaken.

### 2.2 Experimental Procedures

#### 2.2.1 Materials.

2-Hydroxyethyl methacrylate (HEMA) and 2, 3-dihydroxypropyl methacrylate (DHPMA) were donated by Benz R & D (Sarasota, FL, USA). They were used as received without further purification. N-Vinyl-2-Pyrrolidinone (VP) (99.94%) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and purified by vacuum distillation to obtain a colorless liquid according to reference (Jansen et al. 2005). Ethylene glycol dimethacrylate (EGDMA), glucose oxidase (GOX) (EC 1.1.3.4, Type X-S, Aspergillus Niger, 157,500U/g), benzoic acid, gentamicin, ATACS5104 epoxy adhesive, Fluka Selectophore® 81367 polyurethane (PU) and tetrahydrofuran (THF) were obtained from Sigma -Aldrich Co. (St. Louis, MO, USA). 1-phenyl-2-hydroxy-2-methyl-propan-1-one (Benacure 1173) from Mayzon Corporation (Rochester, NY, USA) was used as received. Dextrose (D-glucose), bovine serum albumin (BSA), and glutaraldehyde (50%) were obtained from Fisher Scientific (Pittsburgh, PA). ø 0.125 mm Teflon-covered platinum-iridium (9:1 in weight) and silver wires were obtained from World Precision Instruments, Inc. (Sarasota, FL, USA). High temperature mini round glue sticks were supplied by Adhesive Tech (Hampton, NH, USA). Hematoxylin and eosin were obtained from
Fisher Scientific (Pittsburgh, PA) and trichrome stains were purchased from Newcomer Supply (Middleton, WI).

2.2.2. Synthesis of Poly (HEMA-DHPMA-VP-EGDMA) Hydrogel Series

Various molar ratios of HEMA and DHPMA were weighed and mixed in 20 ml glass vials. 9 mol% of VP, 1 mole% EGDMA and 1.5 mol% initiator Benacure 1173 were added to the solutions (Table 2.1). The solutions were diluted 1:1 with distilled water. After deoxygenation with argon gas, the solutions were injected into hand made glass cells measuring 55mm × 25mm × 1mm. The sides of the cells were secured with a glue gun. The solution filled cells were placed under a UV lamp (Spectronics Corporation, Westbury, NY, USA) at a wavelength of 254nm for 100min. Argon gas was used to purge the system in order to minimize oxygen inhibition of the reaction. Transparent uniform sheets were obtained and these xerogels were swollen in distilled water to yield hydrogels. The water was refreshed every day for one week to remove any unreacted monomers.

The hydrogels were freeze-dried for various studies using a Virtis Freezemobile 12XL freeze dryer (The Virtis Company Inc, Gardiner, NY, USA) to keep the pores inner morphology.
Table 2.1 Feed compositions of synthesized xerogels

<table>
<thead>
<tr>
<th>Mole%</th>
<th>HEMA</th>
<th>DHPMA</th>
<th>VP</th>
<th>EGDMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHPMA0</td>
<td>90</td>
<td>0</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>DHPMA20</td>
<td>70</td>
<td>20</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>DHPMA40</td>
<td>50</td>
<td>40</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>DHPMA60</td>
<td>30</td>
<td>60</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>DHPMA80</td>
<td>10</td>
<td>80</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>DHPMA90</td>
<td>0</td>
<td>90</td>
<td>9</td>
<td>1</td>
</tr>
</tbody>
</table>

2.2.3 Scanning Electron Microscopy (SEM)

The hydrogel samples obtained by the above method were cut and freeze-dried to determine the inner morphology. The morphology of the freeze-dried gels were studied via a Hitachi Scanning Electron Microscope S-800 (Hitachi High Technologies, Pleasanton, CA) with 20nm gold coating by a HummerX sputter coater (Anatech, Ltd, Springfield, VA). The working distance between the sample and electron (WD) was 5mm with 25.0 kV.

2.2.4 Sorption Experiments

Hydrogel samples were cut with a 12mm diameter cork bore to obtain uniform shapes. All sheets were freeze-dried for one day and stored in a desiccator at room temperature. The sorption behavior of hydrogels was monitored by detecting the increase in mass of the samples at different time intervals by Sartorius BP211D balance (± 0.01mg, Sartorius Corporation, Edgewood, NY, USA). In a typical sorption experiment, a pre-weighed dry gel sheet was immersed into water at 24±1°C.
in a Fisher Scientific Isotemp water bath (Pittsburgh, PA). At a prescribed time intervals the hydrogel was taken out of solution and weighed after wiping off the excess water from the surface with Kimwipe paper (Kimberly-Clark Professional).

The sorption degree, SD, of hydrogels was defined as follows:

\[
SD\% = \frac{W_d - W_i}{W_d} \times 100 = \frac{M_t}{W_d} \times 100
\]

(2-1)

Where \( W_d \) is the weight of the dry gel, \( W_i \) is the weight of wet hydrogel at each time interval, and \( M_t \) is the gain in the weight of the dry gel at time \( t \).

The equilibrium water content (EWC) of the hydrogel was determined using the following equation:

\[
EWC\% = \frac{W_\infty - W_d}{W_\infty} \times 100 = \frac{M_\infty}{W_\infty} \times 100
\]

(2-2)

where \( W_\infty \) and \( M_\infty \) are the weight and weight gain of the swollen hydrogel at equilibrium separately.

2.2.5 Differential Scanning Calorimetry (DSC)

All calorimetric data were obtained via a TA Instruments 2920 differential scanning calorimeter (DSC, TA Instruments, New Castle, Delaware). Nitrogen gas was passed through the instrument at a flow rate of 70ml/min. Before measurement the DSC was calibrated from -100℃ to 250℃ at a heating rate of 5℃/min with an indium standard. All the sample masses for DSC ranged from 4mg to 10mg.

For the determination of glass transition temperature, samples were freeze-dried for one day and kept in a desiccator. The samples were quickly weighed, sealed in aluminum pans, and immediately scanned from 30℃ to 200℃.
Samples were cooled and rescanned; the glass transition was determined from the second heating cycle in order to minimize any aging effects. The reported glass transition temperature was determined as midpoint at half heat flow or heat capacity.

To measure the water structure of equilibrium swollen hydrogels, first samples were cut and weighed accurately after wiping off the surface water with Kimwipe paper and immediately sealed in Al pans. The DSC curves were measured heating from -100°C to 20°C with a heating rate 5°C/min. Endotherm areas were determined.

2.2.6 Sensor Preparation

Hydrogel-coated glucose sensors used for this study were based on the coil-type implantable sensor previously described (Yu et al. 2005; Yu et al. 2006). The detail fabrication method of Pt/GOx and Pt/GOx/Epoxy-polyurethane base sensors can be found in those papers. The coating process was achieved by applying a ~ 0.4 µL of 50% hydrogel solution to either the enzyme layer or the epoxy-polyurethane layer, using a 10 µL micropipette, and then curing under an argon atmosphere and a 254 nm UV light for 100 min. Membrane surfaces were observed and photographed using an Olympus BX41 microscope (Quantitative Imaging Co., Canada). The cured hydrogel coating was transparent and firmly attached to the sensor as shown in Figure 2.1.

2.2.7 In vitro Evaluation Method

Electrochemical measurements were performed with Apollo 4000 4-Channel Potentiostats (World Precision Instruments, Inc., Sarasota, FL). Newly prepared
glucose biosensors were conditioned in a 5mM glucose /PBS (NaCl 8.76g, KH$_2$PO$_4$ 3.53g, Na$_2$HPO$_4$ 3.40g, Benzoic acid 2g, water 10000ml) (ionic strength = ~ 0.16M) for at least 2 hours and then continuously polarized at +0.7V vs. Ag/AgCl until a stable background current was reached. The response was determined as the time needed for the sensor’s current to reach 90% of (T$_{90\%}$, min) the maximum current when the glucose concentration changed from 5 mM to 15 mM. The sensitivity (S) was determined using a two-point method and can be calculated by

$$S \text{ (nA/mM)} = \frac{I_{15\text{mM}} - I_{5\text{mM}}}{10}$$

(3)

where $I_{15\text{mM}}$ and $I_{5\text{mM}}$ represent the sensor response currents obtained in 15 mM and 5 mM glucose solutions, respectively. The sensors for long-term observation were stored in PBS. Calibration plots were obtained by stepwise adding 100 mM glucose solutions to 8.0 mL PBS. All measurements were performed at 24±1°C.

2.2.8 In vivo Biocompatibility Studies

The epoxy-PU coated Silastic tubing (Dow Corning, Midland, MI) ($\phi$ = ~ 4 mm, length = ~ 50 mm), a DHPMA 80 bar ($\phi$ = ~5 mm, length = ~50 mm), an epoxy-PU coated glucose sensor and a DHPMA 80 coated glucose sensor were implanted subcutaneously in rats for 28 days. The tissues surrounding the implants were removed at day 28 and processed for histology with hematoxylin, eosin and trichrome stains (Galeska et al. 2001).
**Figure 2.1** Sensor photos show thin layers of hydrogel coating: A. hydrogel on Pt/GOx sensor B. hydrogel on Pt/GOx sensor after swelling C. hydrogel on Pt/GOx/Epoxy-polyurethane base sensors D. hydrogel on Pt/GOx/Epoxy-polyurethane sensors after swelling
2.3 Results and Discussion

2.3.1 Preparation of Hydrogels Sheets and Coatings

A type I photoinitiator, Benacure 1173, undergoes unimolecular bond cleavage upon irradiation in argon atmosphere. It produced well cured samples, including the thin layers used on the sensors (Lewis et al. 1975; Maillard et al. 1983). The water present in the polymerization mixture minimized the heat of polymerization and bubble-free samples were obtained. The amount of solvent in the reaction mixture did not exceed the critical solvent concentration which would result in heterogenous samples (Peppas, 1986). In order to avoid producing bubbles and obtain the homogenous transparent hydrogel, monomers were dissolved in water with the weight ratio around 1:1. Even if there was a little heat produced, it would diffuse readily, and would not yield big bubbles inside the hydrogel. In this work, homogenous hydrogel sheets and coatings of implantable glucose sensor were obtained by UV cured.

2.3.2 Inner Sample Morphology

The scanning electron micrographs shown in Figure 2.2 depict the dramatic effect of DHPMA on morphology. The sample with no DHPMA exhibits irregular pores. At 20% DHPMA the number and regularity of the pores increase. This trend increases up to 80% DHPMA. At 90% DHPMA the structure is less regular, possibly due to the high swelling stresses which can induce tearing in the sample. Coatings for implantable glucose sensors must effectively transport water, glucose
Figure 2.2 SEM micrographs of inner gels (× 350 magnification): D0, D20, D40, D60, D80 and D90
and oxygen. From the morphology of different gels it can be seen that 80% DHPMA gives the most uniform, porous structure and therefore, might be an optimum candidate for permeable sensor coatings.

2.3.3 Sorption Behavior

The sorption versus time profile is shown in figure 2.3. Both the equilibrium water content and the sorption rate increase with DHPMA content. This is due to the extra hydroxy group in DHPMA which increases the hydrophilicity. It has also been shown that increasing DHPMA content can slow the dehydration rate and increase the rehydration rate of the sample (Gates et al. 2003; Pescosolido et al. 1990). This is ideal for the implantable glucose sensors, since dehydration reduces biocompatibility (McConville and Pope, 2001). It has been stated that glucose sensor coatings should exhibit the equilibrium sorption degrees of over 120%, and, preferably over 200% (Van Antwerp et al. 1998). Figure 2.3 demonstrates that the 80% DHPMA, again, is an optimum formulation for sensors.

The water diffusion coefficient can be easily calculated when diffusion controls the transport process. It is well known that sorption behavior in polymers is quite complex (Harmon et al. 1988; Harmon et al. 1987). At one extreme, Fickian diffusion controls kinetics. However, since swelling involves segmental relaxation, the relaxation rate can influence transport. If the relaxation rate is slower than the diffusion rate, relaxation can dominate transport kinetics. In reality, sorption behavior can range from pure Fickian behavior to pure relaxation behavior. In pure
Fickian behavior, sorption versus the square root of time curves are linear up until about 60% of the equilibrium water content is imbibed \( \left( \frac{M_t}{M_\infty} \leq 0.6 \right) \). When relaxation dominates transport, sorption is linear with respect to time. The extent of influence from Fickian diffusion and relaxation is characterized by the time exponent in the following equation (Franson and Peppas, 1983; Alfrey et al. 1966):

\[
\frac{M_t}{M_\infty} = Kt^n \quad (2-4)
\]

Where, \( t \) is time, \( k \) is a constant and \( n \) is the time exponent. The time exponent, \( n \), varies from 0.5 for pure Fickian control to 1 for pure relaxation control. Table 2.2 and Figure 2.4 show that transport kinetics are controlled by diffusion in these hydrogel systems, since \( n \) is close to 0.5 (Wang and Wu, 2005).
Figure 2.3 The swelling degree as a function of time for the different DHPMA fraction hydrogels

Figure 2.4 \( \log \frac{M_t}{M_\infty} \) versus logTime for the different DHPMA fraction hydrogels
Table 2.2 Dynamic Swelling Parameters

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>$\frac{M_t}{M_\infty}$</th>
<th>$R^2$</th>
<th>$D(\text{cm}^2\text{s}^{-1})\times10^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHPMA0</td>
<td>0.57</td>
<td>0.072-0.5690.993</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>DHPMA20</td>
<td>0.52</td>
<td>0.094-0.5570.994</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>DHPMA40</td>
<td>0.47</td>
<td>0.111-0.5540.996</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>DHPMA60</td>
<td>0.45</td>
<td>0.135-0.5320.993</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>DHPMA80</td>
<td>0.41</td>
<td>0.263-0.5890.991</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>DHPMA90</td>
<td>0.32</td>
<td>0.343-0.5680.994</td>
<td>4.5</td>
<td></td>
</tr>
</tbody>
</table>

All sample shapes are similar and the ratio of thickness (L) to diameter ($D_i$) is about 1:10. It is assumed that water diffuses into a planar sheet at a constant rate.

When $\frac{M_t}{M_\infty}\leq0.5$ the relationship between $\frac{M_t}{M_\infty}$ and time will fit equation 5 (Crank, 1978).

$$\frac{M_t}{M_\infty} = \frac{4}{L} \left( \frac{Dt}{\pi} \right)^{0.5}$$

The diffusion coefficient ($D$) values are estimated according to equation (5) and shown in Table 2.2. From Table 2.2 it can be seen that ($D$) increases with DHPMA content. All the swelling kinetics data show that when DHPMA content is increased the swelling ratio and water diffusion rate increased. Since water acts as the diffusion medium between body fluid and the glucose sensor the faster the water transport characteristics of the hydrogel coating the better the detection of the glucose concentration.
2.3.4 DSC Results

Table 2.3 shows that the Tₙₛ of different xerogels composition do vary with DHPMA content. This trend was noted this effect in an earlier study (Gates et al. 2003). The dihydroxypropyl side chain in DHPMA imparts greater free volume, which can facilitate the mobility and decrease the Tₙₛ. However, this effect is offset by the extra hydroxyl group which forms hydrogen bonds in the network structures and restricts mobility.

<table>
<thead>
<tr>
<th>DHPMA%</th>
<th>Tg(℃)(H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) 0</td>
<td>118</td>
</tr>
<tr>
<td>(2) 20</td>
<td>119</td>
</tr>
<tr>
<td>(3) 40</td>
<td>120</td>
</tr>
<tr>
<td>(4) 60</td>
<td>117</td>
</tr>
<tr>
<td>(5) 80</td>
<td>117</td>
</tr>
<tr>
<td>(6) 90</td>
<td>120</td>
</tr>
</tbody>
</table>

It is well known that the biocompatibility of hydrogels not only depends on the equilibrium water content but also on the state of water in the hydrogels (Kim et al., 1980; Mirejovsky et al. 1991; GOxa et al. 2006; Jhon and Andrade, 2004). Some techniques employed to study water structure in hydrogels are NMR (Chowdhury et al. 2004; Capitani et al. 2001), dilatometry and electrical conductivity (Lee et al. 1975; Choi et al. 1977), dielectric relaxation spectroscopy (Kyritsis et al. 1995),
dynamic-mechanical spectroscopy (Lustig et al. 1991) and differential scanning calorimetry (Murphy et al. 1988; Pedley and Tighe, 1979). The classification of water in the hydrogels depends on the techniques used. According to the most common method, DSC, water in the hydrogel is classified as freezing free water, freezing bound water and non-freezing water. Free water does not take part in hydrogen bonding with polymer molecules. It has a similar transition temperature, enthalpy and DSC curves as pure water (Nakamura et al. 1983). Freezing bound water interacts weakly with polymer molecules and has a phase transition temperature lower than 273K. Non-freezing bound water is complexed with the polymer chain through hydrogen bounds and has no detectable phase transition over the temperature range from 200K to 273K (Higuchi et al. 1984). Since the freezing water effects transport and biocompatibility of the hydrogels (Mirejovsky et al. 1991; Goda et al. 2006), it is important to detect water structure inside hydrogel for optimization of material properties in biomedical applications.

Table 2.4 Water structures of different composition hydrogels

<table>
<thead>
<tr>
<th>Sample</th>
<th>EWC%</th>
<th>W_f%</th>
<th>W_nf%</th>
<th>W_f/W_nf</th>
<th>W_f/EWC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td>48.8</td>
<td>21.6</td>
<td>27.2</td>
<td>0.79</td>
<td>44.3</td>
</tr>
<tr>
<td>D20</td>
<td>52.0</td>
<td>30.0</td>
<td>22.0</td>
<td>1.36</td>
<td>57.7</td>
</tr>
<tr>
<td>D40</td>
<td>60.1</td>
<td>39.9</td>
<td>20.2</td>
<td>1.98</td>
<td>66.4</td>
</tr>
<tr>
<td>D60</td>
<td>65.5</td>
<td>50.7</td>
<td>14.8</td>
<td>3.43</td>
<td>77.4</td>
</tr>
<tr>
<td>D80</td>
<td>69.4</td>
<td>56.2</td>
<td>13.2</td>
<td>4.26</td>
<td>81.0</td>
</tr>
<tr>
<td>D90</td>
<td>69.9</td>
<td>41.2</td>
<td>28.7</td>
<td>1.44</td>
<td>41.1</td>
</tr>
</tbody>
</table>
In this work the water structure in equilibrium-swollen hydrogel was studied by DSC. Since the peak shape and the maximum peak temperature of heating and cooling process are dependent on the heating rate (Higuchi and Iijima, 1985; Chan et al. 1992) and different heating and cooling cycles can cause the evaporation of water from the hydrogel and condensation inside the sample pan (Roorda et al. 1988), only the results of the first heating runs were used to calculate relative content of freezing water ($W_f\%$) and the relative content of non-freezing water ($W_{nf}\%$). The $W_f\%$ was calculated by the melting enthalpy of the hydrogel divided by the melting enthalpy of pure water. The observed melting enthalpy is calculated by the area under the endothermic curve. The melting enthalpy of bulk water is taken as 333.3 J/g (Ahmad et al. 1994). $W_f\%$ and $W_{nf}\%$ are calculated by the following equation:

$$W_f\% = W_{free}\% + W_{f-bond}\% = \frac{\Delta H}{\Delta H_w} \times 100 \quad (2-6)$$

$$W_{nf}\% = EWC - W_f\%$$

$\Delta H_w = 333.3 \text{ J/g}$

$\Delta H$ is the observed melting enthalpy. $\Delta H_w$ is the melting enthalpy of bulk water.

The relative content of non-freezing water is obtained by the difference between the equilibrium water content, EWC, and the relative content of freezing water. The EWC, $W_f\%$ and $W_{nf}\%$ are listed in Table 2.4. Figure 2.5 shows the DSC curves obtained during heating run from -100 °C to 20°C.

From Table 2.4 we can see that when the DHPMA content was increased from 0 to 80% the content of freezing water increased and non-freezing water decreased.
The ratio of freezing water to non-freezing water reached a peak at D80. The freezing water increase may be due to greater free volume because of the dihydroxypropyl side chain while the non-freezing water decrease may be due to more hydrogen bonds formed between the side chains, which results in less hydrogen bond formed between water and hydroxyl groups. According to reference (Liu et al. 2000), hydrogel porosity increased with NaCl concentration in the swelling medium. This leads to an increase in freezing water content and a decrease in non-freezing water content and was thought to stem from the increase in porosity, as seen in SEM pictures, and DHPMA content. However, hydrogels without HEMA showed a decrease in freezing water and increase in non-freezing water. From the results it can be seen that 80% DHPMA shows the highest freezing water content. Since the content of freezing water determines the transport properties of the hydrogel (Mirejovsky et al. 1991), it can be expected that 80% DHPMA is the best for transport of water, glucose and oxygen to the biosensor according to reference(Goda et al. 2006) higher freezing water content also results in less protein deposition on the polymer surface. Future studies will characterize the effect of swelling and water structure on mechanical relaxations. Previous publications documented the fact that hydrophilic groups on the polymer backbone influence secondary relaxations and swelling responses(Gates et al. 2003).
Figure 2.5 DSC heating curves of hydrogels with different DHPMA content, performed at the heating rate of 5.0°C min⁻¹
2.3.5 Sensor Performance of Hydrogel-Coated Glucose Sensors

Hydrogels of various compositions were used for coating glucose sensors and the characteristics of the corresponding sensors were examined in vitro. Table 5 gives the measured results of sensitivity (S, nA/mM) and response time (T_{90%}, min) at Day 7 and Day 28. For different compositions of hydrogels a higher percentage of DHPMA resulted in the higher sensitivity and shorter response time in accordance with the results of SEM morphology, swelling behavior and water structure observations.

Among five hydrogels, D0 showed the best long-term stability but its response was too slow. D20 to D60 failed to gave a stable sensor response at day 28. Comparatively, the sensor with a D80 coating showed the best performance. At day 28, the sensor response curve with stepwise glucose increases was still good (Figure 2.6) despite a 40% loss of the sensitivity at day 28.

<table>
<thead>
<tr>
<th>Pt-Ir/GOx/HG (n=4)</th>
<th>D0</th>
<th>D20</th>
<th>D40</th>
<th>D60</th>
<th>D80</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 7</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S (nA/mM)</td>
<td>6.1±2.6</td>
<td>6.3±2.2</td>
<td>10.5±1.7</td>
<td>16.2±0.8</td>
<td>18.4±2.1</td>
</tr>
<tr>
<td>T_{90%} (min)</td>
<td>12.7±2.2</td>
<td>10.5±2.0</td>
<td>8.6±1.2</td>
<td>6.9±1.3</td>
<td>4.6±1.0</td>
</tr>
<tr>
<td><strong>Day 28</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S (nA/mM)</td>
<td>3.9±0.4</td>
<td></td>
<td></td>
<td>Low response</td>
<td>10.9±2.1</td>
</tr>
<tr>
<td>T_{90%} (min)</td>
<td>3.5±1.5</td>
<td></td>
<td></td>
<td></td>
<td>4.3±1.2</td>
</tr>
</tbody>
</table>
Figure 2.6 Representative response curves of a Pt-Ir/GOx/HG-D80 glucose sensor at day 7 and 28, respectively.
Table 2.6 Response characteristics of various hydrogel-EPU-based glucose sensors*
(mean ± SD)

<table>
<thead>
<tr>
<th>Pt-Ir/GOx/EPU/HG (n=4)</th>
<th>Control</th>
<th>D0</th>
<th>D20</th>
<th>D40</th>
<th>D60</th>
<th>D80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 7</td>
<td>S (nA/mM)</td>
<td>3.3±1.1</td>
<td>0.6±0.4</td>
<td>0.6±0.4</td>
<td>1.2±0.8</td>
<td>3.0±1.4</td>
</tr>
<tr>
<td></td>
<td>T(_{90%}) (min)</td>
<td>2.9±1.0</td>
<td>27.0±3.4</td>
<td>24.3±2.7</td>
<td>18.4±7.4</td>
<td>17.4±2.2</td>
</tr>
</tbody>
</table>

*S: response sensitivity of sensor; T\(_{90\%}\) (min): the time to reach 90\% of the maximum response when glucose varies from 5 mM to 15 mM;

Figure 2.7 Calibration plots from a Pt-Ir/GOx/HG-D80 sensor and a Pt-Ir/GOx/HG-D80 sensor. Sampling time was between 4 to 5 min after each stepwise addition of a concentrated glucose solution.
Figure 2.7 shows the calibration lines of a Pt-Ir/GOx/HG-D80 sensor and a Pt-Ir/GOx/HG-D80 sensor. The linear range of Pt-Ir/GOx/HG sensors was in the range of 1 to 15 mM. It was thought that the poor linearity at high concentration could be significantly improved by adding an epoxy-polyurethane membrane in between the enzyme layer and the hydrogel coating. Such a sensor, i.e. Pt-Ir/GOx/EPU/HG, extended the linear range to 40 mM (see Figure 6). Table 2.6 summarized the sensitivity and response time of various Pt-Ir/GOx/EPU/HG sensors. Since the permeability of the epoxy-polyurethane membrane is much lower than that of the hydrogel coating layer, the epoxy-polyurethane membrane played the major role in limiting diffusion. Therefore, when the hydrogel was more permeable, the sensitivity was closer to that of the control sensors (i.e. Pt-Ir/GOx/EPU). However, the additional hydrogel layer caused a significant response delay, especially for D0, D20, D40 and D60. D80 was determined to meet the design requirement of implantable glucose sensors.

2.3.6 In vivo Biocompatibility

PU coated Silastic tube (ϕ = ~ 4 mm, length = ~ 50 mm), 80% DHPMA hydrogel bar (ϕ = ~ 4 mm, length = ~ 50 mm), PU coated glucose sensor and 80% DHPMA hydrogel coated glucose sensor were implanted subcutaneously in rats for 28 days. Figure 2.8 shows the histology results of the PU coated Silastic tube (A), hydrogel bar (B), PU coated glucose sensor (C) and hydrogel coated glucose sensor (D). It can be seen that about 200 µm and more than 500µm in thickness fibrous
capsule formed around the silastic tubing and PU coated glucose sensor with obvious inflammation of the cells. While only 30~60 μm in thickness, the fibrous capsule formed at the hydrogel bar and hydrogel coated sensor, respectively. 80% DHPMA hydrogel can reduce the fibrosis and inflammation and it should make the lifetime of glucose sensor longer. A sensor with a hydrogel coating would not only have all advantages of an epoxy-polyurethane glucose sensor (e.g. long-term stable, wide response range) but also minimize the tissue reactions occurring around the implanted sensor. This study was limited to 28 days. There is no reason to expect that the biocompatibility of this material would change after this period. As previously stated in the introduction, the main innovation of this hydrogel compared to similar materials is the increase in mechanical strength which makes it suitable to coat biosensors for long term implantation.
Figure 2.8. Histology slides: (A) epoxy-polyurethane-coated silastic tubing ($\phi = \sim 4$ mm, length = $\sim 50$ mm), (B) one hydrogel bar ($\phi = \sim 4$ mm, length = $\sim 50$ mm), (C) one PU coated sensor (D) one hydrogel coated sensor (rat 4, left) implanted subcutaneously in rats for 28 days.
2.4 Conclusion

In this study different hydrogels with various DHPMA content were successfully synthesized and coated on to glucose sensors by UV polymerization. The higher freezable water content, swelling rate and uniform porosity that result from high DHPMA content increase the sensitivity and shorten the response time of the glucose sensors. The linear range of a glucose sensor only coated with hydrogel is short. However, it can be improved by coating the epoxy-PU with a layer of hydrogel because PU can control the diffusion of glucose molecules. Since the hydrogel minimizes the fibrosis and inflammation, it shows promise for use in implantable glucose sensors.
CHAPTER 3

USE OF HYDROGEL COATING TO IMPROVE THE PERFORMANCE OF IMPLANTED GLUCOSE SENSORS

Foreword: 8 D80 coated Pt/GOx/epoxy-polyurethane glucose sensors were implanted into 4 rats (2 sensors/rat). The original attempt of in vivo implanted glucose sensors was not successful, because only 25% of the sensors were working at 4 weeks. In order to further improve the efficiency of implanted glucose sensors, another series of novel hydrogels were developed based on copolymer of HEMA and DHPMA.

3.1. Introduction

The material–tissue interaction during sensor implantation, i.e. so-called biofouling which may include protein/platelet deposition, or the attachment of inflammatory response-related cells, is one of the major causes in unpredictable and unexplainable behaviors of implanted glucose biosensors. The performance of implanted biosensors can greatly benefit from the use of more biocompatible outermost coatings such as hydrophilic alginate (Shichiri et al., 1989), phosphorylcholine-modified polyurethane (Yang et al., 2000), 2-methacyryloxyethyl phosphorylcholine-co-n-butyl methacrylate (Nishida et al., 1995; Yasuzawa et al., 2000) and hydrogels (Suri et al., 2003). Hemocompatible synthetic copolymers containing phosphorylcholine significantly improved the performance of the sensors both ex vivo in whole blood and in vivo. Unfortunately, these polymers are mechanically weak and can be easily dislodged in flowing blood or in the subcutaneous tissue and are thereby are not
useful for long-term application. Hydrogels are a kind of network polymer possessing a degree of flexibility very similar to natural tissue due to their significant water content. When in physiological solutions, hydrogels can reduce protein adsorption and the subsequent inflammatory response. In the medical field, hydrogels have been extensively used as scaffolds in tissue engineering and sustained release drug delivery systems (Norton et al., 2005). The properties of some hydrogels make them attractive candidates for use in glucose sensors. A glucose sensitive phenylboronic acid-based hydrogel used pressure stimuli to detect glucose (Lei et al., 2006). Similarly, a polyacrylamide hydrogel-coated cantilever sensor was constructed and responded to swelling which directly correlated with glucose concentration (Ji et al., 2005). Glucose oxidase was immobilized in polyacrylamide gels via crosslinking. Swelling and viscoelastic properties were used along with amperometric measurements to evaluate the materials for use in biosensors (Fernandez and Lopez, 2005). Phenylboronic acid containing hydrogels were shown to exhibit red shifts in holograms which quantify glucose concentrations (Kabilan et al., 2005). Another group of researchers used photonic properties of boronic acid based hydrogels to sense glucose (Alexeev et al., 2004). Berner et al. (1998) determined that the iontophoretic properties of hydrogels can be used to monitor glucose. Certain hydrogels can be doped with glucose-reactive fluorescent dyes and the light that is emitted changes with glucose concentration (Thoniyyot et al., 2006).

Hydrogels can potentially be used as the outermost coating of implanted
glucose sensors to reduce foreign body reaction surrounding the sensor, thereby improving the in vivo sensor performance. Polyethylene oxide-based hydrogels were used as diffusion-limiting membranes in glucose sensors and short-term studies were implemented by subcutaneously implanting hydrogel-coated glucose sensors in Sprague–Dawley rats (Quinn et al., 1997, 1995). Most hydrogels like pHEMA are too weak to maintain their membrane structure and function during implantation, therefore, long-term studies are difficult.

Hydrogels include neutral hydrogels, ionic hydrogels and swollen interpenetrating polymeric networks. However, neutral hydrogels can better meet the sensor coating requirements of biocompatibility and structural stability. A neutral hydrogel, PHEMA and DHPMA (Wichterle and Lim, 1960), was previously developed for contact lenses. This gel was shown to exhibit an increased resistance to dehydration (Gates et al., 2003). The copolymer exhibited high equilibrium water content and good mechanical properties. These copolymers have great potential for producing more durable coatings for glucose sensors.

In this study, novel HEMA-DHPMA-based hydrogels were developed and used to improve the biocompatibility of implantable glucose biosensors. The porosity and water content of the copolymer were improved by the addition of a structural strengthenner, N-vinyl-2-pyrrolidinone (VP; McConville and Pope, 2001). The compositions of monomer mixtures were optimized and the resulting hydrogels were characterized. A selected hydrogel was used for coating an implantable glucose sensor
(Yu et al., 2006). The in vitro and in vivo sensor performance of hydrogel-coated sensors were examined and discussed.

3.2 Experimental

3.2.1. Chemicals and Materials

Monomers HEMA and DHPMA were donated by Benz R & D (Sarasota, FL). Other chemicals including VP, EGDMA and Benacure 1173 were obtained from Sigma–Aldrich (St. Louis, MO). VP was purified by vacuum distillation prior to polymerization according to reference (Jansen et al. 2005). 0.020 inch Silastic tubing (i.d.x.o.d. = 0.51mm×0.94 mm) was purchased from Dow Corning (Midland, MI). Size 3–0 Prolene (polypropylene) were obtained from Ethicon (Somerville, NJ). I.V. catheters (14 ga.) with a needle were obtained from Terumo Medical Corporation (Somerset, NJ). Sprague–Dawley outbred rats (male, 375–399 g) were purchased from Harlan (Dublin, VA). VetBondTM glue came from 3M (St. Paul, MN).

3.2.2. Photopolymerization of Poly(HEMA-DHPMA-VP-EGDMA) Hydrogels

Pure monomers (all liquid state) were first combined to prepare the following four formulations (termed VP0, VP15, VP30 and VP45) listed in Table 3.1. The mixtures were diluted 1:1 with deionized water, 1% (w/w) Photoinitiator (Benacure 1173) was added, and the solutions mixed at room temperature. Polymerization was performed as follows. After purging each solution with argon for 2 min, each solution was transferred into a glass mold (L×W×H=55mm×25mm×1 mm). The
molds were placed under a 254 nm UV light for 100–110 min in an argon atmosphere at 25 °C to polymerize the hydrogels. The hydrogels were removed from the molds and placed into 500 ml of deionized water at 25 °C. The water was changed daily for one week to remove soluble residues.

Table 3.1 Feed composition of synthesized hydrogel

<table>
<thead>
<tr>
<th>Molar ratio</th>
<th>VP</th>
<th>1HEMA:1DHPMA</th>
<th>EGDMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP0</td>
<td>0</td>
<td>99</td>
<td>1</td>
</tr>
<tr>
<td>VP15</td>
<td>15</td>
<td>84</td>
<td>1</td>
</tr>
<tr>
<td>VP30</td>
<td>30</td>
<td>69</td>
<td>1</td>
</tr>
<tr>
<td>VP45</td>
<td>45</td>
<td>54</td>
<td>1</td>
</tr>
</tbody>
</table>

For formation of the hydrogel coating on the glucose sensor tip, 0.4, 0.8 or 1.2 μl of the argon-purged VP30 solution (including additional water and photo-initiator) was pipetted onto the sensor tip. The solution was allowed to spread evenly over the surface of the tip (bare or EPU-coated). After exposing to UV light under argon (as above), the sensors were stored in phosphate buffered saline until further use.

3.2.3. Analysis of Hydrogels

The purified hydrogel samples were cut and freeze-dried for 24 h, and then stored in dessicator at room temperature. The inner morphology of cutting section was examined using an S-800 Hitachi Scanning Electron Microscopy System (Hitachi High Technologies, Pleasanton, CA).
The glass transition temperature \( (T_g) \) was measured using a model 2920 differential scanning calorimeter (DSC, TA Instruments, New Castle, DE) under a pressurized nitrogen atmosphere to avoid sample degradation. In this process, a 4–10mg freeze-dried sample was sealed in aluminum pans. The samples were scanned from 30 to 200 °C at 5°C per min. Each sample was tested at least twice.

Fourier transform infrared spectroscopy (FT-IR) was carried out on the rectangular samples that were dessicated at room temperature after freeze-dried, using a Nicolet Avatar 320 FT-IR Spectrophotometer (Nicolet, Madison, WI) with a 64 scan per sample cycle at a resolution of 4 cm\(^{-1}\).

For the determination of equilibrium water fraction (EWF), hydrogel samples were lyophilized to obtain dry mass \((M_d)\). Xerogels were then swollen in a pH 7.4 phosphate buffer solution (PBS, ionic strength = ~0.16 M) at 37 °C for 2 days. The swollen hydrogels were pat-dried with filter paper and weighed every 2 h until the weight reached a constant value \((M_{eq})\). The water Fraction \((EWF)\) at swelling equilibrium was calculated by:

\[
EWF(\%) = \frac{(M_{eq} - M_d) \times 100}{M_d}
\]  

(3-1)

3.2.4. Sensor Preparation

Glucose sensors were based on coil-type design (Yuet al., 2006)(Figure3.1). Briefly, the coil-type glucose biosensors were prepared by winding the top 10mm of a 40–50mm long platinum wire (\( \Phi \) 0.125mm, including 10% iridium in weight) along a 30-gauge needle to form a coil-like cylinder which was filled with cotton. The enzyme layer was formed over the cylinder by coating an aqueous solution of
10mg/ml GOx, 30–40mg/ml BSA and 0.6% (v/v) glutaraldehyde. In this study, the novel membrane configurations were used on Pt/GOx or Pt/GOx/EPU sensors. The outermost hydrogel coating was formed by applying a 0.4–1.2μl of 50% hydrogel solution to the Pt/GOx or Pt/GOx/EPU sensors as described above. All sensors used in this study were visually inspected for defects under 40× magnification using a dissection microscope (Figure3.2) and then stored in PBS.
Figure 3.1 Schematic diagram of hydrogel-coated sensing element of the glucose electrode. Figure adapted from Ju (Ju, 2006).

(1) Teflon-covered Pt-Ir wire;

(2) Ag/AgCl reference wire;

(3) Collagen scaffold;

(4) Electrically-insulating sealant;

(5) Epoxy-Pu outer membrane;

(6) Enzyme layer;

(7) Stripped and coiled Pt-Ir wire;

(8) Cotton fiber with GOX gel.
3.2.5. *In vitro* Sensor Evaluation

The in vitro performance of the sensors was examined in glucose/PBS to determine the response time, sensitivity and linear range of sensors at different days. The response time represented the required time to reach 90% of the maximum response when the glucose concentration increased from 5 to 10mM. The sensitivity of the Pt/GOx/hydrogel sensors was measured using 5 and 10mM glucose/PBS. The linear range was obtained from a stepwise glucose increase from 2 to 30mM. All test solutions were prepared using pH 7.4 PBS (ionic strength =~0.16M) and amperometric measurements were performed at room temperature at 0.7V vs. Ag/AgCl.

3.2.6. Animals and Surgical Procedures

The sensors were disinfected during polymerization of the hydrogel coating under a 254 nm UV light. Sensors used for implantation were pre-conditioned in
sterile saline for 2 days. The sensor immobilization method for implantation was according to paper (Long et al., 2005) and further improved. Briefly, the sensor wires were covered with a section of 0.02 in. silastic tubing (2 cm in length). An overhand knot then was made in the middle of this section to use as a suturing site (i.e. for anchoring) during implantation and to prevent the sensor moving out of the skin.

For each sensor placement (two/rat), a ~1.5 cm length longitudinal incision was made 1.5 cm laterally to the dorsal midline, 3–4 cm caudally from the neck. A subcutaneous pocket was created by using blunt surgical scissors before sensor wire placement. A 14 ga. I.V. catheter was inserted through the subcutaneous tissue area to the incision from the low back. The needle was withdrawn leaving the cannula in the subcutaneous tissue. The sensor tip and wires were carefully fed into the cannula through the incision. The sensor was secured to the skin by passing a 3–0 Prolene suture through the knot of the silastic tubing covered sensor wires and the incision was closed using 3–0 Prolene sutures. After implantation, a ~4mm length of Silastic tubing containing a ~8mm length of sensor wires was allowed to protrude from the skin.

All protocols were approved by the University of South Florida Institutional Animal Care and Use Committee (IACUC).

3.2.7. In vivo Evaluation Method

During the course of in vivo testing, a continuous flow anesthesia system was used to deliver 1.5% isofluorane to the rats with 1.0 l/min oxygen. A maximum of 8 sensors were continuously monitored for 2–3 h by using two Apollo 4000
potentiostats (4 channels each; World Precision Instruments, Sarasota, FL).

Response currents (nA) were acquired at 1 s intervals every second and dynamic current (nA) vs. time (s) curves for all sensors were simultaneously displayed. After a run-in period of approximately 1 h, a stable signal was obtained from the sensors. Dextrose (0.5–0.8 ml of a 50% (w/v) solution) was administered intraperitoneally using a 27 ga. needle. Figure 3.3 shows the in vivo continuous glucose monitoring procedure. Following the injection, a drop of blood was sampled every 6–8 min from the transected distal rat tail and blood glucose level was determined using a portable glucometer (FreeStyle™; TheraSense, Alameda, CA). The sensor sensitivity was calculated by dividing the current change by the blood glucose difference between the initial (before dextrose injection) and the peak status (after dextrose injection).
Figure 3.3 *In vivo* Continuous Glucose Monitoring Procedure. Figure adapted from Ju’s dissertation
3.3 Results and Discussion

3.3.1. Characterization of Hydrogels

After polymerization, hydrogels VP0, VP15, VP30 and VP45 were transparent and colorless in appearance and had a uniform and smooth surface, indicating that addition of VP did not result in phase separation or deterioration in the uniformity of the cured hydrogels. FT-IR spectrometry (shown in Figure 3.4) revealed specific carbonyl adsorption peaks at ~1735 cm\(^{-1}\) (corresponding to polymerization of HEMA or DHPMA) and ~1655 cm\(^{-1}\) (corresponding to polymerization of VP) (Yaung and Kwei, 1998). As VP concentration was raised, the VP carbonyl peak increased. FTIR results indicate that VP was successfully co-polymerized with HEMA and DHPMA.

The measured glass transition temperature values (\(T_g\)) of VP0, VP15, VP30 and VP45 were 111.88, 121.72, 133.86 and 143.75\(^{\circ}\)C, respectively (shown in Figure 3.5). Due to formation of hydrogen bonds between the carbonyl group in the PVP network and OH groups on the PHEMA or PDHPMA chains, VP effectively enhances the interactions between polymer chains and restricts the mobility of chains (Jin et al., 2006). Consequently, \(T_g\) values increased with increasing VP concentration.

VP improved porosity of the hydrogels. Without VP, the copolymer of HEMA and DHPMA did not show pores in Figure 3.6 while the hydrogels with VP were highly porous. The pore size increased when VP increased in the range of 0–45\%.
According to higher magnification SEM photos (Figure 3.7), it can be seen that nets start to appear inside the gel on the surface of the pores from VP15 and VP30. There are more nets inside VP30 than VP15. When it is magnified to 10K times, it can be seen that some big pieces of structures combined with nets present on the surface of the pores. The diameter of net string is about 50nm. However, there are no nets on the surface of pores of VP45.

Equilibrium water fraction $EWF$ values for VP0, VP15, VP30 and VP45 were 146%, 149%, 166% and 217% (wt%), respectively. In consideration of biocompatibility, the hydrogel with a large $EWF$ and large pores would be preferred.

In order to detect the durability of these novel hydrogels, hydrogel samples ($n = 4$/hydrogel) were stored in water at room temperature for 4 weeks. Fortunately, no significant weight loss was observed. However, VP45 spontaneously broke into several smaller pieces. From SEM pictures (Figure 3.6 and 3.7) nets are present inside the gel on the surface of the pores of VP15 and VP30. These nets may strengthen polymer matrix with high water content. Since there are no nets on the surface of pores of VP45, VP45 was observed fragility probably due to swelling stresses as a result of the high water content. Balancing the properties of water content, porosity and durability of various hydrogels, VP30 was selected for coating sensors for more in-depth in vitro/in vivo performance testing.
Figure 3.4 FTIR of four freeze-dried samples (the thickness of samples are around 0.5mm, taken by reflected light)
Figure 3.5 DSC of four freeze-dried samples
Figure 3.6 A SEM of sample VP0

Figure 3.6 B SEM of sample VP15
Figure 3.6 Scanning electron microscopy images of four freeze-dried hydrogel samples (magnification: 350×, bar = 50μm).
Figure 3.7 A SEM of sample VP15 (magnification \( \times 1000 \))

Figure 3.7 B SEM of sample VP30 (magnification \( \times 1000 \))
Figure 3.7 C SEM of sample VP30 (magnification × 10K)

Figure 3.7 D SEM of sample VP30 (magnification × 100K)

Figure 3.7 Scanning electron microscopy images of VP15 and VP30 freeze-dried hydrogel samples with different magnification
3.3.2. In vitro Performance of Pt/GOx/VP30 Sensors

In order to improve the long-term biocompatibility of the sensors in vivo, the hydrogel coating must be thick enough to be durable. On the other hand, if the coating is too thick and/or relatively less porous, the sensor response will be very slow. The wet coating thickness was estimated by the volume of the coating solution ($V_c$). Volumes of 0.4, 0.8 and 1.2 μl of VP30 solution on the enzyme layer of Pt/GOx sensors ($n = 4$). In this case, the hydrogel layer served as the diffusion-limiting membrane. Compared to the control sensors (Pt/GOx), the hydrogel layer did not improve the sensor response linearity; neither did it significantly reduce sensitivity except for the thicker coating (Table 3.2). The calibration plots from Pt/GOx/VP30 sensors with coating layers formed from 0.4, 0.8 and 1.2μl of VP30 solutions are shown in Figure 3.8. The thicker coating did not improve linearity but reduced sensitivity. After the sensors had been in water for 28 days, all coatings were still firmly attached to the sensors but the sensitivity decreased ~50%.
Table 3.2 Influences of Coating Thickness on Sensor Response

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Coating Volume (µL)</th>
<th>S (nA/mM)</th>
<th>T&lt;sub&gt;90%&lt;/sub&gt; (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt/GOx</td>
<td>0.0</td>
<td>48.2±6.3</td>
<td>0.22±0.07</td>
</tr>
<tr>
<td>Pt/GOx</td>
<td>0.4</td>
<td>47.6±2.8</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>Pt/GOx/VP30</td>
<td>0.8</td>
<td>45.4±1.5</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>Pt/GOx/VP30</td>
<td>1.2</td>
<td>34.7±1.8</td>
<td>1.9±0.3</td>
</tr>
<tr>
<td>Pt/GOx/EPU</td>
<td>0.0</td>
<td>3.0±2.1</td>
<td>1.4±1.1</td>
</tr>
<tr>
<td>Pt/GOx/EPU</td>
<td>0.4</td>
<td>3.0±1.6</td>
<td>3.4±0.9</td>
</tr>
<tr>
<td>Pt/GOx/EPU/VP30</td>
<td>0.8</td>
<td>2.9±2.2</td>
<td>6.4±3.0</td>
</tr>
<tr>
<td>Pt/GOx/EPU/VP30</td>
<td>1.2</td>
<td>2.7±0.6</td>
<td>8.1±2.2</td>
</tr>
</tbody>
</table>

Figure 3.8 Calibration plots for Pt/GOx and Pt/GOx/VP30 glucose sensors.
Data are the means of measurements from 4 sensors at day 3. Error bars are ±standard error.
3.3.3 *In vitro* Performance of Pt/GOx/EPU/VP30 Sensors

Due to the poor linearity, Pt/GOx/VP30 sensors might not be able to meet the basic requirement of in vivo measurements for accuracy and precision. Though non-linear calibration can be performed in an effort to improve measurement reliability, a better solution is to add an additional diffusion-limiting membrane in between the hydrogel layer and the enzyme layer, for example, a low permeability epoxy-polyurethane membrane (Yu et al., 2006). Such sensors with hydrogel coating, i.e. Pt/GOx/EPU/VP30, were prepared using similar methods as those used for preparing Pt/GOx/VP30 sensors and tested in glucose/PBS. It was found that hydrogels could firmly attach to the epoxy-polyurethane membrane. The sensitivity was decreased to about 10% of the sensitivity of Pt/GOx/VP30 sensors, which did not change with an increase in hydrogel coating (Table 3.2). This was because the epoxy-polyurethane was much less permeable than the hydrogel and played the decisive diffusion-limiting role. The linearity of the sensor’s response was significantly improved by the epoxy-polyurethane membrane and was at least in the range of 2–30mM. The sensor response was dramatically delayed when the hydrogel coating was thicker (the response time is proportional to the square root of membrane thickness (Baronas et al., 2003). For 0.4, 0.8 and 1.2 μl of hydrogel coating, the response time was 3.4±0.9, 6.4±3.0 and 8.1±2.2 min, respectively. Thus, further experiments were carried out using the sensor with 0.4 μl of VP30 coating.

The Pt/GOx/EPU/VP30 (0.4μl) sensors did not show a decline in either sensitivity or
linearity over 28 days (Figure 3.9). The coating was still visible and firmly attached to the sensor after 28 days.

**3.3.4. In vivo Performance of Pt/GOX/EPU/VP30 Sensors**

Eight Pt/GOx/EPU/VP30 (0.4 μl) sensors were subcutaneously implanted in four rats (two sensors/rat) and the in vivo sensitivity of each sensor was tested at days 7, 14, 21 and 28. The testing results are summarized in Table 3.3. All sensors kept functioning in the first 3 weeks and produced an in vivo response sensitivity of ~4 nA/mM. At day 28 after implantation, 3 of 8 implanted sensors still performed. It has been reported that 68 Pt/GOX/EPU sensors were tested in vivo and the sensor survival at day 21 was 13.5% (Long et al., 2005). This suggests that the addition of hydrogel coating apparently improves the long-term performance of the implanted glucose sensors.

The improvement might be attributable to the excellent biocompatibility of the hydrogel coating. Representative histological sections, prepared from tissue surrounding a Pt/GOx/EPU/VP30 sensor and a Pt/GOx/EPU sensor at day 28 after implantation, are shown in Figure 3.10. Only a few inflammatory cells were observed in the tissue surrounding hydrogel-coated sensors (Figure 3.10B). Without hydrogel coating, many inflammatory cells appeared (Figure 3.10A). The thickness of the fibrous tissue around the Pt/GOx/EPU/VP30 sensor was 50–100μm, much less than the thickness of 100–500μm which was routinely observed in tissue surrounding Pt/GOx/EPU sensors (Long et al., 2005). The reason for the functional failure of the other five sensors at day 28 in this study is unknown. Histological results did not
show any apparent differences between the tissue surrounding the surviving sensors and the tissue surrounding the failed sensors.

Table 3.3 *In vivo* sensor sensitivity of Pt/GOx/EPU/VP30 sensors (n=8)

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Average ± 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>12.0</td>
<td>5.5</td>
<td>2.6</td>
<td>2.1</td>
<td>4.6 ± 1.2</td>
</tr>
<tr>
<td>Day 14</td>
<td>4.4</td>
<td>7.3</td>
<td>1.5</td>
<td>5.8</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>Day 21</td>
<td>6.6</td>
<td>4.2</td>
<td>1.5</td>
<td>5.6</td>
<td>5.3 ± 0.7</td>
</tr>
<tr>
<td>Day 28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.2 ± 0.7</td>
</tr>
</tbody>
</table>

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Figure 3.9 Calibration plots for Pt/GOx/EPU/VP30 (0.4ul) sensors at day 3, 7, 16 and 28. Data are the means of measurements from 4 sensors. Error bars are ±standard error.
Figure 3.10 Hematoxylin and eosin-stained sections of tissue surrounding glucose sensors implanted subcutaneously in rats for 28 days. The photo (A) shows the capsular tissue formed around a Pt/GOx/EPU sensor. The photo (B) shows the capsular tissue formed around a Pt/GOx/EPU/VP30 (0.4μl) sensor.
3.4. Conclusion

This study has provided a feasible approach to design and select the properties of the copolymer for coating implantable biosensors. For the first time, animal experiments were used to demonstrate that a hydrogel coating was effective in minimizing tissue reactions surrounding implanted minimally invasive needle-type glucose biosensors. With a hydrogel outermost coating and an appropriate diffusion-limiting layer, significant improvements of the in vivo performance of implanted glucose sensors can be anticipated.
CHAPTER 4
EFFECT OF NOVLE HYDROGEL COMPOSITION ON TARGETED
DEXAMETHASONE-21 PHOSPHATE DISODIUM SALT DELIVERY

4.1. Introduction

When implants such as biosensors, pacemakers and bioartificial organs are implanted in the body, acute inflammation can occur within seconds or may be delayed for days. Without suppressive agents, chronic inflammation and fibrosis usually follows. The implants will lose normal functions with these tissue reactions (Hickey et al. 2002). According to recent reports (Norton et al. 2006; Patil et al. 2004; Yoon et al. 2003; Klueh et al. 2007), the local release of DX can suppress the acute inflammation around the biosensors and may improve biocompatibility and prolong sensor lifetime. The dexamethasone concentration around the implant depends on the drug release characterization from the matrix and must be high enough to prevent inflammation (Moussy et al. 2006). The drug release from equilibrium swollen hydrogel can be affected by both drug and materials used (Karlgard et al. 2003). Norton et al (Norton et al. 2005; Gallardo et al. 2001) used HEMA-VP-PEG hydrogel as a matrix for DX release, but there is no report about the effect of hydrogel compositions on DX releasing. It has been reported that polymer structure and composition greatly influenced drug release, so it was a matter of importance to focus
on the effect of hydrogel composition on the targeted delivery of DX-21.

In this phase of the research, a serious of porous poly(HEMA-DHPMA-VP) matrixes are prepared in the membrane form according to previous two chapters. The water soluble DX-21 was loaded into the hydrogel followed by the study to determine drug load and release. The simple HPLC method was developed to detect both DX and DX-21 using the same mobile phase.

4.2 Experimental

4.2.1 Chemicals

2-Hydroxyethyl methacrylate (HEMA) and 2, 3-dihydroxypropyl methacrylate (DHPMA) were donated by Benz R & D (Sarasota, FL, USA) and used as received without further purification. N-Vinyl-2-Pyrrolidinone (VP) (99.9+%) was purchased from Sigma-Aldrich Co.( St. Louis, MO, USA) and purified by vacuum distillation to obtain a colorless liquid (Janson et al. 2005). Ethylene glycol dimethacrylate (EGDMA), Dexamethasone-21 phosphate disodium salt (98%, powder) and monobasic potassium phosphate ACS grade from Sigma –Aldrich Co. (St. Louis, MO, USA) were used as received. Methanol HPLC grade from Fisher Scientific (Fair Lawn, NJ, USA) and Benacure 1173 from Mayzon Corporation (Rochester, NY, USA). Phosphate buffered saline (PBS) 10X solution from Fisher Scientific (Fair Lawn, NJ, USA) was diluted to 1X with pH 7.4±0.1.
4.2.2 Drug loading by Equilibrium Partitioning

The homemade swollen hydrogels were cut by the core bore (diameter, 5.73mm) and freeze-dried for one day to obtain xerogel pellets which are kept in the desiccator until used.

Xerogel pellets were weighed, wrapped in the net and placed in individual vials with 3mL of 25mg/mL dexamethasone-21 phosphate disodium (DX-21) solutions. The pellets were immersed in the solutions which were stirred and incubated in 37°C oil bath for 24 hours.

4.2.3 In vitro Release Study

Drug-loaded hydrogels were removed from drug solution. The excess solution on the surface of the hydrogels was removed by using Kimwipes.

Drug loaded hydrogel were wrapped again in the net and placed into 3mL fresh PBS solution which was stirred and incubated in 37°C oil bath. PBS at pH7.4±0.1 at 37°C resembles the environment of the drug inside the human body. At different time intervals, hydrogels were removed and placed in fresh PBS solutions to keep the sink condition. The drug solutions were kept in the refrigerator prior to detect the DX-21 and DX concentration. Three in vitro release studies were performed under the same conditions for each hydrogel sample. The means were calculated and graphed for each different time interval.
4.2.4 HPLC Analysis of DX-21 and DX Release

4.2.4.1 Preparation of Standard Solution

An accurately weighed quantity of DX-21 was dissolved in different mobile phase (Table 4.1) to obtain a known concentration of about 535.20 \( \mu \text{g/mL} \). Stock solution was diluted to obtain 5.35, 10.70, 21.41, 42.82, 53.52, 64.22, 85.63 and 107.04 \( \mu \text{g/mL} \) solution.

A known amount of DX-21 was weighted and dissolved with mobile phase to obtain a known concentration of 98.80 \( \mu \text{g/mL} \). The stock solution was diluted to obtain 79.00, 59.30, 49.40, 39.50, 19.75, 9.88 and 4.94 \( \mu \text{g/mL} \) solution.

4.2.4.2 HPLC System

The concentration of the solution was monitored by HPLC with Shimadzu system controller, UV-vis detector, and liquid chromatograph. The column is Shimadzu premier C18 5um 150*4.6mm. The wavelength was 241nm, and the flow rate was 1 mL/min.

4.2.4.3 Selection of the Mobile Phase

Different mobile phases were prepared according to table 4.1 and degassed by ultrasonic. The mobile phase ran about half hour until the base line was stable. First, 39.5 \( \mu \text{g/ml} \) DX and 42.82 \( \mu \text{g/ml} \) DX-21 were separately injected. Second, the mixture of 10ml 79.00 \( \mu \text{g/ml} \) DX and 10ml 85.63 \( \mu \text{g/ml} \) DX-21 was injected. De-ionized water is used to clean the tube and column for two hours. After that a
new mobile phase ran about one hour before the drug solution was injected. The same procedure was repeated to test different mobile phase.

4.2.4.4 Calibration Curve and Sample Assay

The peak area under the curve was plotted to the standard concentration to obtain the calibration line. The formula derived from the line was used to calculate the drug concentration of the samples.

The samples were injected to the 20uL auto valve. Every sample was injected at least twice. If the error was beyond 5%, a third injection was done. The concentration of the solution was taken as the average value. The mass of the drug equalled the average of the triple experiments.

4.3. Results and Discussion

4.3.1 The Selection of the Mobile Phase

Dexamethasone -21 phosphate disodium (DX-21)(Figure4.1), prodrug of dexamethasone, can convert to dexamethasone(DX) (Figure4.2) in biological fluids in vitro(Blackford et al. 2000). During the release experiment, some DX-21 molecules convert to DX too. Different mobile phases have developed to determine DX-21 and DX separately (Blackford et al. 2000). It was too costly and time consuming to use different mobile phase to detect DX and DX-21 separately; therefore, a single mobile phase that could be used to detect both was necessary. Table 4.1 shows different mobile phases are used to separate DX-21 and DX.

According to Table 4.1, it is seen that DX-21 is eluted earlier than DX because
DX-21 is more polar than DX since reverse HPLC was used. When the polarity of mobile phase decreases, retention times of DX-21 and DX are extended. When the ratio of methanol to water arrives at 6:4, DX-21 and DX can be separated. They can further separated by mobile phase of methanol: water (5:5). However, retentions time of DX-21 (2.850 and 3.175) are so short that they are close to the dead time of the column which is about 2min. When the 0.01M KH$_2$PO$_4$ is added to the mixture, it makes the retention time of DX-21 longer, while the retention time of DX shorter. Both of the two mobile phases of methanol:water (6:4) and methanol :water(5:5) can separate DX-21 and DX too. Furthermore, the peak of DX-21 can be separated from dead volume of column. In order to save time, methanol: water (6:4) with 0.01M KH$_2$PO$_4$ was preferred. Figure 4.3 shows HPLC profiles of DX-21 and DX with mobile phase of methanol:water(5:4, 0.01KH$_2$PO$_4$).

![Figure 4.1 structure of Dexamethasone 21-phosphate disodium salt(DX-21)](image)

Figure 4.1 structure of Dexamethasone 21-phosphate disodium salt(DX-21)
**Figure 4.2** Structure of dexamethasone

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>Retention time DX-21 (min)</th>
<th>Retention time DX (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% methanol</td>
<td>2.075</td>
<td>2.117</td>
</tr>
<tr>
<td>Methanol : water=7:3</td>
<td>2.658</td>
<td>3.392</td>
</tr>
<tr>
<td>Methanol:water=6:4</td>
<td>2.850</td>
<td>6.725</td>
</tr>
<tr>
<td>Methanol: water=5:5</td>
<td>3.175</td>
<td>17.342</td>
</tr>
<tr>
<td>Methanol:water=5:5(0.01MKH$_2$PO$_4$)</td>
<td>9.942</td>
<td>16.625</td>
</tr>
<tr>
<td>Methanol:water=6:4(0.01MKH$_2$PO$_4$)</td>
<td>4.533</td>
<td>6.558</td>
</tr>
</tbody>
</table>
Figure 4.3 HPLC profiles with mobile phase Methanol:water=6:4(0.01MKH$_2$PO$_4$):

A.DX-21  B. DX  C. DX-21 and DX

Table 4.2 Regression parameters for DX and DX-21

<table>
<thead>
<tr>
<th>formula</th>
<th>$R^2$</th>
<th>$n^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DX-21</td>
<td>3E-5x-1.0425</td>
<td>0.9986</td>
</tr>
<tr>
<td>DX</td>
<td>1E-5x+0.0707</td>
<td>0.9880</td>
</tr>
</tbody>
</table>

$n^*$ is the number of experiments: seven samples with three times injection each
Table 4.2 shows the calibration formulas of the DX-21 and DX, $y=3E^{-5}x-1.0425$ and $y=1E^{-5}x+0.0707$ separately, x is the area under the peak, and y is drug concentration. The concentrations of DX-21 and DX are calculated by these two formulas with the area under the peak.

### 4.3.2 Drug Loading by Equilibrium Partitioning

DX has often been loaded to hydrogels by putting certain amount of DX in the monomer solution before polymerization because it is not water soluble (Norton et al. 2005). With this kind of method, the obtained hydrogel can’t be purified after polymerization due to the loss of drug, and the drug may not stable during the polymerization. For the current research effort, DX-21, pro-drug of DX-21, was chosen for its high water solubility. It has been reported that highly hydrophilic hydrogels are useful for the release of water soluble drugs that can be immobilized in the hydrophilic matrix by physical entanglement (Barbu et al. 2005). Furthermore, hydrogels can be loaded with water soluble drugs by partitioning it into the polymer matrix when the purified xerogel swells in the drug water solution after polymerization (Kim et al. 1992).
Table 4.3 drug loading percents & EWCs of hydrogels with DX-21

<table>
<thead>
<tr>
<th>sample</th>
<th>Load(%)&lt;sup&gt;a*&lt;/sup&gt;</th>
<th>EWC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td>4.75</td>
<td>52.8</td>
</tr>
<tr>
<td>D20</td>
<td>8.95</td>
<td>64.4</td>
</tr>
<tr>
<td>D40</td>
<td>11.16</td>
<td>71.8</td>
</tr>
<tr>
<td>D80</td>
<td>15.73</td>
<td>77.2</td>
</tr>
<tr>
<td>VP0</td>
<td>13.3</td>
<td>73.5</td>
</tr>
<tr>
<td>VP15</td>
<td>13.4</td>
<td>73.7</td>
</tr>
<tr>
<td>VP30</td>
<td>21.2</td>
<td>74.6</td>
</tr>
<tr>
<td>VP45</td>
<td>20.1&lt;sup&gt;b*&lt;/sup&gt;</td>
<td>75.6</td>
</tr>
</tbody>
</table>

<sup>a*</sup>Load(%)=Mass of total DX-21/Mass of Xerogel

<sup>b*</sup>VP45 tears during releasing experiment

The DX-21 loading percent is calculated by mass of cumulated release amount of DX-21 and DX divided by mass of xerogel. When xerogel is immersed in the drug solution, drug enters to the polymer due to three types of driving forces: a drug concentration gradient, a polymer stress gradient and the osmotic forces (Brazel and Peppas, 1999). Therefore, a higher degree of swelling is caused by the polymer stress gradient and osmotic forces which allow greater amounts of drug imbibed in the polymer matrix. From above table 4.3, it can be seen that DX-21 loading percent increased with the swelling degree of hydrogels in each series.

According to Karlgard et al.’s work, their contact lenses only up-took about 67±13ug DX-21 and the release could not be evaluated due to the low concentration of drug released. The lowest maximum uptakes observed for DX-21 can be
explained by the high aqueous solubility of DX-21 which would remain preferentially in solution rather than within the lens material (Karlgard et al. 2003). According to results of table 4.3, the drug uptakes of different formulations of hydrogels are much more than Karlgard et al.’s research. The high aqueous solubility of DX-21 does not appear to be the reason for the lowest uptakes of DX-21. This result is consistent with Peppas’s results regarding hydrophilic drugs. It is reported that the amount of drug loaded into xerogel is a function of the partition coefficient between the drug in solution and the gel itself, hydrophilic drugs which are more soluble in the loading solutions are loaded to higher concentration into polymer samples (Brazel and Peppas, 1999).

4.3.3 Drug Release

After surgical implantation of the glucose sensors, the acute inflammatory response normally takes place within seconds. During the initial stage, proteins and inflammatory cells adsorb to the sensor surface. Phagocytic cells (e.g., neutrophils, monocytes, and macrophages) then surround the device within minutes to hours in an effort to destroy it. Such membrane biofouling is detrimental to sensor function resulting in restriction of analyte diffusion to the sensor and/or degradation of the sensor membrane (Shin and Schoenfisch, 2006). If the acute inflammation cannot be suppressed, the chronic inflammation will be sustained from weeks to lifetime. This may result in the forming of fibrosis around the implanted glucose sensors and enable the implanted glucose sensors lose the valid function (Shin and Schoenfisch, 2006). In order to prevent the acute inflammation and prolong the lifetime of the implanted
glucose sensors, both penetration rate and concentration of anti-inflammatory drug around the tissue should be high enough to suppress the inflammation (Moussy et al. 2006). According to the results from figure 4.4 and 4.5, all hydrogels showed a high initial release, followed by slow, long term release during the next hours to days. This function is believed to be good for the implanted glucose sensors to suppress the acute inflammation and chronic inflammation.

Drug release kinetics from hydrogels can be expressed by the following equation (4-1):

$$\frac{M_t}{M_\infty} = kt^n \quad (4-1)$$

Where $M_t$ is the amount of release at time t, $M_\infty$ is the initial drug loading, $\frac{M_t}{M_\infty}$ is the fraction of drug released, t is the release time, n, drug release exponent, and k is a constant incorporating the structural and geometrical characteristics of the release device. It has been reported that n=1 is Case II release kinetics and n=0.5 corresponds to the Fickian release kinetics (Ritger and Peppas, 1987). The values of n were derived from the linear regression slopes of the release profiles ($M_t/M_\infty < 0.6$) using above equation shown in Figure 4.5. It shows that n is about 0.5 which indicates the release mechanism is Fickian diffusion.

Average DX-21 release rates from hydrogels can be expressed by the diffusion coefficient. All sample shapes are similar and the ratio of thickness (L) to diameter (D_i) is about 1:6.5. It is assumed that water diffuses into a planar sheet at a constant rate. When $\frac{M_t}{M_\infty} \leq 0.5$ the relationship between $\frac{M_t}{M_\infty}$ and time will fit
The diffusion coefficient (D) values were estimated according to equation (5) and shown in Figure 4.6 and Table 4.4. From Table 4.4 it can be seen that average DX-21 release rates from DHPMA series hydrogels increases with DHPMA content due to higher equilibrium water content, initial DX-21 loaded percent and more pores. The higher initial drug loaded percent causes higher concentration gradient between hydrogel and fresh PBS solution which acts as the driving force for the drug diffusion. The pores of polymers work as a tunnel for drug transport. Since water is the vehicle to transport the drug out of polymer, higher water content will facilitate drug diffusion. Factors influencing drug releasing behavior is related to the drug uptaking behavior: concentration gradient, equilibrium water content and pores which are dictated by polymer composition.

For VP series hydrogels, average release rates of VP15 and VP30 are lower than VP0 and VP45. According to SEM figures 3.4, there are nets that appear on the surface of pores. These nets may work to block DX-21 transport out of the hydrogel. There are more nets on the surface of VP30 pores than in VP15. The average release rate of VP30 is also slower than VP15. This confirms that the polymer inner-morphology is another factor that greatly influences drug diffusion.
Figure 4.4 A initial burst release

Figure 4.4 B low level long term release

Figure 4.4 DX-21Cumulative release profiles from DHPMA hydrogels
Figure 4.5 A initial burst release

Figure 4.5 B low level long term release

Figure 4.5 DX-21Cumulative release profiles from VP hydrogels
Figure 4.6 Drug release kinetics $\log(M_t/M_0)$ versus log Time

A. DHPMA series

B. VP series
Table 4.4 DX-21 release exponents and coefficients

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>$R^2$</th>
<th>$D \times 10^8 \text{cm}^2\text{s}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td>0.4394</td>
<td>0.9958</td>
<td>2.60±0.08</td>
</tr>
<tr>
<td>D20</td>
<td>0.4964</td>
<td>0.9970</td>
<td>13.80±0.13</td>
</tr>
<tr>
<td>D40</td>
<td>0.4177</td>
<td>0.9987</td>
<td>21.76±1.03</td>
</tr>
<tr>
<td>D80</td>
<td>0.4780</td>
<td>0.9969</td>
<td>62.36±4.04</td>
</tr>
<tr>
<td>VP0</td>
<td>0.5649</td>
<td>0.9980</td>
<td>38.37±1.12</td>
</tr>
<tr>
<td>VP15</td>
<td>0.5022</td>
<td>0.9995</td>
<td>33.70±2.54</td>
</tr>
<tr>
<td>VP30</td>
<td>0.4422</td>
<td>0.9999</td>
<td>27.67±0.23</td>
</tr>
<tr>
<td>VP45</td>
<td>0.4193</td>
<td>0.9999</td>
<td>52.30±2.72</td>
</tr>
</tbody>
</table>
4.4. Conclusion

The mobile phase methanol:water=6:4(0.01MKH$_2$PO$_4$) could separate DX and DX-21 at the same run so it is available to detect both DX and DX-21 by a simple HPLC method.

DX-21 was successfully loaded into a series of hydrogels by equilibrium partitioning. The drug loaded percents show that D80 could uptake the maximum DX-21 due to high equilibrium water content and more pores. The drug release kinetics shows that DX-21 releasing mechanism from novel hydrogels is Fickian diffusion. The factors influence drug releasing behavior is relative to drug uptaking behavior: concentration gradient, inner-morphology and water content which are decided by polymer composition.
CHAPTER 5

WATER STRUCTURE IN HYDROGELS

5.1. Introduction

The study of water structure in the hydrogels can assist in determining the biocompatibility of the hydrogel and affect the transportation of the molecules between the matrix and intermediate. An investigation of water structure in a polymer yields valuable information on the sorption, diffusion and permeation properties of molecular species in hydrophilic polymers. (Ping et al. 2001) Most research on water structure in hydrogels has focused on the water in hydrogels using deionized water or PBS solution as media (Goda et al. 2006; Liu and Huglin, 2003; Ping et al. 2001; Ahmad and Huglin, 1994). Research conducted by Stevenson and Gates compared water structures in hydrogels at equilibrium in different media such as water and PBS (Stevenson and Sefton, 1988; Gates and Harmon, 2001). However, there is no report about water structure in hydrogels which swell to reach equilibrium when immersed in drug solution.

Water structure in hydrogels can be detected by differential scanning calorimetry (DSC) (Murphy et al. 1988; Pedley and Tighe, 1979), NMR (Chowdhury et al. 2004; Capitani et al. 2001), dilatometry, electrical conductivity (Lee et al. 1975; Choi et al. 1977), dielectric relaxation spectroscopy (Kyritsis et al. 1995), and
dynamic-mechanical spectroscopy (Lustig et al. 1991). The most common method is DSC. Using DSC, water in a hydrogel is classified as free water, freezing bound water and nonfreezing water. Free water does not take part in hydrogen bonding with polymer molecules. It has a similar transition temperature, enthalpy and DSC curves as pure water (Nakamura et al. 1983). Freezing bound water interacts weakly with polymer molecules and has a phase transition temperature lower than 273K. Non-freezing bound water is complexed with the polymer chain through hydrogen bonds and has no detectable phase transition over the temperature range from 200K to 273K (Higuchi et al. 1984).

It was previously discussed that equilibrium water content of the hydrogel is one important factor affecting drug diffusion. It is known that freezing water content is essential for predicting efficient diffusion of salts and macromolecules (Pedley D.G. and Tighe 1979). Hydrogel coatings loaded with DX-21 will be implanted and come in contact with different body fluids which contain many small water soluble molecules. When these small molecules diffuse into hydrogels and DX-21 molecules diffuse out of hydrogels, the water content and structure in hydrogels may change too. Therefore, it is very important to investigate the water structure in hydrogels at equilibrium in small molecule media and DX-21 solution. In this chapter, 1HEMA:1DHPMA copolymer and 3 VP-HEMA-DHPMA copolymers were used to study the water structure. PBS was chosen as the media for small molecules because it is similar to body fluid. The water structures of these hydrogels were studied by DSC.
5.2. Experimental

5.2.1. Chemicals

Four different polymers were prepared as described in chapter 3: one HEMA-DHPMA copolymer and three VP-HEMA-DHPMA copolymers. Dexamethasone-21 phosphate disodium salt (98%, powder) ACS grade was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA) was used as received. Phosphate buffered saline (PBS, 11.9mM Phosphate, 137mM Sodium Chloride, 2.7mM Potassium Chloride) 10X solution from Fisher Scientific (Fair Lawn, NJ, USA) was diluted to 1X with pH 7.4±0.1.

5.2.2. Instrumentation

All water structure data were obtained by measuring heat flow with a TA Instruments 2920 differential scanning calorimeter (DSC). DSC measures heat flow and temperature between a sample and reference as a function of time and temperature. The reference is an empty hermetic aluminum pan of similar weight to that of the sample pan. The DSC was calibrated from -100°C to 200°C at 5°C/min heating rate for baseline and temperature using an indium standard.

Hydrogels were analyzed by cooling the sample from 20°C to -80°C, and a one minute isothermal period at -80°C, and heating the sample from -80°C to 20°C at 5 °C/min. The water fractions for hydrated samples were reported as the water mass over xerogel mass. The total integrated area of the respective endotherm peak represents the melting enthalpy. The melting enthalpy was plotted as a function of
total water fraction, and the amount of nonfreezing water was determined by the water fraction intercept of the linear plot.

5.2.3. Method

The drug loading and releasing method are the same as previously discussed. The equilibrium water fraction (EWF) was determined by the mass of water divided by the mass of xerogel.

Freeze-dried were equilibrated swollen in de-ionized water, 1X PBS solution, 25mg/ml DX-21 solution at atmospheric conditions, 23℃ and 55% relative humidity.

The samples analyzed by DSC were cut from the swollen hydrogels with #1 cork bore and placed in media to reach equilibrium. For the partially hydrated samples, some water was allowed to evaporate and then the samples were sealed in the pans to equilibrate. The equilibrium samples were quickly weighed to ±0.01mg and immediately analyzed by DSC. The sample mass ranged from 5 to 15mg. After DSC analysis, the samples were removed from the hermetic pans, oven dried to obtain the dry polymer mass. The water mass was determined by subtracting the xerogel mass from the mass of the hydrated DSC sample.

5.3. Results and discussion

5.3.1 Equilibrium Water Fraction of Hydrogel Before and After DX-21 Release

The equilibrium water fraction of hydrogels were determined when swollen to equilibrium in DX-21 water solution and when DX-21 loaded hydrogels released
DX-21 completely in PBS solution at 37 ℃. This data are listed in Table 5.1.

From Figure 5.1 it can be seen that when DX-21 is released, the hydrogels shrink. Before the release of the drug, the equilibrium water fraction of hydrogel is more than after the release. From this it was apparent that when DX-21 is released from the hydrogel, some water also moves out of the hydrogel and causes shrinkage. It can be concluded that not only the composition of hydrogel, but also solutes affect the water content of the hydrogel. Further research will focus on studying the water structure in hydrogel at the equilibrium state in different media.
Figure 5.1 Photos of hydrogel loaded with DX-21(A) and hydrogel released DX-21(B)

Table 5.1 Equilibrium water fractions of DX-21 loaded hydrogels at equilibrium before and after the release of the drug in PBS solution at 37°C (means ± SD, n=3)

<table>
<thead>
<tr>
<th></th>
<th>EWF(with DX-21)</th>
<th>EWF(released DX-21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP15</td>
<td>2.91±0.17</td>
<td>1.49±0.11</td>
</tr>
<tr>
<td>VP30</td>
<td>4.25±0.09</td>
<td>2.17±0.23</td>
</tr>
</tbody>
</table>
5.3.2 Water Structure of the HEMA-DHPMA Copolymer

HEMA-DHPMA copolymer was made by 1:1 molar ratio of HEMA and DHPMA. Table 5.2 shows EWFs of HEMA-DHPMA copolymer swollen in different media.

Table 5.2 EWF and NFWF of 1HEMA:1DHPMA copolymer

<table>
<thead>
<tr>
<th>sample</th>
<th>EWF</th>
<th>NFWF</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>1.7465</td>
<td>0.5806</td>
</tr>
<tr>
<td>PBS</td>
<td>1.6913</td>
<td>0.4814</td>
</tr>
<tr>
<td>DX-21</td>
<td>2.7737</td>
<td>0.4239</td>
</tr>
</tbody>
</table>

According to table 5.2 results, HEMA-DHPMA copolymer exhibits a lower EWF in PBS solution than in deionized water. This is different from the results reported by other researchers (Stevenson, 1988; Gates, 2001). Stevenson found that EWFs of polyHEMA, and poly(HEMA-co-MMA) were much higher in PBS than in distilled water. Gates reported that HEMA-DHPMA copolymer exhibited the same equilibrium water content in de-ionized water as well as in saline solution. These results are not consistent which may imply that the equilibrium water content of hydrogel in PBS solution depends on the gel composition and crosslink ratio.

HEMA-DHPMA copolymer imbibed more equilibrium water in the DX-21 solution than in PBS solution and de-ionized water. This may be due to incorporation of large molecule DX-21 which weakens the secondary bonds within the hydrogel, enlarging the distance between the polymer chains and causing an uptake of water. Thus the equilibrium water content of hydrogel in DX-21 is much
more than in PBS solution and water. This result is similar to the above result when DX-21 released from hydrogel along with water.

HEMA-DHPMA copolymer at equilibrium and at various states of partial hydration was analyzed by DSC (Figure 5.2). When hydrogels were swollen in de-ionized water, two crystallization exotherm peaks, assigned as $T_{c1}$ at higher temperature and $T_{c2}$ at lower temperature, are shown in cooling curves. The integrated area of lower temperature exotherm is greater than the higher temperature at equilibrium in deionized water. At initial dehydration stage, a third exotherm peak is seen at $T_{c3}$ which is lower than $T_{c1}$ and $T_{c2}$, and both $T_{c1}$ and $T_{c2}$ are the same as equilibrium. When the material continued desorption, all crystallization temperatures $T_{c1}$, $T_{c2}$ and $T_{c3}$ decreased. These results are consistent with previous research (G. Gates and J. Harmon, 2001).

When hydrogels were swollen in PBS solution, there is one crystallization exotherm ($T_{c1}$) with a lower temperature shoulder at equilibrium. The shoulder progressively separates from the $T_{c1}$ and moved to lower temperature during desorption.

There is only one crystallization exotherm ($T_{c1}$) at equilibrium during initial dehydration in DX-21 solution. $T_{c1}$ moved to lower temperature and one new crystallization peak ($T_{c2}$) appeared when dehydration process continued.

In summary, the crystallization of water inside the hydrogel appears to be dependent of the water fraction of hydrogel. At high water fraction, only one
transition peak is present, and more transitions appear as water fraction decreases. At higher water fractions, there is only one sharp peak indicating the perfect crystallization of water. With lower water fraction, one or two more crystallizing peaks appeared. The reduced swelling and shrinkage during the process of dehydration can restrict the mobility of the water due to stronger interactions with polymer or water. This makes it harder to form perfect crystals, so broader peaks appeared in lower temperatures instead of sharp peaks.

There are two transitions observed in the heating curves at different water fractions when HEMA-DHPMA copolymer is swollen in water and PBS solution. The temperature $T_{m1}$ (transition near 273K) was assigned for free freezing water, and temperature $T_{m2}$ for freezable bound water (transition near 263K) (Ahmad and Huglin, 1994). From the transitions of different water fractions swollen in DX-21, it can be seen that when EWF is over 2.36, the two peaks merge progressively to become a broad peak. Such a broad endothermic peak was also observed in other hydrophilic polymers (Ping et al. 2001; Wycisk and Trochimczuk, 1992; Tasaka et al. 1988; Zhang et al. 1989; Fushimi et al. 1991; Hatada et al. 1982)

Ahmad M. B. reported that the appearance of the second transition at $T_{m2}$ seemed to be dependent of the amount of water absorbed by the hydrogels while there is no evidence for $T_{m1}$ to deviating from 273 K. Ping reported that the melting point of freezable bound water increased linearly with the water content in PVA hydrogels and explained that the lowering of the freezable bound water melting point was due to
the increase in the overall hydrogen bonding ability of the polymer (Ping et al. 2001). Baker reported that the thermal transition temperature for free freezing water was found to decrease with decreasing water content (Fushimi et al. 1991). For the present results, both peak maximum temperatures $T_{m1}$ and $T_{m2}$ decreased with decreasing water fraction. These heating results are consistent with our cooling results, because in the cooling process imperfect ice crystals formed during the dehydration process, were unstable compared to the perfect crystals and melt at lower temperatures.

A decrease in separation ($T_{m1}-T_{m2}$) of peak maximum temperatures with increasing EWC has been reported by (Higuchi and Iijima,1985), but the separation did not decrease consistently with increasing EWC was also reported by Ahmad M. B. (Ahmad,1994). These reports were not in agreement with each other, which may be due to different polymer compositions with different EWC. In present study, the separation was studied based on the same formulation with different water contents. The results showed that separation ($T_{m1}-T_{m2}$) (Table 5.3) increased with increasing water fraction contrary to what was reported by Higuchi (Higuchi and Iijima,1985).
Table 5.3 Water melting points of hydrogels at different water fractions

<table>
<thead>
<tr>
<th></th>
<th>WF</th>
<th>$T_{m1}(\degree C)$</th>
<th>$T_{m2}(\degree C)$</th>
<th>$T_{m1}-T_{m2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP0(water)</td>
<td>1.74</td>
<td>5.62</td>
<td>-5.13</td>
<td>10.75</td>
</tr>
<tr>
<td>VP0-1(water)</td>
<td>1.58</td>
<td>3.45</td>
<td>-5.67</td>
<td>9.12</td>
</tr>
<tr>
<td>VP0-2(water)</td>
<td>1.20</td>
<td>2.03</td>
<td>-5.84</td>
<td>7.87</td>
</tr>
<tr>
<td>VP0-3(water)</td>
<td>0.88</td>
<td>-0.22</td>
<td>-6.13</td>
<td>5.91</td>
</tr>
<tr>
<td>VP0(PBS)</td>
<td>1.69</td>
<td>3.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP0-1(PBS)</td>
<td>1.51</td>
<td>3.14</td>
<td>-7.63</td>
<td>10.77</td>
</tr>
<tr>
<td>VP0-2(PBS)</td>
<td>1.41</td>
<td>1.84</td>
<td>-8.15</td>
<td>9.99</td>
</tr>
<tr>
<td>VP0-3(PBS)</td>
<td>1.27</td>
<td>0.61</td>
<td>-8.67</td>
<td>9.28</td>
</tr>
<tr>
<td>VP0-4(PBS)</td>
<td>0.89</td>
<td>-0.22</td>
<td>-8.93</td>
<td>8.71</td>
</tr>
<tr>
<td>VP0(DX21)</td>
<td>2.77</td>
<td>6.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP0-1(DX21)</td>
<td>2.36</td>
<td>4.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP0-2(DX21)</td>
<td>1.62</td>
<td>2.96</td>
<td>-6.20</td>
<td>9.16</td>
</tr>
<tr>
<td>VP0-3(DX21)</td>
<td>1.14</td>
<td>-7.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP0-4(DX21)</td>
<td>0.81</td>
<td>-0.24</td>
<td>-8.37</td>
<td>8.13</td>
</tr>
</tbody>
</table>
VP0 (water) EWF = 1.74

VP0-2 (water) W£3 = 1.20
VPO-2(PBS) WF = 1.41

VPO-4(PBS) WF = 0.89
VP0-1(DX21) WF=2.36

VP0-2(DX21) WF=1.62
Figure 5.2 DSC cooling and heating curves for 1HEMA:1DHPMA copolymer at various stages during desorption of deionized water, PBS solution and DX-21
Since the nonfreezing water fraction can be defined as the limiting value of water fraction at zero enthalpy of fusion (Vaquez et al. 1997), the enthalpy (integrated area) of melting endotherms from the DSC data was used to determine the nonfreezing water fraction by extrapolation. Linear plots of the total endotherm area as a function of water fraction are presented in figure 5.3, 5.4, 5.5. The water fraction intercept was used as the fraction of nonfreezing water (NFWF) and shown in Table 5.2. This is different from Gates’s result which reports that nonfreezing water fractions in water and PBS solution are almost the same. This may be due to different PBS solution and different crosslink ratio of 1HEMA:1DHPMA copolymer.
Figure 5.3 Linear plot of total integrated endotherm area versus total water fraction for the 1 HEMA:1 DHPMA copolymer swollen in deionized water.

Figure 5.4 Linear plot of total integrated endotherm area versus total water fraction for the 1 HEMA:1 DHPMA copolymer swollen in PBS solution.

Figure 5.5 Linear plot of total integrated endotherm area versus total water fraction for the 1 HEMA:1 DHPMA copolymer swollen in DX-21 solution.
5.3.3. Water Structure of VP-HEMA-DHPMA Copolymer Hydrogels

Based on 1HEMA:1DHPMA copolymer, different molar ratios (15, 30, 45) of VP were copolymerized with 1HEMA:1DHPMA monomers to obtain VP-HEMA-DHPMA copolymer, VP15, VP30, VP45. Equilibrium water fractions of VP15, VP30 and VP45 were determined by mass of water divided by mass of xerogel and listed in Table 5.4. Water structures of VP-HEMA-DHPMA copolymer swollen in de-ionized water, PBS solution and DX-21 solution were studied by DSC. Linear plots of the total endotherm area as a function of water fraction are presented in figure 5.6, 5.7, 5.8. The water fraction intercept was used as the fraction of nonfreezing water (NFWF) and shown in Table 5.6. Freezing water fraction was determined by subtracting nonfreezing water fraction from equilibrium water fraction (Table 5.7).

All VP-HEMA-DHPMA copolymers exhibited highest equilibrium water fractions in DX-21 and lowest in PBS solution. This result is the same as the 1HEMA:1DHPMA copolymer. The low equilibrium water content of hydrogel is usually improved by copolymerizing more hydrophilic monomers. It seems that large water soluble salts such as DX-21 also can increase the equilibrium water content of hydrogel.

Table 5.5 shows values of the difference of water mass between the hydrogels at equilibrium in DX-21 and PBS solution divided by mass of DX-21 loaded in hydrogel. For DX-21 all values are about 9.6 which is independent of polymer compositions. This offers a new and simple method to estimate the drug loading amount in hydrogels.
Table 5.4 EWF of VP-HEMA-DHPMA copolymer

<table>
<thead>
<tr>
<th>sample</th>
<th>PBS</th>
<th>water</th>
<th>DX-21</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP15</td>
<td>1.6947</td>
<td>1.8122</td>
<td>2.8036</td>
</tr>
<tr>
<td>VP30</td>
<td>1.8709</td>
<td>2.0100</td>
<td>2.9346</td>
</tr>
<tr>
<td>VP45</td>
<td>1.8890</td>
<td>2.2428</td>
<td>3.1004</td>
</tr>
</tbody>
</table>

Table 5.5 Values of Δwater mass(g) / DX-21mass(g)

<table>
<thead>
<tr>
<th>Hydrogel</th>
<th>Δwater mass(g) / DX-21mass(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP15</td>
<td>9.6</td>
</tr>
<tr>
<td>VP30</td>
<td>9.6</td>
</tr>
<tr>
<td>VP45</td>
<td>9.6</td>
</tr>
</tbody>
</table>
Figure 5.6 Linear plot of total integrated endotherm area versus total water fraction for the VP15 copolymer swollen in water, PBS and DX-21 solution.
Figure 5.7 Linear plot of total integrated endotherm area versus total water fraction for the VP15 copolymer swollen in water, PBS and DX-21 solution
Figure 5.8 Linear plot of total integrated endotherm area versus total water fraction for the VP15 copolymer swollen in water, PBS and DX-21 solution
Table 5.6 NFWF of VP-HEMA-DHPMA copolymer

<table>
<thead>
<tr>
<th>sample</th>
<th>PBS</th>
<th>water</th>
<th>DX-21</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP15</td>
<td>0.4581</td>
<td>0.4120</td>
<td>0.4756</td>
</tr>
<tr>
<td>VP30</td>
<td>0.4291</td>
<td>0.3791</td>
<td>0.4798</td>
</tr>
<tr>
<td>VP45</td>
<td>0.4301</td>
<td>0.3186</td>
<td>0.5112</td>
</tr>
</tbody>
</table>

Table 5.7 FWF of VP-HEMA-DHPMA copolymer

<table>
<thead>
<tr>
<th>sample</th>
<th>PBS</th>
<th>water</th>
<th>DX-21</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP15</td>
<td>1.2366</td>
<td>1.4002</td>
<td>2.3280</td>
</tr>
<tr>
<td>VP30</td>
<td>1.4418</td>
<td>1.6309</td>
<td>2.4548</td>
</tr>
<tr>
<td>VP45</td>
<td>1.4589</td>
<td>1.9242</td>
<td>2.5892</td>
</tr>
</tbody>
</table>

When the hydrogels are at equilibrium in PBS solution and water, non-freezing water content decreased with the increase of the VP content even though the whole water content increase. A similar decrease has also been found for the poly(VP-MMA) hydrogels (Liu and Huglin, 1995). Liu tried to explain that the decrease of nonfreezing water content was due to the increase of water content according to different swelling time. This was an obvious fact because the same formulation swells at different time when nonfreezing water content is expressed relative to the mass of the hydrogel. However, this explanation is not reasonable when nonfreezing water content is expressed relative to the mass of xerogel. This explanation also does not explain the relation of the nonfreezing water content and VP
content, thus there is no definitive explanation for this trend. However, following are two possible explanations.

One explanation for the observed trend may be that when VP content increased, more H-bond formed between the polymer chains as evident by the increase of the glass transition temperature (chapter 3). Since nonfreezing water is the H-bond water formed between water and polymer chain, the inter-chain H-bond reduced the total available sites to form H-bond with water molecules. Thus, the amount of non-freezing bond water decreases with increasing VP content.

Another possible explanation for present polymers is that different average number of non-freezing water molecules per site. From the following table, it can be seen that average number of non-freezing water molecules per VP is 4.2, per HEMA is 4.1 and per DHPMA is 6.4. Since the molar ratio of HEMA and DHPMA is 1:1, so the average number of non-freezing water molecules per (HEMA-DHPMA) is 5.25 which is larger than VP. Thus when VP content increased, the number of non-freezing water molecules decreased which lead to the decrease of nonfreezing water content.
Table 5.8 Molar ratios of non-freezing bound water in different polymers

<table>
<thead>
<tr>
<th>Polymers</th>
<th>N_{nf}/N_p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVP*</td>
<td>4.2</td>
</tr>
<tr>
<td>PHEMA*</td>
<td>4.1</td>
</tr>
<tr>
<td>PDHPMA*</td>
<td>6.4</td>
</tr>
</tbody>
</table>

*PHEMA and PDHPMA results were calculated from the reference (Gates and Harmon, 2001)

*PVP result was obtained from the reference (Ping et al. 2001)

From table 5.6 it also can be seen that all VP contained samples showed higher nonfreezing water content in PBS solution than in de-ionized water which is different from 1HEMA:1DHPMA copolymer. Without VP, the salt ions interfere with the OH groups on the polymer chain thereby reducing the number of H-bonding sites available to water. VP in the polymer chain blocks the salts from interfering with the H-bonding sites thus allowing water to H-bond with the polymer. Thus more H-bonding sites are created and higher nonfreezing water content was seen.

When VP contained gels were swollen to reach equilibrium in DX-21, hydrogels exhibited more FWF and NFWF than in de-ionized water and PBS solution. The additional nonfreezing water content could be from H-bonding water with the DX-21 since DX-21 molecules contain polar groups such as O, F, and OH. When more DX-21 molecules entered into the polymer matrix, they also bring in more free water to the matrix. The incorporation of large DX-21 molecules can strengthen the distance of the polymer chain and let more freezable water enter into the matrix.
5.4. Conclusion

Equilibrium water fraction of drug loaded hydrogel is bigger than that of drug released hydrogel, which is confirmed by the shrinkage of hydrogel. This result implies that both the composition of the hydrogel and the solutes can affect the water structure.

1HEMA:1DHPMA copolymer and VP-HEMA-DHPMA copolymers imbibed highest equilibrium water fraction in DX-21 solution, lowest equilibrium water fraction in PBS solution. During dehydration process, crystallization exotherms and melting endotherms of 1HEMA:1 DHPMA copolymer in all different media moved to lower temperatures due to the shrinkage of hydrogel causeing imperfect ice crystals to form, and a decrease in separation (T_{m1}-T_{m2}).

For VP-HEMA-DHPMA copolymer, the non-freezing water fraction decreases with an increase of VP at equilibrium state in water and PBS solution even though the equilibrium water fraction increased. This trend is explained by the decrease of H-bond sites with water and less molar ratio of nonfreezing water per molecule when compared to 1HMEA:1DHPMA. Both non-freezing water fraction and freezing water fraction of VP contained hydrogels are higher in DX-21 solution than in PBS and water. The information offers a new way to increase the low water content hydrogel by swelling xerogels in media containing large water soluble molecules. Since DX-21 molecules contain polar groups such as OH, F and O, it can bring some H-bond water into the matrix. When the distance between the polymer chains is
increased, more free water will be incorporated.

Since the ratio of transporting water mass to DX-21 mass is 9.6 which is independent of hydrogel compositions, the DX-21 load can also be estimated by the difference of equilibrium water content of hydrogel swollen in DX-21 solution and PBS solution.
CHAPTER 6

SUMMARY AND SUGGESTIONS FOR FUTURE STUDY

6.1. Summary

State of the art implantable glucose sensors do not work reliably and have a rather short life after implantation (Moussy, 2002). This in vivo loss of function is caused by tissue reactions surrounding the sensor such as fibrosis and inflammation (Mang et al, 2005). It is believed that the performance of implanted glucose sensors can greatly benefit from the use of more biocompatible outer most coatings. Therefore, in order to improve the lifetime of implantable glucose sensors, two different novel series of hydrogel coatings were designed, synthesized and characterized for use on implantable glucose sensors.

During this effort novel hydrogel polymers with various DHPMA content were prepared, characterized, and coated onto implantable glucose sensors and then tested in vitro and in vivo. The effects of 2,3-dihydroxypropyl methacrylate (DHPMA) on the swelling, morphology, glass transition($T_g$), and water structure were studied. The results show that the degree of swelling increases with increasing DHPMA content. Scanning electron microscopy (SEM) studies identified uniform, porous structures in samples containing 60-90 mole % DHPMA. Glass transition temperatures did not change significantly with DHPMA content, but the ratio of
freezing to nonfreezing water tended to increase with DHPMA content. Sensors coated with different hydrogels were prepared and in vitro evaluations were performed. The 80% DHPMEA hydrogels exhibited optimum sensitivity, response and stability when coated directly onto the sensor or top of a polyurethane (PU) layer. The histology results show that 80% DHPMA samples exhibit reduced fibrosis and inflammation. Eight D80 coated Pt/GOX/epoxy-polyurethane glucose sensors were implanted into 4 rats (2 sensors/rat). The original attempt of in vivo implanted glucose sensors was not successful, because only 25% of the sensors were working at 4 weeks. In order to further improve the efficiency of implanted glucose sensors, another series of novel hydrogels were developed based on copolymer of HEMA and DHPMA. The porosity and mechanical properties of the hydrogels were improved using VP and EGDMA. The results of SEM, DSC, and FT-IR analysed show that the hydrogel (VP30) produced from a monomeric mixture of 69% (1HEMA:1DHPMA), 30% VP and 1% EDGMA (mol%) had a more uniform pore structure and net structure, high water content at swelling equilibrium (EWF = 166% by mass) and acceptable mechanical properties. Two kinds of VP30-coated sensors, Pt/GOx/VP30 and Pt/GOx/epoxy-polyurethane (EPU)/VP30 sensors were examined in glucose solutions during a period of 4 weeks. The Pt/GOx/VP30 sensors produce large response currents but the response linearity was poor. Therefore, further studies were focused on the Pt/GOx/EPU/VP30 sensors. With a diffusion-limiting epoxy-polyurethane membrane, the linearity was improved (2–30 mM) and the response time was within 5
Eight Pt/GOx/EPU/VP30 sensors were subcutaneously implanted in rats and tested once per week over 4 weeks. All of the implanted sensors kept functioning for at least 21 days and 3 out of 8 sensors still functioned at day 28. Histology revealed that the fibrous capsules surrounding hydrogel-coated sensors were thinner than those surrounding Pt/GOx/EPU sensors after 28 days of implantation.

It was discussed that the biocompatibility of glucose sensors was improved by hydrogel. However, there is still a small amount of inflammation and fibrosis occurring within the tissue around the implanted glucose sensors after 4 weeks. According to recent reports (Norton et al, 2006; Patil et al, 2004; Yoon et al, 2003; Klueh et al, 2007), the local release of DX can suppress the acute inflammation around the biosensors and may improve biocompatibility and prolong sensor lifetime. In order to further suppress the biofouling process, Dexamethasone-21 phosphate disodium salt (DX-21) was incorporated to the hydrogel to do the targeted drug delivery research. DX-21 was successfully loaded into a series of hydrogels by equilibrium partitioning. The drug release kinetics shows that DX-21 releasing mechanism from novel hydrogels is Fickian diffusion. The factors influence drug releasing behavior is relative to drug uptaking behavior: concentration gradient, inner-morphology and water content which are decided by polymer composition. All hydrogels showed a high initial release, followed by slow, long term release during the next hours to days. This function is believed to be good for the implanted glucose sensors to suppress the acute inflammation and chronic inflammation.
During the release experiment, some DX-21 (Figure 4.1) molecules convert to DX (Figure 4.2). Different mobile phases have been developed to determine DX-21 and DX separately (Blackford et al., 2000). It was too costly and time consuming to use different mobile phase to detect DX and DX-21 separately; therefore, a single mobile phase methanol:water=6:4(0.01MKH₂PO₄) that could be used to detect both was created.

The structure of water in the hydrogel can determine the biocompatibility of the hydrogel and affect the transportation of the molecules between the matrix and intermediate. The investigations on the structure of water in a polymer can give valuable information on the sorption, diffusion and permeation properties of molecular species in hydrophilic polymers (Ping et al., 2001). The water structure in hydrogels swollen in different media including water, PBS and DX-21 solution were investigated. Results show that equilibrium water fraction of drug loaded hydrogel is larger than that of drug released hydrogel, which is confirmed by the shrinkage of hydrogel. This result implied both the composition of hydrogel and solutes can affect the water structure in the hydrogel.

1HEMA:1DHPMA copolymer and VP-HEMA-DHPMA copolymers imbibed highest equilibrium water fraction in DX-21 solution, lowest equilibrium water fraction in PBS solution. This information offers a new way to increase the low water content of hydrogel by swelling xerogels in large water soluble molecules media. Since the ratio of transporting water mass to DX-21 mass is 9.6 which is
independent of the hydrogel composition, the DX-21 load can be estimated by the
difference of equilibrium water content of hydrogel swells in DX-21 solution and PBS
solution.

During dehydration process, crystallization exotherms and melting endotherms
of 1HEMA:1 DHPMA copolymer in all the different media moved to lower
temperatures due to the shrinkage of hydrogel which causes imperfect ice crystals, at
the same time separation ($T_{m1} - T_{m2}$) decreased too.

For VP-HEMA-DHPMA copolymer, the non-freezing water fraction decreases
with VP increases at equilibrium state in water and PBS solution even though the
equilibrium water fraction increases. This trend is explained by the decrease of
H-bond sites with water and less molar ratio of nonfreezing water per molecule.
Both non-freezing water fraction and freezing water fraction of VP contained
hydrogels are higher in DX-21 solution than in PBS and water.

6.2. Suggestions for Future Works

The efficiency of implanted glucose sensors has been enhanced by novel
hydrogel coatings. However, there is still some inflammation processes and fibrosis
that occurs to the tissue around implanted glucose sensor. The drug delivery in vitro
study shows that DX-21 loaded hydrogel should effectively suppress acute
inflammation and chronic inflammation. In a future study, drug loaded hydrogel
coated glucose sensors will be implanted into rats and determine the performance of
the implanted glucose sensors.

It has been reported that controlled neovascularization around sensor by angiogenic growth factors such as VEGF can improve the performance of implanted glucose sensors (Klueh et al.2003,2005). The pores structures inside the hydrogel can be useful for locating nanoparticles or microspheres for controlled drug release system. The future goal of more studies will be to incorporate VEGF loaded microspheres or nanoparticles to these novel pores matrix. At last a dual release system for both DX-21 in the hydrogel and VEGF in microspheres will be realized. It has been reported that DX can lead to an anti-angiogenesis effect along with an anti-inflammatory response. The initial burse release of DX-21 from this new dual release system can suppress the acute inflammation. VEGF will be released slowly form microspheres and nanoparticle accompanied by low level release of DX-21 which can not greatly affect the angiogenesis.

Since these novel hydrogel coatings improved the biocompatibility of implanted glucose sensors, they have the potential use for other implantable biosensors. A future study can also be focused on the modification of these novel hydrogel coatings for other implantable biosensors.
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APPENDICES
Appendix A: Chapter 2, DSC curves

Figure A-1. DSC curve for glass transition of D0 sample

Figure A-2. DSC curve for glass transition of D20 sample
Appendix A (continued)

Figure A-3. DSC curve for glass transition of D40 sample

Figure A-4. DSC curve for glass transition of D60 sample
Appendix A (continued)

Figure A-5. DSC curve for glass transition of D80 sample

Figure A-6. DSC curve for glass transition of D90 sample
Appendix A (continued)

Figure A-7. DSC curve for water structure of D0 with 48.8% EWC

Figure A-8. DSC curve for water structure of D20 with 52.0% EWC
Figure A-9. DSC curve for water structure of D40 with 60.1% EWC

Figure A-10. DSC curve for water structure of D60 with 65.5%
Figure A-11. DSC curve for water structure of D80 with 69.4% EWC

Figure A-12. DSC curve for water structure of D90 with 69.9% EWC
Figure B-1 DSC curve for water structure of VP15 swollen in water with 1.8122 EWF

Figure B-2 DSC curve for water structure of VP15 swollen in water with 1.6144 EWF
Appendix B (Continued)

Figure B-3 DSC curve for water structure of VP15 swollen in water with 1.3636 EWF

Figure B-4 DSC curve for water structure of VP15 swollen in water with 1.0245 EWF
Appendix B (Continued)

Figure B-5 DSC curve for water structure of VP15 swollen in water with 0.5908 EWF

Figure B-6 DSC curve for water structure of VP15 swollen in PBS with 1.6947 EWF
Appendix B (Continued)

Figure B-7 DSC curve for water structure of VP15 swollen in PBS with 1.3817 EWF

Figure B-8 DSC curve for water structure of VP15 swollen in PBS with 1.0981 EWF
Figure B-9 DSC curve for water structure of VP15 swollen in DX-21 with 2.8036 EWF

Figure B-10 DSC curve for water structure of VP15 swollen in DX-21 with 2.3937 EWF
Figure B-11 DSC curve for water structure of VP15 swollen in DX-21 with 1.3192 EWF

Figure B-12 DSC curve for water structure of VP15 swollen in DX-21 with 2.0100 EWF
Appendix B (Continued)

Figure B-13 DSC curve for water structure of VP30 swollen in water with 1.6053 EWF

Figure B-14 DSC curve for water structure of VP30 swollen in water with 1.0348 EWF

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Appendix B (Continued)

Figure B-15 DSC curve for water structure of VP30 swollen in water with 0.8266 EWF

Figure B-16 DSC curve for water structure of VP30 swollen in water with 0.5072 EWF
Appendix B (Continued)

Figure B-17 DSC curve for water structure of VP30 swollen in PBS with 1.8709 EWF

Figure B-18 DSC curve for water structure of VP30 swollen in PBS with 1.5754 EWF
Appendix B (Continued)

Figure B-19 DSC curve for water structure of VP30 swollen in PBS with 1.1570 EWF

Figure B-20 DSC curve for water structure of VP30 swollen in PBS with 0.9071 EWF
Figure B-21 DSC curve for water structure of VP30 swollen in PBS with 0.6337 EWF

Figure B-22 DSC curve for water structure of VP30 swollen in DX-21 with 2.9346 EWF
Appendix B (Continued)

Figure B-23 DSC curve for water structure of VP30 swollen in DX-21 with 2.3633 EWF

Figure B-24 DSC curve for water structure of VP30 swollen in DX-21 with 1.5244 EWF
Appendix B (Continued)

Figure B-25 DSC curve for water structure of VP30 swollen in DX-21 with 1.1222 EWF

Figure B-26 DSC curve for water structure of VP30 swollen in DX-21 with 0.7178 EWF

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Appendix B (Continued)

Figure B-27 DSC curve for water structure of VP45 swollen in water with 2.2428 EWF

Figure B-28 DSC curve for water structure of VP45 swollen in water with 1.5505 EWF
Figure B-29 DSC curve for water structure of VP45 swollen in water with 1.2132 EWF

Figure B-30 DSC curve for water structure of VP45 swollen in water with 0.8414 EWF
Appendix B (Continued)

Figure B-31 DSC curve for water structure of VP45 swollen in PBS with 1.8890 EWF

Figure B-32 DSC curve for water structure of VP45 swollen in PBS with 1.6677 EWF
Figure B-33 DSC curve for water structure of VP45 swollen in PBS with 1.3592 EWF

Figure B-34 DSC curve for water structure of VP45 swollen in PBS with 1.2318 EWF
Figure B-35 DSC curve for water structure of VP45 swollen in PBS with 0.7746 EWF

Figure B-36 DSC curve for water structure of VP45 swollen in DX-21 with 3.1004 EWF
Figure B-37 DSC curve for water structure of VP45 swollen in DX-21 with 2.1888 EWF

Figure B-38 DSC curve for water structure of VP45 swollen in DX-21 with 2.1169 EWF
Figure B-39 DSC curve for water structure of VP45 swollen in DX-21 with 1.8013 EWF
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Chunyan Wang received a Bachelor of Science in Chemistry Education from Shandong Normal University (China) in 2000 and a M.S. in Polymer Chemistry from Fujian Normal University (China) in 2003. After earning M.S., she worked as a research scientist at the Natural Product Research Center in the National Oceanic Administration (China) until summer 2005. She entered the Ph.D. in Chemistry Program at the University of South Florida in fall 2005 and worked in the Polymer Materials Lab under the supervision of Dr. Julie Harmon.