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To the Graduate Council:

I am submitting herewith a thesis written by James F. Patton entitled "Ellipsometric Measurement of Swelling Properties Associated With pH Responsive Hydrogels in Solution." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemistry.

Michael J. Sepaniak, Major Professor

We have read this thesis and recommend its acceptance:

S. D. Gil, Brian Zhao

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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We have read this thesis and recommend its acceptance:

Acceptance for the Council:

Vice Chancellor and Dean of Graduate Studies

Thesis
2004
P387

Ellipsometric Measurement of Swelling Properties Associated With pH Responsive Hydrogels in Solution

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

James F. Patton

December 2004

Dedication

This thesis is dedicated to my family.

Their love and support has been

my inspiration.

Acknowledgements

The years of study I have spent here at the University of Tennessee have provided a unique opportunity to work with many devoted individuals. I would like to thank Dr. Michael J. Sepaniak for his guidance, support and allowing me to be a member of his research group throughout the course of my post-graduate education. I would also like to thank Dr. Fred M. Schell for accepting me into the Department.

I have had the privilege to work with many gifted colleagues, especially Chris Tipple, Lance Riddle, Maggie Connatser, Kathleen Giesfeldt, Marcos Dejesus, Pampa Dutta, Nickolay Lavrick, Adam Mullenix, and most recently Dr. Jack Steehler all truly devoted to chemistry, education, and research. The input to my work and teaching that these individuals offered made possible my success.

The support and love of my family provided me the initiative to return to academia. They are truly the driving force behind all the other factors that were required for me to succeed in graduate school.

Abstract

Previous investigations have demonstrated that aqueous solutions containing hydrogels using 2-hydroxyethyl methacrylate (2-HEMA) as one of the monomer components swell reversibly under variable conditions of pH, temperature, and ionic strength, based upon weight gain ratios. A more unique approach will be employed to measure swelling properties associated with pH responsive hydrogels in buffered solutions, namely ellipsometry. Traditionally ellipsometry has been used to measure thickness of materials on a nanoscale dimension under ambient conditions. Finally, more novel approaches of employing hydrogel swelling response properties will be investigated; including response to glucose and enzyme loading schemes. The goal of our project will be to evaluate the feasibility of utilizing ellipsometry as a means to measure swelling properties based upon changes of thickness of very thin film substrates. This method will allow fast, real-time, remote measurements, and will provide information required for extending the usefulness of ellipsometry to measure micro-cantilever (MC) sensing abilities.

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Chapter 1

Introduction

Early uses of hydrogels for medical applications include tissue repair[1], contact lens material[2] and surgical implant devices[3]. The reason for such widespread medical use relates to hydrogels ability to adapt to physiological media, and the similarity of this material to actual living tissue. Specifically, hydrogel material has been used for hernia repair mesh known as Ivalon, and has been considered for possible organ repair material including spleen and kidney. The ability for hydrogels to expand and contract under tonic conditions has made them an attractive material for contact lens applications. Hydrogels have been adapted for extended use contact lens material. Asher et al[4] have even suggested a lens material for glucose sensing of tear fluid.

Additional applications for hydrogel material have been in the field of microactuating, and drug delivery systems. Beebe et al.[5] have extensively studied micro-actuating efforts employing hydrogel material. These efforts involved coating stabilized microstructures with hydrogel material, which surround openings in a preformed valve-like system of channels. In response to pH changes the hydrogel would swell or contract permitting or restricting flow of a pH balanced solution. The advantage of using hydrogel devices over micrometer scale mechanical devices is fast volume change in response to stimuli; such as pH change, without the need for an external power source.

Kost et al.[6] have focused on drug delivery systems incorporating hydrogel material. The unique ability for hydrogel contraction, or expansion allows delivery of drugs to specific sites. For instance caffeine release experiments[7] in which caffeine was imbedded within the hydrogel matrix and released based on changes in local environment. Further applications in efforts such as insulin release ability in response to changes in glucose levels[6], and controlled release of pesticides[8] offer unique solutions to maintaining constant levels of desired compounds within a localized environment. Peppas et al.[9] have fabricated a micro cantilever (MC) as a pH sensor. Thundat et al.[10] have measured chromate ion using MC devices, while the Sepaniak group has been investigating novel materials to use as coatings for MC applications[11,12,13]. Therefore a study in the measurements of hydrogel responsiveness in both varying pH and environments of varying analyte composition shall be explored; this study will provide information to support future MC applications for hydrogels as responsive thin film material.

Micro cantilevers offer an outstanding platform for chemical and biological sensing devices. They have an excellent dynamic response in a very small package[10]. The sensitivity of MC towards minute quantities is superior to that of traditional quartz crystal microbalances (QCM) and surface acoustic wave (SAW) transducers[14]. Figure 1 illustrates the dimensions of a typical silicon MC. A unique characteristic of MC devices is their ability to undergo bending due to molecular adsorption or binding induced change in surface tension. This is achieved by confining the adsorption to one side of the MC.

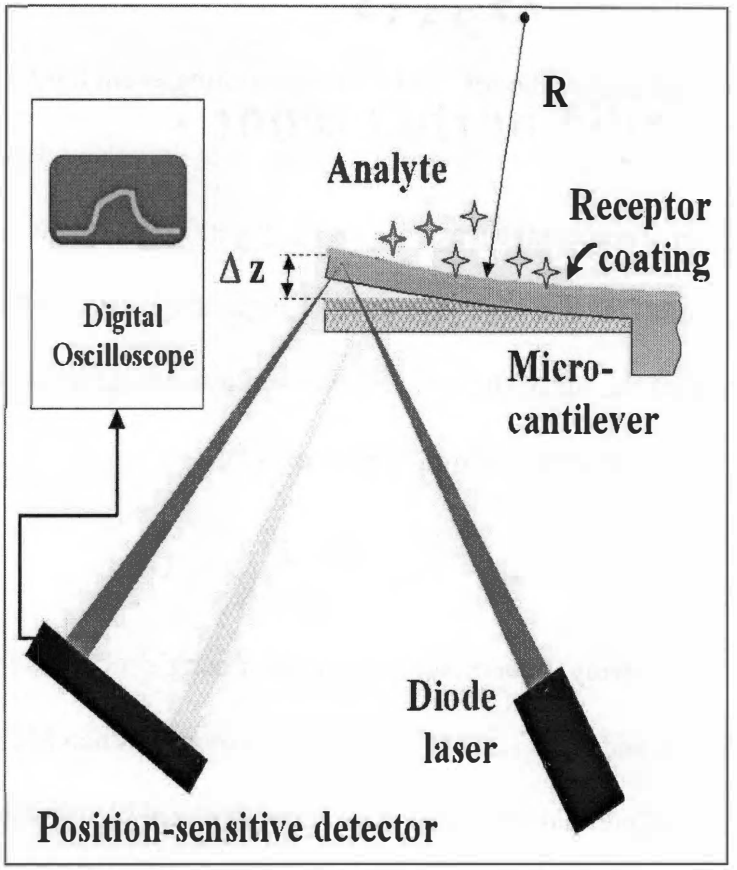


Figure 1. Diagram of Micro Cantilever and Tip Deflection Measurement. Dimensions: Length: 400 μm, Width: 35 μm, Thickness: 1 μm

For instance if a modified side of the MC undergoes a swelling event the MC will bend in a direction to oppose the applied stress. Stimuli responsive hydrogels undergo swelling events in response to environmental changes. Thin hydrogel films applied to a MC device will therefore cause the MC to bend in response to changes in the local environment.

The deflection of the tip of the MC, z_{max}, resulting from differential stress on its opposite sides, $\Delta\sigma$, is described by the Stoney equation (1);

$$z_{max} = \frac{3l^2(1-\upsilon)}{Et^2}\Delta\sigma \qquad (1)$$

where υ and E are, respectively, the Poisson's ratio and Young's modulus for the MC, and l and t are the length and thickness of the MC, respectively. When MC*s* are used as chemical sensors, this differential stress can be generally assured by using asymmetric MC*s* where one side of the MC interacts preferentially with the target analyte(s) through absorption into a thin chemical film on that surface. The untreated, opposite side is essentially passive with respect to the measured analytes creating a situation that produces a differential stress[15]. Tip deflections are measured by the displacement of a laser beam reflected off the free end of the cantilever (Figure 1).

The ability to measure nano-scale properties of hydrogels rather than bulk properties will provide information required to determine the feasibility of incorporating these gels into thin films. Thin films coated on MC devices will respond quickly to local environmental changes. This response involves changes in the dimension of the thin film, specifically thickness changes; thus films on a nanometer dimension respond quickly to these environmental changes, as we have demonstrated. Films coated on MC devices

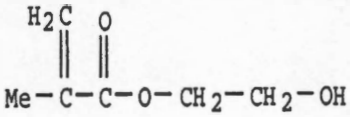
swell or collapse in response to changes in local environmental pH, temperature, or ionic strength. This swelling will provide a measurable response parameter specific to the concentration of a target analyte in a localized environment. Ellipsometry will provide a means to measure the responsiveness of very thin films, which may differ from that of bulk observations.

1.1 Hydrogel Materials And Preparation

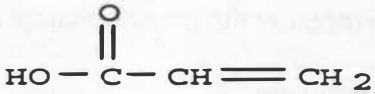
Hydrogels are hydrophilic, three-dimensional, cross-linked polymeric networks[7] synthesized by reaction of one or more monomers. The strength of such networks are due to density of cross-linking agent as well as contributions provided by hydrogen bonding or strong van der Waals forces between chains. These polymeric networks have the unique ability to swell but not dissolve when brought into contact with an aqueous environment. Typically hydrogels are composed of a functional monomeric unit, crosslinking agent and photoinitiator. By choosing appropriate monomers and other additives one can fashion hydrogels to swell or contract in response to changes in pH, analyte concentration, ionic strength, and temperature.

Control of the amounts of crosslinking agent allows one to control the structural integrity of the gel to fit experimental needs. A common comonomer system (Figure 2) includes 2 hydroxy ethylmethacrylate (HEMA), a hydrophilic compound which allows the hydrogel to expand in aqueous environments, and a pH responsive comonomer. A widely used pH responsive comonomer is acrylic acid. The crosslinking agent often

2-HEMA



Acrylic Acid



Ethylene Glycol Dimethacrylate (EGDMA)

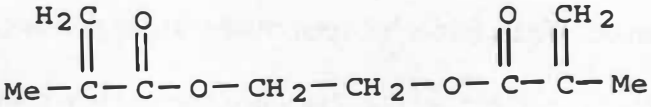


Figure 2. Hydrogel composition. Monomers 2 hydroxyethyl methacrylate and acrylic acid. Cross-linking agent ethylene glycol dimethacrylate.

chosen and used in our work is ethylene glycol dimethacrylate (EGDMA). The most prominent structural feature is the presence of the double bond in each component, thus allowing free radical photo polymerization to be accomplished through the use of the photo initiator alpha-alpha dimethoxy alpha phenyl acetophenone (Irgacure 651).

A method described by Beebe[5] uses 16-weight percent acrylic acid, 80-weight percent HEMA, 1-weight percent EGDMA, and 3-weight percent Irgacure 651. The gel we prepared by this method was tested on both large scale (bulk sampling) and nano-scale dimension by spin-coating on silicon slides provided by Wafer World Inc. The coated slide was exposed to 254 nm of ultra-violet radiation for ten minutes to induce cross-linking. This bulk gel was pH responsive. At high pH the acrylic acid would deprotonate thus causing a charge separation upon the gel backbone leading to an expansion of the gel. Figure 3 illustrates the pH responsiveness of a HEMA co AA hydrogel network. More detail to describe the complete mechanism will be addressed later in this thesis. The hydrophilicity of the HEMA enhanced the swelling characteristic by taking up a large volume of the aqueous medium.

1.2 Ellipsometry

A schematic of the instrumentation involved with ellipsometry is depicted in Figure 4. This schematic shows that a source of radiation probes a sample through a flow cell, which is positioned upon a sample stage Figure 5 shows the instrumental arrangement. Theoretical details on the principles of ellipsometry can be found in

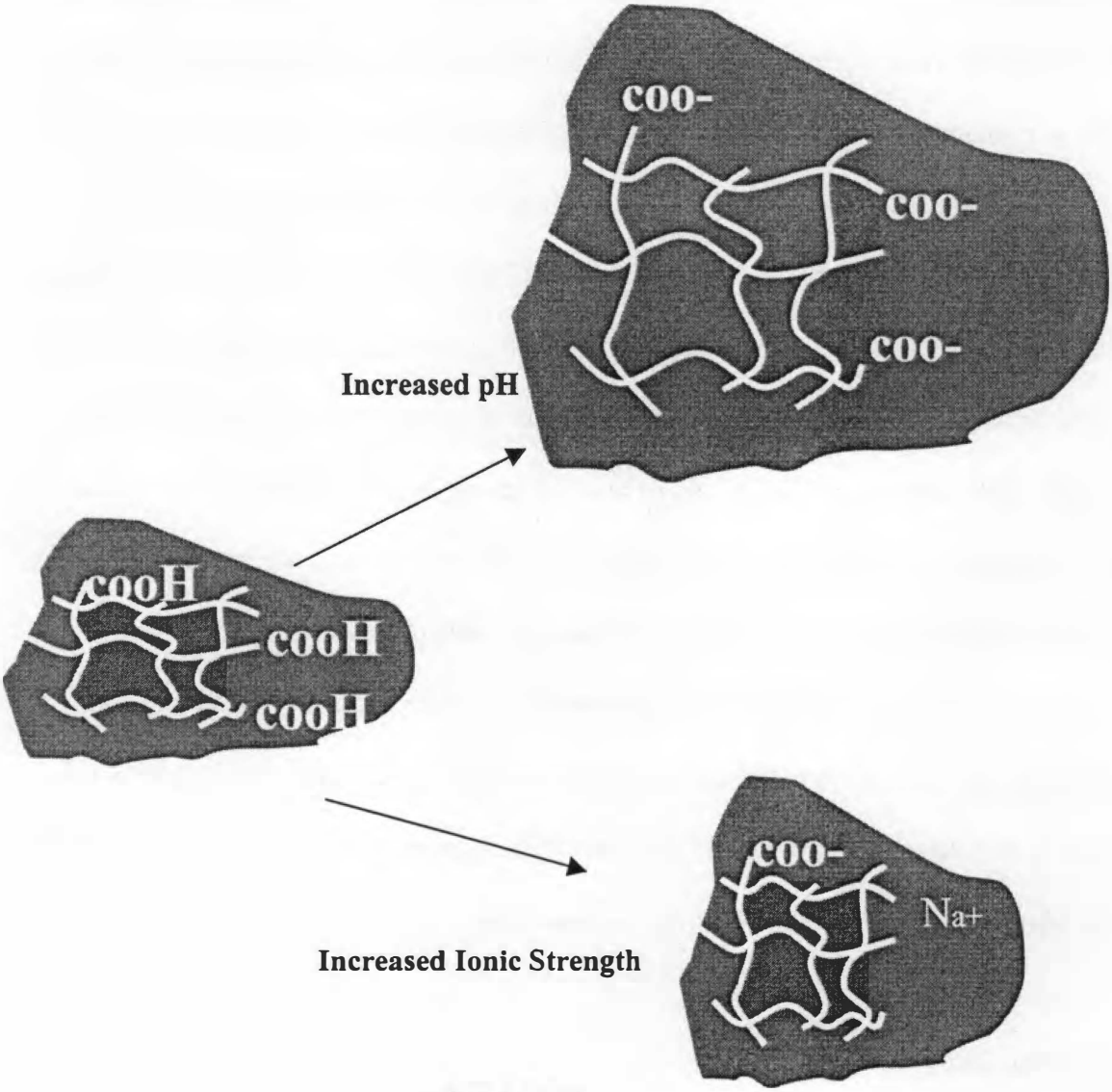


Figure 3. Swelling response mechanism of HEMA co AA hydrogel. This diagram shows effect of local pH, and increased ionic strength.

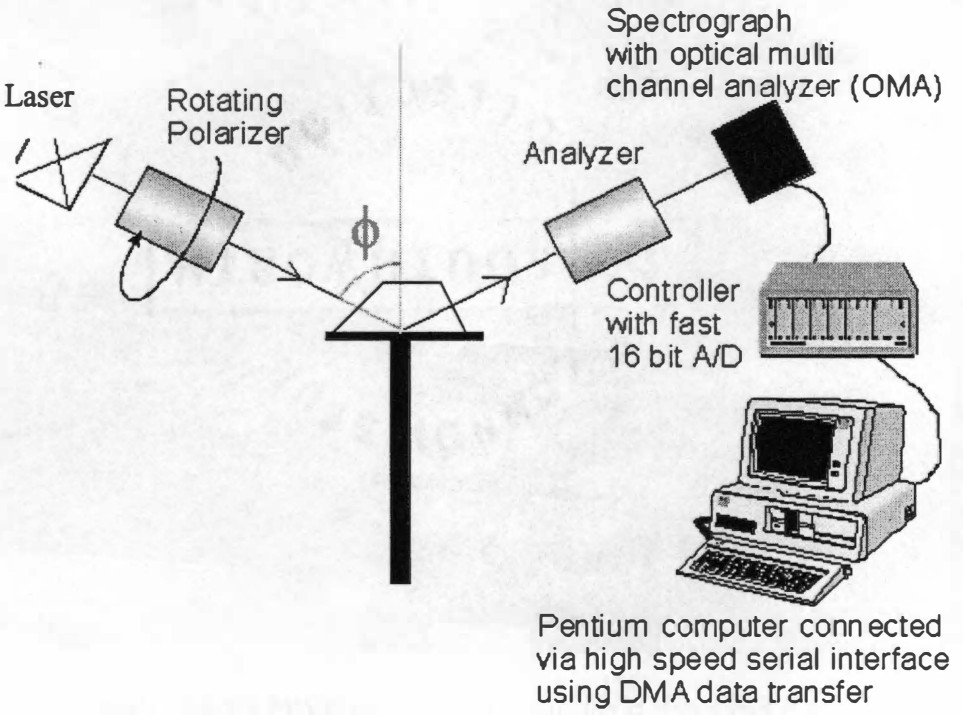


Figure 4. Schematic EL X-02c High Precision Ellipsometer.

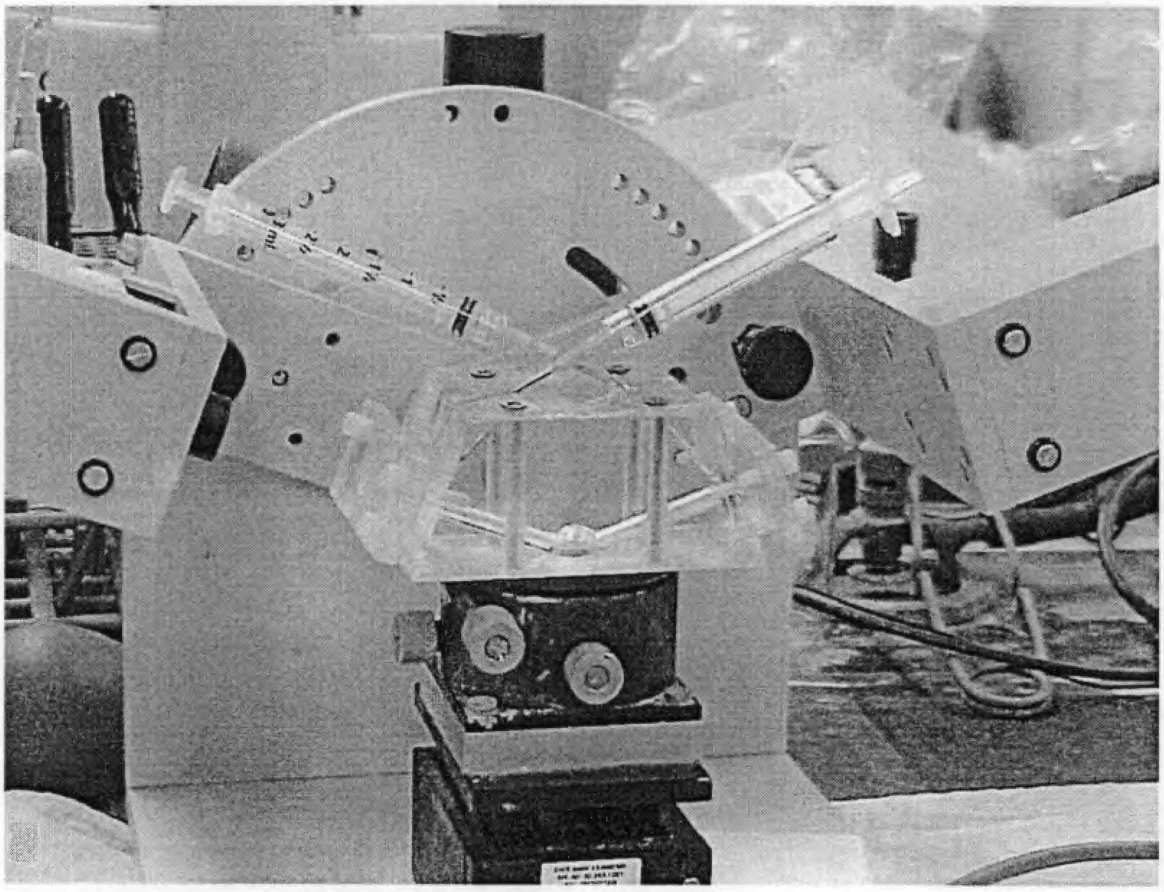


Figure 5 Flow cell positioned upon Ellipsometer sample stage.

literature [16]. Very briefly ellipsometry measures two angularly based optical parameters, Δ and Ψ. There is a phase difference between parallel and perpendicular components of an incoming wave of radiation. Δ is defined as the phase difference occurring upon reflection and can range from 0° to 360°. The amplitude of these two components may change upon reflection, allowing P and S to be the ratios of the outgoing wave and incoming wave amplitudes for these components respectively. This allows one to define Ψ as:

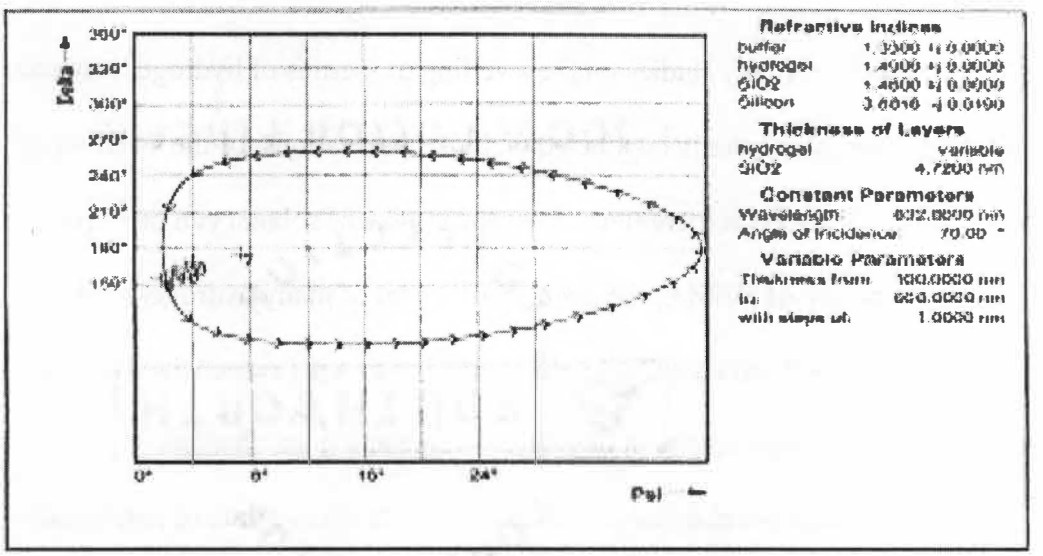
$$\tan \Psi = P/S \qquad (2)$$

Ψ can assume any value between 0° and 90°, and is the angle whose tangent is the ratio of the absolute value of the total reflection coefficients. Δ and Ψ are related by the fundamental equation of ellipsometry, allowing ρ to be the complex ratio of P and S; the reflection coefficients, and j to be the mathematical expression for imaginary numbers ($\sqrt{-1}$) an expression relating the two parameters Δ and Ψ can be expressed as:

$$\rho = \tan \Psi \, e^{j\Delta} \text{ or } \tan \Psi \, e^{j\Delta} = P/S \qquad (3)$$

These formulas reflect the mathematical modeling that is used by the software, which is interfaced with the ellipsometer. These parameters are then used to measure material properties of thin films including thickness and refractive index. Therefore ellipsometry does not measure thickness directly, however by choosing proper conditions

based upon refractive index, and a reasonable range of thickness one can make meaningful calculations. Each set of refractive index, and range of thickness can be saved in a database provided by the software interfaced with the ellipsometer. This combination of refractive index and range of thickness provide a simulation model, which can be used to calculate a thickness of the sample being investigated. A sample of the data generated is depicted in Figure 6. This response curve was generated for thin films under aqueous conditions. This model illustrates the elliptical nature of the previously described components of light. Delta axis is the change in phase difference that occurs upon reflection. Deltas value can from zero to 360° resulting in the elliptical nature of the response curve. The PSI axis is the angle whose tangent is the ratio of the magnitudes of the total reflection coefficients. The PSI value can be from zero to 90°. Each individual calculation based on the response curve returns a calculated thickness (bottom data of Figure 6), using the refractive index of each layer (upper right), and the user-defined thickness parameters.



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No.	Δ/°	Ψ/°	α/°	λ/nm	T/nm	Information
6	164.4344	1.8156	70.00	632.80	411.3587	p=32.05um=1.47
7	171.0305	7.3709	70.00	632.80	955.4251	p=47.2.10p,n=1.33
8	168.5078	4.1110	70.00	632.80	1020.7045	p=47.3.02p,n=1.34
9	165.0445	3.5006	70.00	632.80	1030.7040	p=47.4.36p,n=1.33
10	161.0220	2.8341	70.00	632.80	1067.5772	p=49.4.40p,n=1.33
11	159.1640	2.6106	70.00	632.80	1081.0661	p=49.4.50p,n=1.33

Figure 6. Ellipsometric response curve and data generated for HEMA co AA hydrogel in varying pH environments.

Chapter 2

Ellipsometry As A Means To Measure Changes Of Thickness In pH Responsive Hydrogels

2.1 Introduction

There have been many studies on the swelling properties of hydrogel materials[5,7,10]. These studies have been mainly conducted on bulk observations, of the swelling of samples. The resulting mass increments from the uptake of solvent can be attributed to the hydrophilic nature of HEMA, the major component of many hydrogel networks. This phenomenon combined with the Coulombic repulsion of a pH responsive co-monomer in appropriate pH conditions results in dramatic volume and mass changes.

However, a more novel approach to study the thin-film regime of nano-scale dimension has been accomplished by our project. By using the mass increment observations of solvent uptake, and the observable decrease in refractive index associated with pore filling of solvent, ellipsometric models where designed which measured changes in thickness of pH responsive hydrogels in a aqueous environment. This method of measurement will allow fast, real-time, in-situ measurements of thin films providing information needed for sensing devices such as MCs, biocompatible membranes, and micro drug delivery actuating devices. This method will save time and money, providing fast real time results owing to its remote and robust capabilities. It will also provide a platform for future uses in measurements of other interesting thin film materials including calixarenes, cyclodextrans, and molecularly imprinted polymers (MIP) or as suggested by Peppas et al[17], a combination of MIP's and hydrogels.

2.2 Experimental

Initial experiments involved observation of changes in mass of hydrogel material in varying pH environments. HEMA (Sigma), acrylic acid, EGDMA(both supplied by Aldrich), and Irga Cure (Ciba) were prepared as previously described[5]. Polymerization was accomplished by exposure of the HEMA co AA to 254 nm UV light for ten minutes. The solid gels were cut into small sections and weighed (OHAUS Galaxy 110, Analytical Balance). These sections were then allowed to soak in pH 3 or pH 9 buffer solutions. The samples were then blotted dry and re-weighed. Acrylic acid based monomer slides were prepared by spin-coating the polymer onto a silicon wafer. Each slide was exposed to a different pH solution 3, 5, 7 and 9. This was accomplished by injecting approximately 2mL of the buffer into a flow cell that was mounted on the stage of our ellipsometer (EL X-02C, High Precision Ellipsometer, DRE Ratzeburg , Germany). The ellipsometer was then employed to calculate film properties including thickness and refractive index. To measure initial refractive index of the gels a refractometer (Abbe-3L Refractometer, Bausch & Lomb, Rochester, NY) was used to measure the gels in liquid state resulting in a reported refractive index of approximately 1.45. In a polymerized state the ellipsometer measured refractive index of approximately 1.50 for our hydrogel model.

Additional experiments were conducted to compare the effects of ionic strength on the swelling characteristics of pH responsive hydrogels. Hydrogels again were prepared as described. Weight gain and thickness calculations of hydrogel swelling were conducted in varying environments of NaCl concentration.

2.3 Results And Discussion Of pH Responsive Experiments

Initial experiments required first measuring mass changes in pH 9 sodium phosphate buffer solution of bulk samples of acrylic acid co HEMA polymer samples using the following relationship:

$$\text{Weight Gain} = \text{mass}_f/\text{mass}_o \qquad (4)$$

Mass_f is the final mass of the polymer after exposure to buffer; mass_o is the initial mass of the polymer before buffer exposure, the dry state. The pH 3 samples showed small changes in mass relative to the semi-dry state due to solvent uptake by HEMA alone. The pH 9 samples showed a nearly three times mass increment owing to both HEMA's hydrophilic nature and charge separation upon the hydrogel backbone due to deprotonation of the acrylic acid monomer.

After determining an appropriate range of mass increment the ellipsometer can be employed to measure changes in thickness in response to the changes in pH. At pH 3 the refractive index remained around 1.5, and when exposed to pH 9 the gel's refractive index decreased to nearly 1.33, according to ellipsometric modeling. This decrease in refractive index provides good evidence that swelling is occurring. In the swelled state pore formation and filling of solvent results in lowering of refractive index[18] as previously noted, this proposed model results in the gel's refractive index approaching that of the aqueous environment.

By using the observable increase in mass, and decrease in refractive index ellipsometric models were designed to measure changes in thickness. An example of such a response curve with data is found in Figure 6. This figure represents actual experimental data generated for acrylic acid functionalized hydrogels. Close attention to this figure shows that under low pH conditions (pH 5) the thickness is 411 nm, with a refractive index of 1.47. As pH is increased to 7 the thickness increases to 955 nm while the refractive index decreases to 1.35. Finally, as pH is increased to 9 the thickness is calculated as greater than 1060 nm, as the refractive index approaches that of water 1.33.

This method of using observable phenomena provided a means to test the pre-designed models. This range of pH was above and below the pKa of acrylic acid ~ 4.5, and also provided a range of neutral pH conditions. We should expect a swelled and deswelled event since the hydrogel backbone will have a charge separation at higher pH, and minimal if any charge separation at low pH. Figure 7 shows calculated thickness and varying refractive index for each slide at the noted pH. pH 3 showed slow collapse of the film, with a comparable increase in refractive index. The first data point appears as an outlier, however this was the initial calculated thickness, the latter two calculations reflect the change in refractive index and collapse of the film thickness. The pH 5 sample showed a slow increase in thickness with a constant decrease in refractive index. pH 7

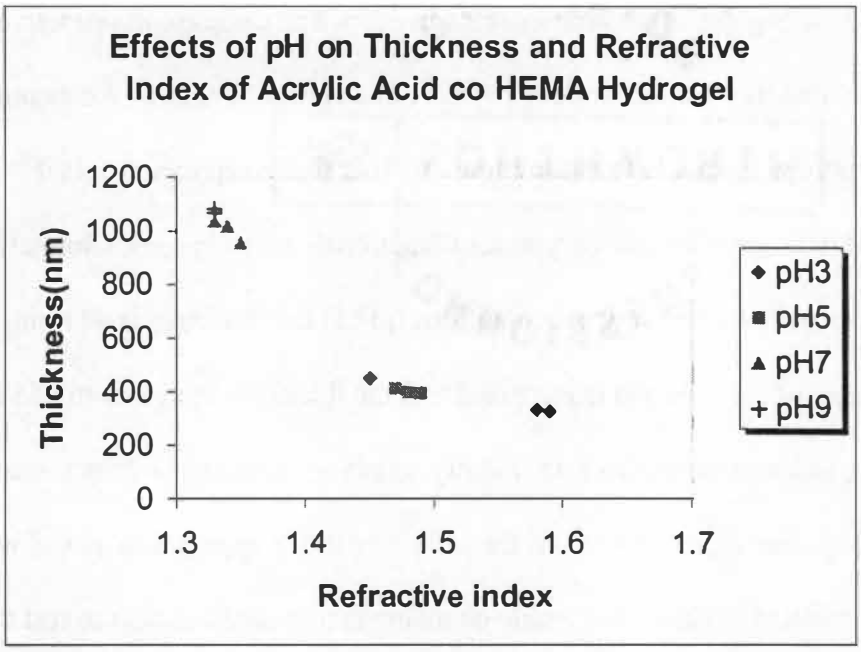


Figure 7. Thickness and refractive index data for HEMA co AA hydrogel under varying pH conditions.

and 9 showed more dramatic changes in swelling as the refractive index stabilized at 1.33. Bulk samples were soaked overnight in the same buffer solutions. pH 3 and 5 experienced minimal change while pH 7 and 9 showed a 5 and 4 times increment respectively, this may reflect the effect of higher ionic strength interactions(Table 1). pH 9 should have a higher ionic strength thus a smaller swelling response as reported in the data, ionic strength will be discussed later in this thesis. This experiment provides strong evidence that ellipsometric models can be designed to measure thickness changes on nano-scale dimensions. This also provides a means to create models that can be used for unknown sampling conditions, providing a remote capability to the instrument.

There are three important parameters to consider for successful thin film applications. First, is the ability to tailor hydrogel material to respond to minute changes in pH, thus lowering the detection limit of this method. Second, structural integrity of the network must be realized for coating a MC device. Finally, the pH response of the material must be reversible, in a timely fashion. Thus allowing a reproducible response in long term usage.

The first goal, responsiveness of thin films to minute changes in local pH may be attained by increasing the amount of functional monomer (AA), or by decreasing the amount of cross-linking agent (EGDMA). By varying amounts of acrylic acid 9%-15%-16%-24% with constant crosslinking agent (1%) exposed to pH 9 buffer, mass increments of 2.4, 2.7 and 3.2 were observed (Table1). A sample containing 3% EGDMA showed 2.4 times mass increment opposed to 1% EGDMA showing a 2.8 times mass increment (Table 1) when comparing the same percentage of cross-linking agent. Further

Table 1. HEMA co AA hydrogel swelling response under varying conditions of pH and percentage of acrylic acid.

Conditions	Mass dry (g)	Mass wet (g)	Ratio
pH 3 (9%AA)	.154	.160	1.0
pH 3 (16% AA)	.013	.014	1.1
pH 3 (16% AA)	.019	.030	1.6
pH 3 (24% AA)	.126	.130	1.0
pH 9(9%AA)	.126	.300	2.4
pH 9 (9%AA)	.319	.766	2.4
pH 9 (15% AA)	.291	.788	2.7
pH 9 (16% AA)	.013	.041	3.2
pH 9 (1% EGDMA)	.0424	.119	2.8
pH 9 (3% EGDMA)	.126	.304	2.4
pH 9(.5%EGDMA)	.0148	.0595	4.0
pH 7(.5%EGDMA)	.0121	.0625	5.2

experiments reveal that 0.5% cross-linking agent of acrylic acid based monomers swell nearly 5 times initial mass when exposed to pH 9 buffer.

These results suggest that altering cross-linking agent, or functional monomer will allow control of swelling properties. Increments in acrylic acid seem to show favorable response to a change in local pH, thus this should allow one to enhance detection limits. By decreasing cross-linking agent the elastic properties of the hydrogel matrix may increase allowing a greater swelled state and overall better response to changes in local environmental pH. However the mechanical stability of the gel may suffer from decreased cross-linking agent. When cross-linking agent EGDMA is decreased from 1% to 0.5% a decrease in mechanical strength is observed. For example, a 10 gram mass metal weight was able to insert itself into the lower concentrated EGDMA gel whereas the higher concentrated EGDMA does not allow incorporation of the mass, instead the mass was supported by the gel.

Reversibility of the pH responsiveness can be fashioned after an oscillatory response. The "valve-like" action reported for microactuation in studies conducted by Lee et al.[7], and Beebe et al.[5], were repeated using ellipsometer to measure thickness changes. The results show good agreement with these prior studies. In low pH (less than pH 4), the gel showed little swelling, as pH increased (greater than pH 7), the swelling response increased by a factor greater than 3 times initial thickness. This swelling, deswelling was observable through several cycles (Table 2 and Figure 8). Besides thickness, refractive index also showed an oscillatory response increasing as thickness

Table 2. HEMA co AA hydrogel oscillation swelling behavior; thickness and associated refractive index changes.

PH	Thickness(nm)	Refractive Index
Air	281	1.485
3	412	1.485
9	931	1.335
9	863	1.348
3	413	1.485
9	990	1.335
9	941	1.340
3	402	1.485
9	975	1.340
9	947	1.343

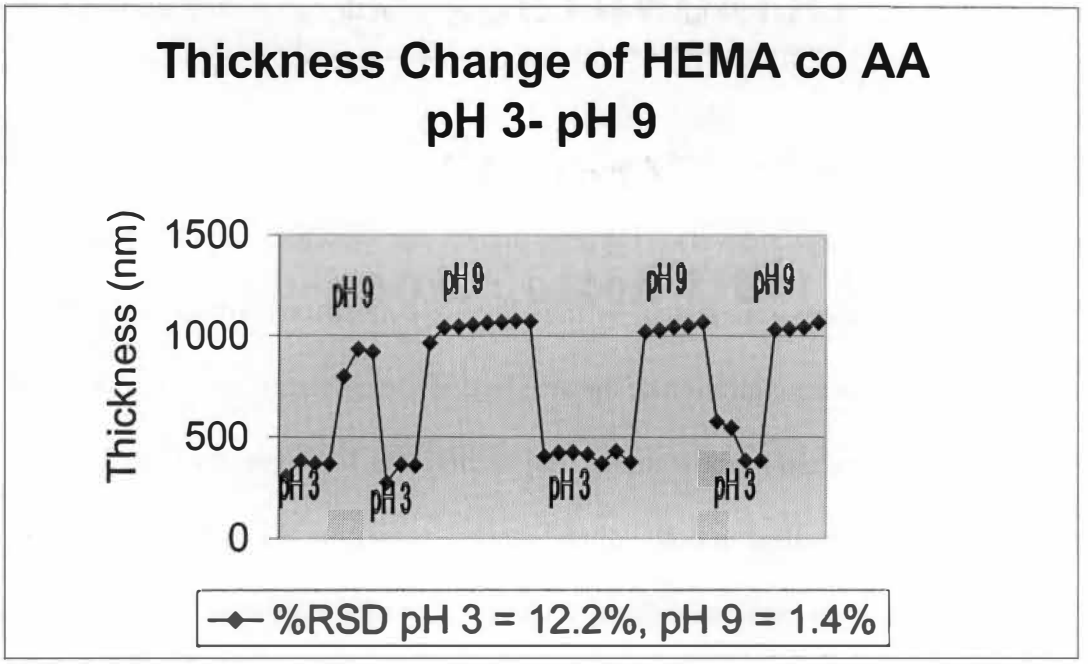


Figure 8. Oscillatory response of HEMA co AA hydrogel. Measuring reversibility of response.

decreased, and decreasing towards the refractive index of the local environment as the thickness increased.

Ionic strength is another environmental issue to address when studying the swelling characteristics of hydrogel networks. Figure 3 depicts the dynamics of pH and increasing ionic strength of local environments. The migration of counterions within the local environment results in an inhibition of the swelling of the hydrogel network; the swelling due to Coulombic repulsion is now minimized in this state. Changes in pH, as I have demonstrated above, resulted in both volume increments within the hydrogel network, and measurable changes in the calculated thickness. The effects of ionic strength on the previous parameters were investigated. Ionic strength experiments were conducted on HEMA co AA hydrogel polymers. At pH 7, both weight gain and ellipsometric calculated thicknesses were compared. The gel slabs were exposed to varying concentrations of NaCl (0-1.2 M). The slabs were then weighed after overnight exposure. The same hydrogel composition was spin-coated on to silicon wafers. These slides were exposed to pH 7 buffer under varying conditions of NaCl concentrations (0-1.2 M). The results show good agreement between the bulk sample and the thin film nano-scale samples and are compared in Figure 9. As ionic strength is increased, the swelling characteristic of the network is minimized. Both a decrease in weight gain and decrease in thickness are observed.

The advantages of using ellipsometry have been described; remoteness, robustness, and fast real-time in-vitro measurement capabilities. Ellipsometry also provides more

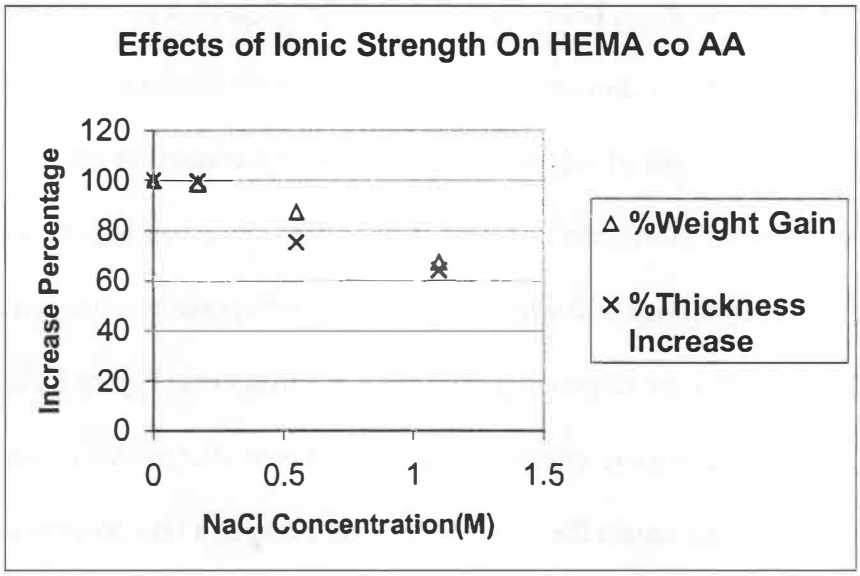


Figure 9. Weight gain vs. thickness of HEMA co AA hydrogel. Ionic strength shows similar effect on weight gain and thickness response.

detailed information into the mechanism of the responsiveness of thin-film material. Oscillatory swelling and deswelling events measured using ellipsometry are comparable to other groups weight gain observations[7]. However, our method provides additional insight to the phenomena. First, refractive index changes suggest a pore-forming step that occurs as thickness increases. Solvent migration into the pore results in a lowering of refractive index closer to that of solvent. Second, as pH is increased to pH 9 an initial increase in thickness is followed by a slight decrease. This observation provides information into the dynamics of the process. This may reflect the counter-ion migration, sodium ions of the buffer are responding after protonation occurs (Figure 3). Initially a charge separation creates a more swelled condition as the smaller proton dissociates, eventually the sodium ion enters the local medium resulting in a less pronounced swelled state, as charge is neutralized. The results for ionic strength also show good correlation between methods. Weight change and thickness change show similar trends as sodium chloride concentration is increased (Figure 9).

Another major advantage as we have discussed is increased sensitivity. Figure 6 shows that as pH changes from 5 to 7, the calculated thickness changes from 400nm to 1000 nm or 75% change per pH unit. If one wished to measure a change of .001 pH units, this would result in .075% swelling response. If the length of our MC is 400 micrometers then the actual swelling required to measure .001 change in pH units can be treated as follows:

$$.075\% = (\text{swelling response}/400 \text{ micrometers}) \times 100\% \quad (5)$$

This results in a 300 nm deflection response of our MC. Considering that normal noise associated with MC measurements is 10 nm, a very large signal to noise ratio is possible. The swelling involved for a 400 nm thick gel would only be 30 nm. Ellipsometry has the ability to measure changes in thickness as small as 1 nm. Therefore, ellipsometry would provide an excellent tool to quickly measure the swelling properties required for MC applications. Bulk weight gain experiments can be used to support MC applications. However, nano-scale behavior of very thin films may deviate from these bulk observations, thus it is advantageous to use a tool such as ellipsometry to investigate very thin film responsiveness.

The major disadvantages of our method include complex theory behind ellipsometry, which would require advanced knowledge by the user in the event of technical difficulties. Other disadvantages include alignment of the laser which is somewhat time consuming for the novice. These disadvantages are overcome by prolonged use of the instrument. It would also be valuable to be able to measure continual changes in pH. If a continous flow cell could be employed the time factor required for laser alignment would be diminished. This would allow a more detailed study in the mechanism and the kinetics of hydrogel responsiveness.

Chapter 3

Novel Analyte Sensing Applications: Enzyme Based Systems

3.1 Glucose Response Experiments

Several groups have studied hydrogel response to glucose exposure[4,7,19]. This application focused primarily on insulin release in response to changing glucose environments. Our project is concerned with the realm of biosensing materials in the form of thin film applications to MC devices (Figure 1). A swelling, deswelling response to glucose exposure will provide a means to measure cantilever-bending response to glucose in a physiological environment. This will allow a remote means of measuring glucose, which may be beneficial to those suffering from such diseases as diabetes.

The mechanism we have chosen involves using a similar hydrogel network as described in our pH experiments. However; opposed to a HEMA co AA comonomer system, the glucose responsive gel requires a HEMA, 2 dimethyl amino ethylmethacrylate (DEAMA). This gel shows weight gain in low pH environment (Tables 3 and 4). And measurable thickness changes in comparable pH (Table 5). Included in this network is glucose oxidase, and catalase. Glucose oxidase is required to convert glucose to gluconic acid, which in turn lowers the pH of the local environment within the gel network. The DEAMA accepts a proton in the low pH environment, the resulting positive charge on the polymer

Table 3. HEMA co DEAMA hydrogel swelling response, pH vs cross-linker composition in a wide range of pH.

HEMA co DEAMA Swelling Response ($Mass_f/Mass_o$)

PH	1.4%EGDMA	0.7%EGDMA
4.6	2.8	3.2
7.1	2.8	3.3
9.2	1.2	1.2

Table 4. HEMA co DEAMA hydrogel swelling response varying pH, constant cross-linker composition in a narrower range of pH closer to physiological conditions.

HEMA co DEAMA Swelling Response 0.7% EGDMA ($Mass_f/Mass_o$)

pH	Swelling Response
4.6	3.4
6.9	2.9
7.2	2.7
7.4	2.4

Table 5 HEMA co DEAMA hydrogel film thickness response varying pH. Example of ellipsometer data of film thickness response of basic monomer.

PH	Thickness(nm)
Air	332
4.6	1221
7.4	1036
9.0	1024

backbone which undergoes Coulombic repulsion much like the HEMA co AA, again resulting in a swelled state. This polymer network is more sophisticated than the HEMA co AA network, owing too a more intricate response mechanism, depicted in Figure 10. As glucose is introduced into the local environment interaction with polymer occurs. In Figure 10 (a) glucose initially reacts with glucose oxidase, resulting in the production of gluconic acid Figure 10 (b). The complete mechanistic steps are outlined in equations 6-7. Catalase is incorporated to overcome the oxygen requirement for glucose oxidase to function; it also assists in overcoming the peroxide side reaction, which inhibits the following process:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{Gluconic acid} + H_2O_2 \quad (6)$$

The oxygen requirement and hydrogen peroxide inhibition can be overcome by the use of catalase, which results in the following reaction:

$$H_2O_2 \xrightarrow{\text{catalase}} {}^{1}/_{2}O_2 + H_2O \quad (7)$$

3.2 Enzyme Loading Experimental Conditions

Samples were prepared according to a published method[6]. Briefly, for glucose response experiments, HEMA co DEAMA (Aldrich) gels were incorporated using EGDMA as cross-linking agent, Irga Cure as photoinitiator, glucose oxidase as the active enzymatic

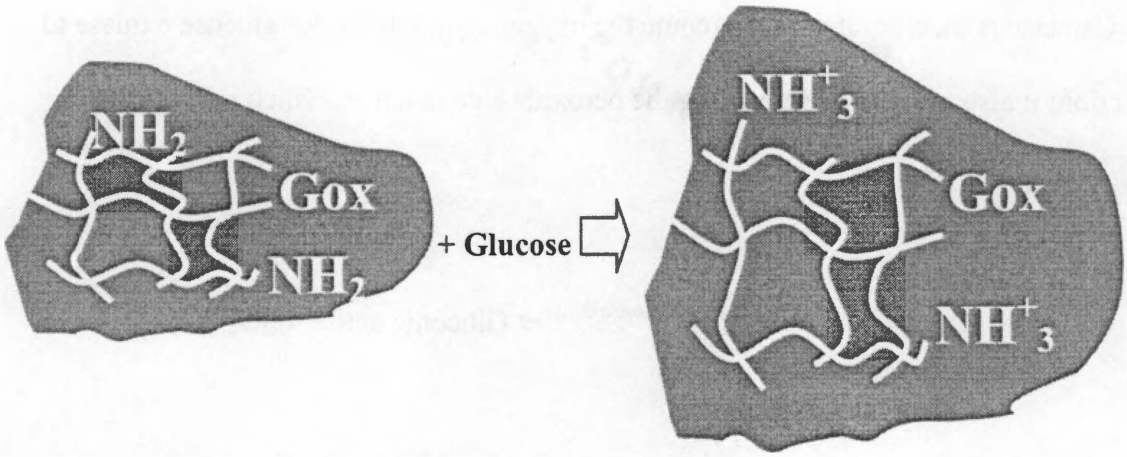


Figure 10. Glucose Response Mechanism Incorporating Glucose Oxidase.

component, Aspergillus niger as the catalase (enzyme and catalase supplied by Sigma) and ethylene glycol (Aldrich). For urea response experiments (see below) HEMA co AA, EGDMA, Irga Cure, ethylene glycol and Urease (Sigma) were incorporated.

The initial experiments required measuring the response of the HEMA co DEAMA in acidic environments, while varying cross-linker EGDMA to find the most effective swelling response. Both weight gain and thickness calculations were conducted initially on gels prepared by copolymerizing HEMA and DEAMA. After determining appropriate conditions, pH and cross-linking ratio glucose response experiments could be conducted on the combined network of HEMA co DEAMA, EGDMA, glucose oxidase, catalase, ethylene glycol, and photo-initiator.

Small gel slabs were weighed and exposed to varying concentrations of either glucose (glucose response experiments), or urea (urea response experiments). These samples were blotted dry and re-weighed. Weight gain data was then calculated for each sample. Silicon slides were then coated and exposed to similar concentrations of glucose or urea. Weight gain data and thickness calculations were compiled and compared for each experimental scenario.

3.3 Discussion Of Glucose Response Experiments

Table 3 shows the results of using pH 4.6, 7.1, and 9.2 while varying the EGDMA from 1.4% to 0.7%. These results suggest that the HEMA co DEAMA show a pronounced swelling response in pH less than 9.2. The 0.7% EGDMA appears to be the most dramatic system as expected. However; the range of pH response required further experimentation to determine the response in a narrower range of pH changes (near physiological conditions). Therefore a further experiment using 0.7% EGDMA was conducted with the following results listed in Table 4. From these results it appears that, as pH decreases swelling will increase, below that of physiological pH (7.4) and near that of the pKa of gluconic acid (3.6), and in the active range of glucose oxidase which is reported as pH = 5.6[20]. Table 5 demonstrates the ability to calculate thickness changes of the basic monomer DEAMA in acidic environments.

The next experiment was used to measure weight gain in the gel network incorporating all components as previously described. The substrate used for comparison studies included the following; D + glucose, beta D + glucose, sucrose, and deionized water. The following results were observed and are listed in Table 6 and Figure 11.

The discrepancies between trial #1 and trial #2 may be related to the use of tetrahydrofuran (THF). The sample was prepared fresh for trial#1, after sitting for 2 days trial #2 was executed. THF may have caused oxidase decomposition, thus altering results. Trial #3 was run adding THF right before testing. The oxidase that was used should be specific for beta D + glucose, however; it appears that a kinetic issue may be affecting the

Table 6. Glucose swelling response varying sugar. Glucose responsive hydrogels weight gain data when exposed to varying environments of sugar and deionized water.

HEMA co DEAMA Swelling Response ($Mass_f/Mass_o$)

Substrate	Trial#1	Trial#2	Trial#3
D+glucose	5.0	4.4	3.9
Beta D+glucose	1.8	2.9	4.1
Sucrose	1.7	1.8	--
Deionized Water	1.9	1.6	1.5

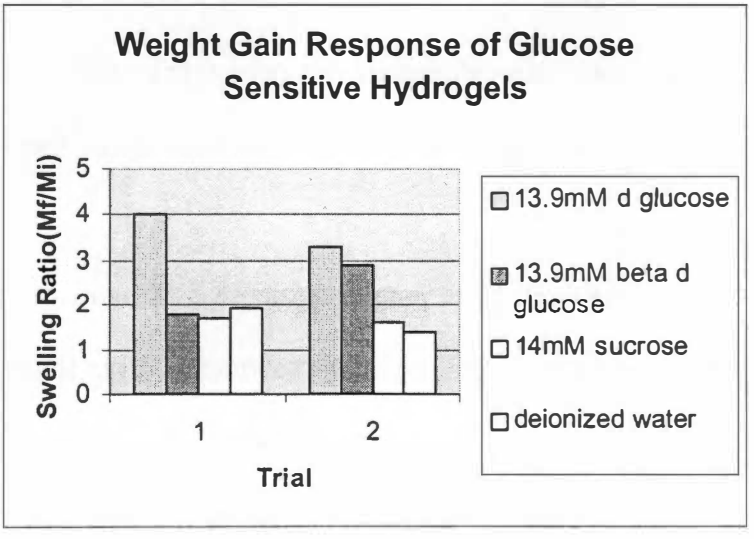


Figure 11. Weight gain results of glucose responsive hydrogels. Comparison of analyte response.

observed results. The D + glucose is an open ring structure which is actually an intermediate in the mechanism of beta D + glucose oxidation to gluconic acid, the mechanism may be outlined as such:

Beta D+ glucose→ ring opens→ **D+glucose→**glucox→**gluconic acid** (8)

What may be occurring during trial #1 is that D + glucose is converted to acid faster thus larger swelling effect. During trial #2 and trial #3 the rates of conversion are approaching one another as decomposition of the oxidase continues. The exposure time for trial #1 is 2.5 hours while the exposure time for trial #2 was 5 hours. The first trial shows better response for the straight chain form owing to ring structure requiring more time to open to the straight chain conformation, the second trial supports this observation. The size difference between the beta D+ and D+ conformation may also be a factor in the response of swelling. Perhaps the strait chain conformation may be able to diffuse through the gel more efficiently leading to a faster swelling event compared to the bulkier ring conformation.

Upon establishing the swelling response of glucose oxidase loaded hydrogels, silicon slides were prepared as in the pH response experiments. Film thickness was calculated using the ellipsometer results are listed in Figure 12. Five trials were conducted; the first three trials showed only minimal response. Some of the problems may be rationalized as follows; trial #1 shows a somewhat favorable response, however increased glucose concentration did not result in greater swelling response. This may

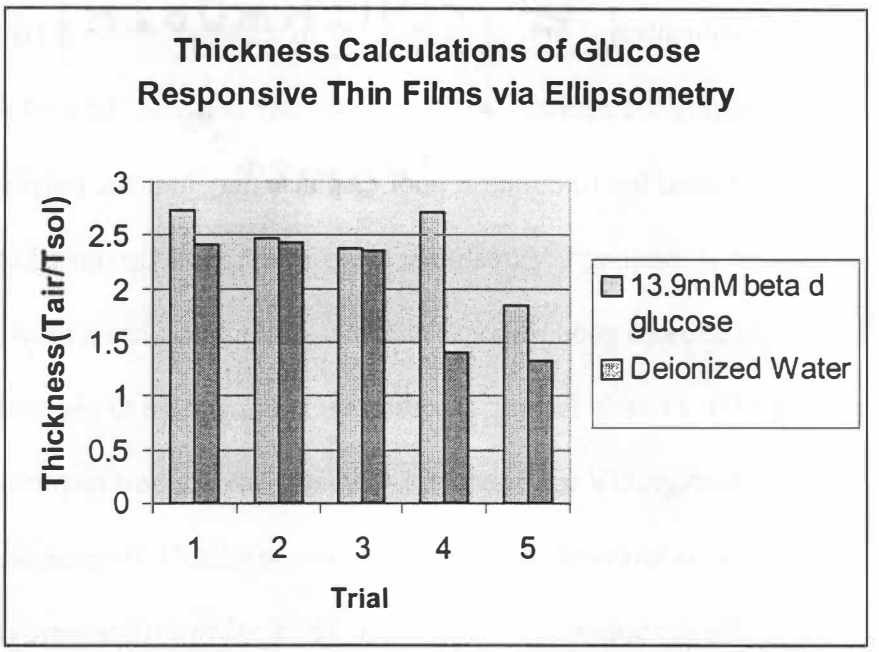
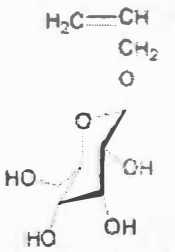


Figure 12. Thickness calculations of glucose responsive thin films using ellipsometry.

reflect either leaching of oxidase, or saturation of glucose response. Trial #2 showed poor response, this may be indicative of storing the polymer in tetrahydrofuran (THF) for three days resulting in denaturing the enzymes within the polymer network. Trial #3 was fresh polymer that was UV treated for 10 minutes; poor response may indicate polymer is incompletely dissolved or too long UV treatment again leading to a denatured enzyme.

Two final trials showed good response as UV treatment was lowered to 5 minutes and samples were used in a timely fashion. Therefore it is imperative to choose proper conditions for sample storage, UV treatment and solvent to invoke best response. High temperatures in the lab may have also contributed to poor results. During the semester break temperatures in the lab approached 30 Celsius. The final two trials were executed by cooling the glucose solution overnight.

Comparison experiments were attempted. Using allyl glucoside (AG) an allylic glucose derivative (Figure 13) and the enzyme concanavalin A(ConA) attempts were made at mimicking a response mechanism reported by Hoffman et al[24]. In this mechanism (Figure 14) Hoffman utilized a poly (2-glucosyloxyethyl methacrylate)(GEMA)(Figure 15) ConA complex that created a collapsed gel state due to the glucose moieties upon the polymer backbone binding to the ConA. Exposure to glucose the ConA released the GEMA, following competitive binding to the free glucose within the local environment causing the gel to expand. GEMA being commercially unavailable prompted me to find a similar compound that would have pendant glucose groups off of a polymeric backbone that could be functionalized within a hydrogel



Molecular structure of AG

Figure 13. Structure of Allyl Glucoside.

Source: Xu, *Langmuir*, **2003**, 19, 6869

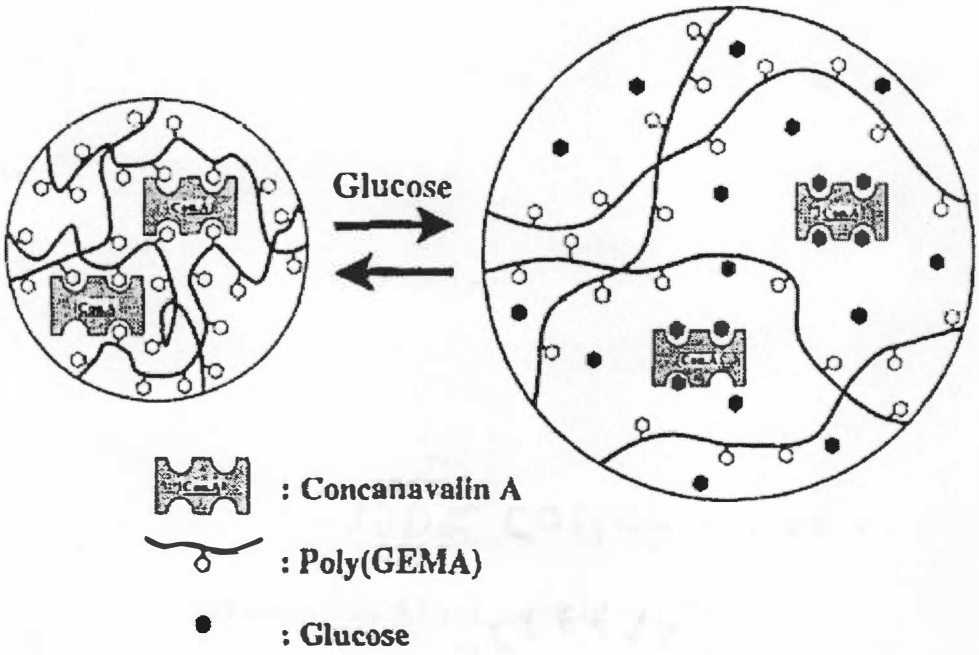


Figure 14. Concanavalin A GEMA mechanism. Con A GEMA complexation inhibited by presence of glucose resulting in swelling of polymer network.

Source: Hoffman, *Macromolecular Chemical Physics*, **1996**, 197, 1135

GEMA:

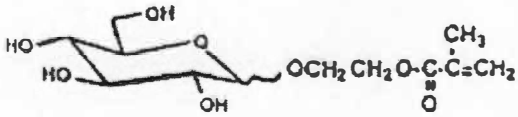


Figure 15. Structure of 2 glucosyl oxy ethyl methacrylate.

Source: Hoffman, *Macromolecular Chemical Physics*, **1996**, 197, 1135

network. With the assistance of Dr. David C. Baker I was able to attain allyl glucoside (AG). The pendant glucose group on the polymer (compare Figures 13and 15) should result in a similar response mechanism comparable to the GEMA ConA mechanism reported by Hoffman. Unfortunately this was not realized. The major problems were due to incompatible solvent systems, and inability to functionalize our hydrogel system.

Attempts were also conducted at using AA monomer and observing if a de-swelling event would occur. This mechanism would compare if swelling of the basic monomer DEAMA or de-swelling of the acidic monomer AA would be more responsive. However; the AA monomer showed no de-swelling events. The pH was probably not low enough to protonate the AA exposed backbone.

The ability to measure the responsiveness of thin films using ellipsometry in support of MC applications is the long term goal of our project. I have demonstrated the ability to calculate the changes of thickness associated with glucose responsive hydrogels using ellipsometry. Dr. Nickolay Laverick has conducted similar experiments using glucose responsive hydrogels coated on "macro-levers"; the results are depicted in Figure 16.The micron deflection suggests a size factor of the lever being much larger than the scale we wish to investigate in future work. Therefore we have demonstrated the ability to calculate very thin film responsiveness and the ability to measure cantilever deflection in response to local environmental changes in glucose concentration.

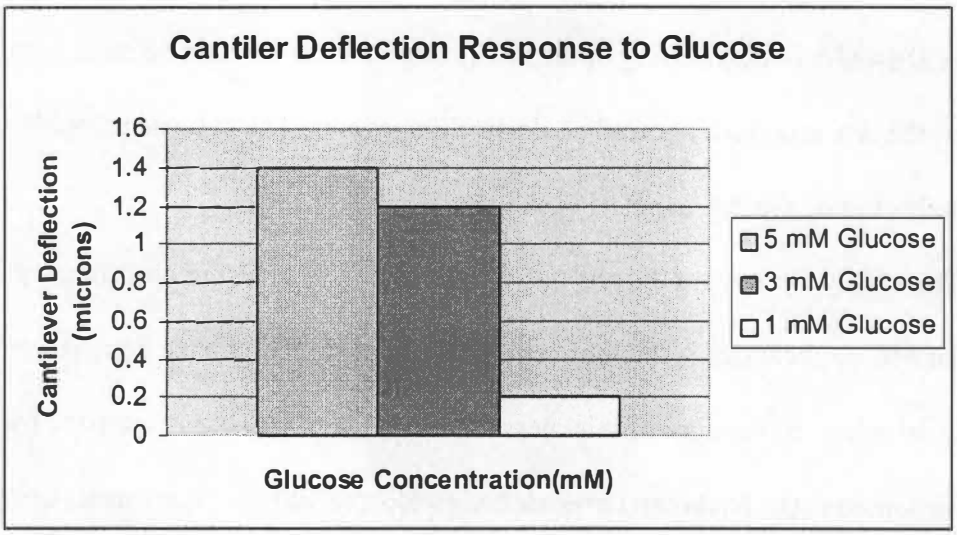


Figure 16. Cantilever Deflection Response to Glucose.

3.4 Urease Results And Discussion

My last project was an investigation of another enzyme-loading technique. This techniques has been employed for novel detection schemes including cholesterol detection[22], and the detection of organophosphate[23]; a major component of pesticides and neurotoxins. An effective technique to test more sophisticated enzyme loading methods will be the detection of Urea in the presence of Urease, the mechanism is as follows;

$$\text{Urea} + H_2O \rightarrow \text{Urease} \rightarrow 2\ NH_3 + CO_2 \quad (9)$$

The formation of ammonia will cause the local environments pH to increase. Again a HEMA co AA should deprotonate thus causing a swelled condition of a hydrogel network in response to Coulombic repulsion along the gel backbone.

Using the same method of polymer preparation as described by Beebe[5] from previous experiments for HEMA co AA will allow formulation of hydrogel networks to be tested under varying conditions of urea. These conditions include comparing responsiveness of polymers that contain urease versus polymers that are free of the enzyme. Likewise, testing environments that are free of urea versus those that contain ~ 80mM urea concentration. The same method of measuring weight gain of polymer slabs and thickness change of spin coated silicon slides was used to determine the success of this experiment. Denaturing of enzyme is of utmost importance; thus choosing

appropriate experimental conditions is vital. These conditions include solvent selection and uv-treatment time. Finally, ionic strength and pH must be carefully monitored to ensure responsiveness is due to urea alone.

The initial experiments were conducted to test the effect of urease-loaded hydrogel compared to hydrogel with no urease. Figure 17 displays the initial results of weight gain experiments. The response is greatest for higher pH environment, owing to acrylic acid monomer response alone, and possibly due to urease being most active in pH of 7.2[24]. Increasing the amount of urease by a factor of ten a response in deionized water is now observed, this is most likely due to the presence of urease. Choosing a solvent for dissolving the urease while not damaging the enzyme was the next obstacle. James B. Sumner[25] has reported that alcohols at low temperature were useful in urease extraction. However; Sumner further demonstrated that acetone provided a much-improved method of extraction. This improvement resulted in the first method of enzymatic crystallization. Therefore by changing solvent from ethanol to acetone better overall response is observed; notably pH 3 buffer now shows responsiveness. It was also reported by Sumner[25]; that urease is slowly deactivated in environments below pH of 4. Therefore acetone either precipitates the urease, strongly activates the enzyme even at low pH as my results suggest (Figure 17), or alters any impurities that adversely affect the enzyme[25], perhaps these factors may have contributed to better results when using acetone as my solvent. This observation addresses the need to carefully choose appropriate solvents when working with enzymes. It appears that the less polar acetone is

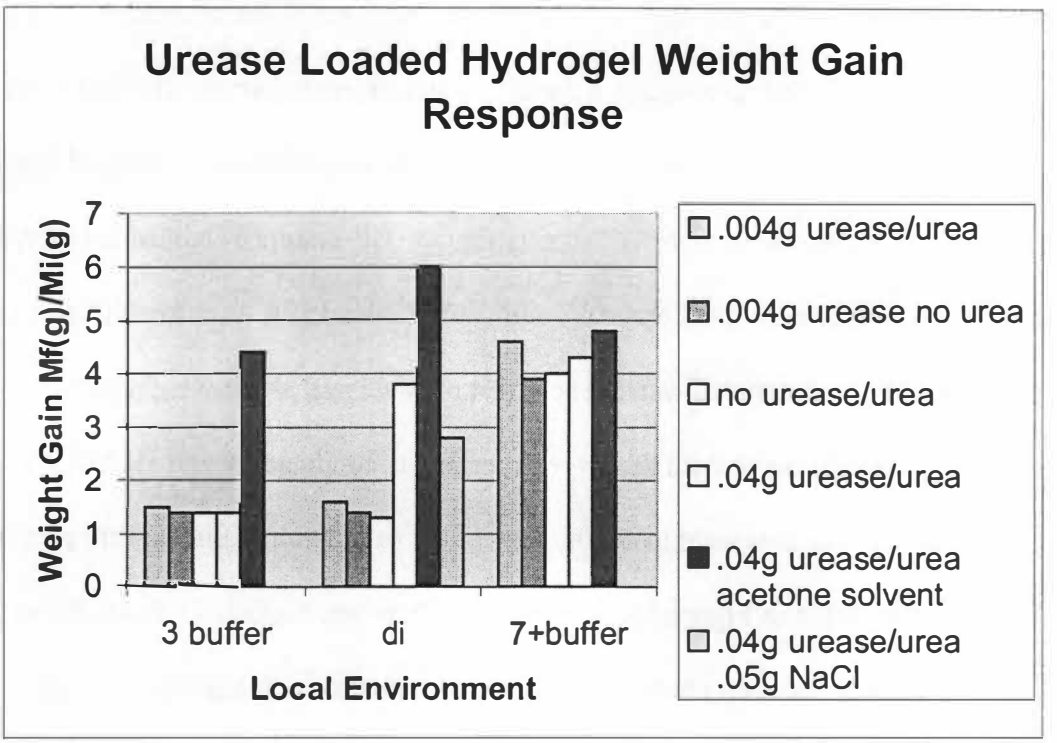


Figure 17. Urease loaded hydrogels weight gain results in varying environments of buffered and deionized water. (Top of Key to bottom reads left to right).

a more compatible solvent for urease. Perhaps the ethanol is either denaturing the fragile enzyme, or the pH of the solvent itself could be playing a role in the mechanism. Another possible explanation is that the enzyme is simply leaching out of the network.

The greater observed response in deionized water prompted an experiment to test the dependence of local environmental ionic strength. It appears that the buffered system may be less responsive due to increased ionic strength. The results of controlled increase of the ionic strength of deionized water are shown in Figure 15. A 43 mM solution of NaCl was prepared in deionized water. The effect of NaCl stifled the weight gain response. These results agree with the prior experiments conducted using HEMA co AA.

Ellipsometry was now used to conduct thickness calculations. Slides were prepared and the results are listed in Figure 18. A comparison between Figures 17 & 18 shows very good agreement between observations of the two methods. Weight gain results provided the information needed to choose sampling environments, since deionized water showed best results I have chosen deionized water with 83 mM urea compared to deionized water alone. I have also conducted ionic strength experiments to compare with the weight gain results. Again refractive index changes accompany thickness changes, increased refractive index for the collapsed state, and the converse true for the swelled state.

The results show that the thickness increased for 83 mM urea environments in agreement with the weight gain results. Also, the deionized water samples alone did not

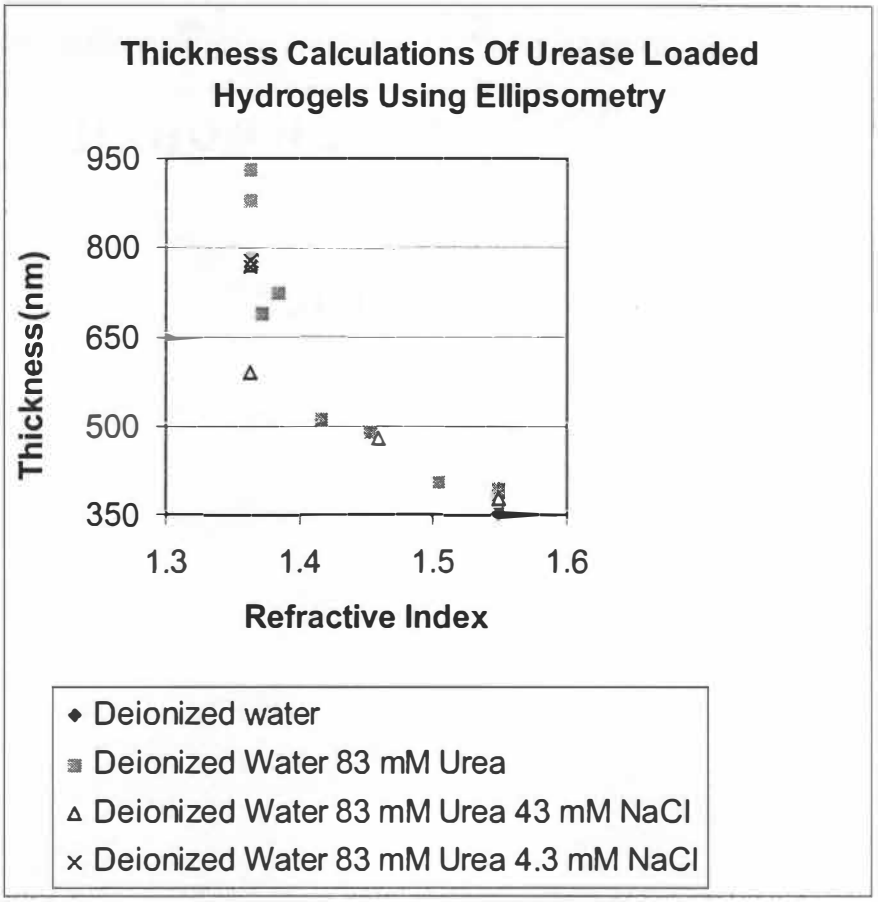


Figure 18. Urease loaded hydrogels calculated thickness results using ellipsometry. Effects of both urea and ionic strength are compared. The Deionized Water/Urea samples are plotted for a series of measurements. Initial measurements show large refractive index with minimal swelling. Eventually a swelled state is reached, accompanied by a decrease in refractive index. Comparing with Deionized Water/Urea/43mM NaCl, a less pronounced swelled state is observed.

show any swelling as the refractive index remained around 1.55. The 43 mM NaCl sample showed a decrease in response parameters. It should be noted that the data for this response is decreasing, that is the points are shifting towards higher refractive index and decreased thickness.

Chapter 4

Conclusions And Future Directions

Previous investigations have demonstrated that hydrogels, which are three dimensional polymer networks; respond to changes in local environmental conditions such as pH, temperature, and ionic strength. The ability for these gels to conform to these changing conditions based upon shape, permeability, and host release renders them useful for many applications including drug release, micro-actuating, and as I have demonstrated analyte responsive thin films. HEMA being hydrophilic is extremely useful for many physiologically important applications including wound dressings, and in-vivo drug delivery devices. Our project provides a means for measuring the property of shape, specifically swelling; on a nano-scale dimension. The ability to measure at this dimension will provide information required for determining the utility of such systems in a timely and remote fashion in support of MC applications. Timeliness related to fast response, which is a primary feature of such systems, and remoteness that will allow measurements in the field; thus providing real-time results. The measurements made using ellipsometry will allow the researcher to determine if swelling response will be useful for actuating devices, such as micro-fluidics, or if the material will be responsive enough to use in sensing applications, such as MC devices. Arwin[26] has described the favorable features of ellipsometry for sensing applications as possessing the in-situ advantage, the possibility to work with non-labeled molecules, and high thickness resolution.

Observations based upon experimental results suggest that hydrogel material can be tailored to respond to changes in pH, ionic strength, and varying concentrations of

specific substrates such as glucose. The amount of cross-linking agent (EGDMA) will allow the researcher to alter hydrogel architecture to either show enhanced response or increase in structural integrity. Ellipsometry can be used to measure parameters required for calculating thickness on a nano-scale dimension, perhaps this will allow ellipsometry to be used as a means to measure responsiveness of thin films, rather than using ellipsometry as a secondary measuring device to verify thin film parameters. What we have demonstrated is that models can be designed based upon observable mass increments, and changes in refractive index. The disadvantages of ellipsometry include operator preparation of the instrument, and creating models based upon physical observations.

An inherent disadvantage in using thin films especially in enzyme loading mechanisms is the delicate nature of the enzyme. Denaturing of the enzyme, and leaching of the enzyme from within the network are two most problematic areas to address. We have dealt with denaturing issues by carefully choosing solvent and temperature to enhance conditions in favor of most responsive situations. The ability to prevent leaching of enzymes may be provided by tethering the enzyme within the gel matrix. One such attempt is described by Park et al[27], this method uses covalent bonding of ConA immobilized within a hydrogel network, thus preventing leaching of the ConA.

Future studies may also reveal information on unique compound applications such as calixarenes and cyclodextrans, and other polymer methods including molecularly imprinted polymers, or as Peppas et al[17] have proposed hydrogels incorporated within molecularly imprinted polymers.

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