SWELLING PROPERTIES OF PHENYLBORONIC ACID CONTAINING HYDROGELS

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Dedication

This thesis/project is dedicated to my family,

Seongki Kim, Soon Gil Han, and Jingul Kim.

Abstract

Glucose-sensitive hydrogels have been of interest for developing a glucose sensor for management of diabetes. In this thesis, swelling behavior and mechanical properties of glucose-sensitive hydrogels containing phenylboronic acid (PBA) were investigated. Swelling studies were conducted at different pH values and at different sugar concentrations. At pH values lower than the pKa of PBA, the hydrogel swells with increased glucose concentration due to progressive charging of the polymer chains. At pH values higher than the pKa of PBA, extra reversible crosslinks form in the hydrogel due to complexation of PBA sidechains on separate polymer chains with glucose molecules, which causes hydrogel shrinking to occur. By incorporating a tertiary amine, reversible crosslinking by glucose occursat physiological pH,7.4. Modeling of pH and fructose effects on the swelling of MPBA-*co*-AAm hydrogels was also researched. Extended Flory-Rehner-Donnan-Langmuir (FRDL) models were applied to our data, and good fits were obtained. Auxiliary experiments to validate the models were carried out. Compression tests provided a value for crosslink density that was not consistent with that determined by the model fits to swelling data.. Osmotic deswelling experiments using poly(N-vinyl-pyrrolidone) (PVP) were also carried out to challenge the FRDL models. This work provides both experimental and theoretical input to the development of novel glucose sensors based on PBA-based hydrogel swelling.

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1. Introduction

1.1. Research Objectives

Diabetes mellitus is a condition in which control of blood sugar is compromised, either because the body doesn't produce insulin properly (Type 1), or because cells don't respond to the insulin that is produced (Type 2) [1]. According to statistics collected by the American Diabetes Association in 2007, 23.6 million children and adults in the United States (7.8% of the population) have diabetes [2]. Type 2 diabetes is the most common, affecting 90 to 95% of the U.S. diabetic population. Without proper treatment, diabetes can cause many complications such as heart disease, stroke, high blood pressure, blindness, kidney disease, and neuropathy [1].

For long term management of diabetes accurate measurements of blood glucose levels are essential. Blood glucose levels in diabetics exhibit large swings throughout the day. However, current widely used devices such as test strips and glucose meters give only discrete time information about blood glucose level, possibly missing fluctuations involving sudden increase or decrease in glucose level [3,4]. Recently developed enzyme based sensors can offer continuous information, but they are problematic due to instability of the enzyme, fouling under physiological conditions, and inflammation and infection that result from breaching the skin with a needle [5]. Therefore, development of alternative glucose sensing systems is desirable.

In this thesis, efforts to investigate the swelling mechanism of glucose sensitive hydrogels, which swell and shrink according to glucose concentration, for further development of a glucose sensor for diabetes will be described. Sensors based on this

principle, which do not require enzymes, might overcome some of the disadvantages of current glucose monitoring systems.

1.2. Background

1.2.1. Blood Glucose Monitoring Systems

Adequate treatment of diabetes is important in preventing or delaying many complications, and this demands reliable glucose sensing techniques. Conventional blood glucose monitoring systems require the patient to apply a drop of blood, obtained by pricking his or her finger on a test strip, which is then inserted into a meter that displays the glucose level. The patient must perform this procedure many times a day in order to get information about blood glucose level, and based on this information the patient determines when to inject insulin [3,4]. However, this procedure provides only information at discrete times. Also, this procedure is uncomfortable, and requires skill and compliance on the part of the patient.

Recently continuous blood glucose monitoring systems (CGMS) have been developed using enzyme based sensors. These systems typically rely on the enzymatic oxidation of glucose via glucose oxidase (GOX). GOX converts glucose to gluconate in the presence of water and oxygen molecules, and generates hydrogen peroxide and hydrogen ion as products. Hydrogen peroxide can be sensed by an electrode, and it directly reflects the amount of glucose in blood [6-8]. However, there are several disadvantages with this system, including instability of the enzyme, antigenicity, poisoning, and the need for frequent calibration against blood glucose obtained by finger

stick. Also, the enzymatic and electrochemical processes are subject to interference by endogenous and exogenous compounds [6].

Besides blood glucose monitoring systems, development of insulin pumps for implantable drug delivery has been an interest of many researchers. Currently available miniature insulin pumps are wearable and present only minor inconvenience. These pumps have a reservoir containing insulin and a control system that determines the flow rate of insulin through a transcutaneous catheter, and it is necessary to refill the reservoir periodically [9,10]. Therefore, miniature closed loop insulin delivery systems, sometimes called "artificial pancreases", should be quite feasible if a reliable blood glucose monitoring system is developed.

1.2.2. Glucose-Sensitive Hydrogel Based Systems

Hydrogels are three-dimensional polymeric networks that swell in aqueous solution without dissolution. With manipulation of chemical structure, hydrogels can be designed to undergo volume changes in response to environmental stimuli such as temperature, pH, and electric field [11,12]. In particular, glucose sensitive hydrogels that swell or shrink according to blood glucose concentration are very useful for developing a glucose sensor [13-16]. With this in mind, many glucose sensitive hydrogel based systems have been developed for sensing glucose levels and modulating insulin delivery.

Combining glucose oxidase with pH-sensitive hydrogels is an approach that has been widely investigated in glucose sensing by many researchers [17,18]. When glucose oxidase converts glucose to gluconic acid, pH in the hydrogel drops. In a glucose

oxidase loaded hydrogel membrane, as a result, insulin is released by swelling of the hydrogel. Thus, such hydrogels can control insulin release in response to glucose concentration. However, there are many problems in enzyme stability such as denaturation, degradation, and poisoning.

Concanavalin A (Con A) has also been frequently used in glucose sensing systems because of its glucose-binding property [19-21]. Con A-containing hydrogels can undergo sol-gel phase transitions depending on glucose concentration in the environment, because Con A plays a role as a crosslinker. A highly specific interaction between glucose and Con A is used to form crosslinks between pendant glucose-containing polymer chains. Because of the noncovalent interaction between glucose and Con A, the formed crosslinks are reversible. As glucose molecules diffuse into the hydrogel, they compete with the pendant glucose molecules and bind to the Con A sites, releasing the crosslinks and causing the hydrogel to swell. Insulin release can then be controlled as a function of glucose concentration in the environment. However, leakage of Con A degrades device function and is a source of toxicity.

In addition to the studies that utilize proteins, such as glucose oxidase or Con A molecular imprinting technique have been applied to the fabrication of glucose sensitive hydrogels [22-25]. The most common method of molecular imprinting is polymerization of functional monomers and a crosslinking agent in the presence of a target molecule as a template. Subsequent removal of the template molecule is accomplished by extraction or hydrolysis, leaving recognition sites that are complementary in size, shape, and chemical functionality to the template molecule. In the application to glucose sensitive hydrogels

the template molecules will be glucose molecules. As glucose molecules bind to the active sites or specific chemical groups of the hydrogel, the overall ionic character, or hydrophilicity, and thus swelling of the hydrogel are changed, regulating permeability of the hydrogel. However, glucose imprinting, which is noncovalent, still has many problems including site heterogeneity and lesser selectivity compared to covalent imprinting.

Introduction of phenylboronic acid (PBA) is another approach to construct a glucose sensitive hydrogel based system. We focus on the PBA-containing glucose sensitive hydrogels in the next section.

1.2.3. Phenylboronic Acid Glucose-Sensitive Containing Hydrogel

Phenylboronic acid (PBA) and its derivatives have been used for glucose sensing due to their ability to form complexes with polyols in aqueous solution [16,26-29]. Figure 1.1 shows the mechanism by which PBA responds to sugar molecules. Under alkaline conditions boron, a Lewis acid with unfilled sp^2 orbitals, becomes negatively charged by Lewis acid/base reaction with hydroxide ion (Lewis base). Sugar molecules with planar

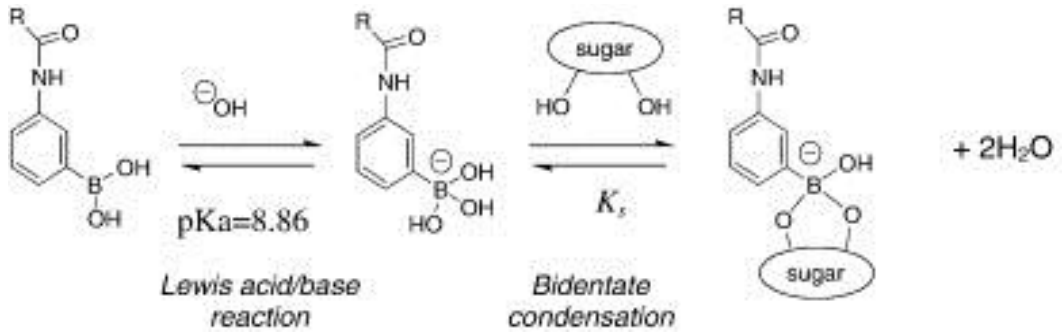

Figure 1.1. Complex between PBA moiety and a sugar molecule in aqueous solution [30]**.**

diols condense with this negatively charged tetrahedral complex and stabilize the charged form.

At physiological pH both glucose and other sugars, such as fructose, are able to complex with the PBA moiety, and glucose does not bind as avidly as fructose. Thus, PBA lacks glucose specificity at physiological pH. However, at pH 10, above the pKa of PBA, almost all PBA moieties are already charged. Two sets of planar diols in a glucose molecule can complex with PBA moieties on separate chains, forming a bridge, as shown in Figure 1.2. Other sugars such as fructose can bind to only one PBA moiety [31-33]. The 2:1 mode is therefore selective to glucose. Lowering pKa of PBA so that is already highly charged at pH 7.4 would enable this glucose selective mode to be exploited at physiological pH.

Two strategies have been considered to lower the pKa of PBA. One is to introduce electron withdrawing groups such as nitro, sulfonyl and carboxyl groups on the benzene ring. The other strategy is to place amine groups near the PBA moiety [16,26,28].

In the latter case, amine comonomer is copolymerized with PBA containing monomer, leading to a random copolymer structure. The lone pair on the nitrogen on this neighboring amine group acts similarly to the hydroxide ion in the Lewis acid/base

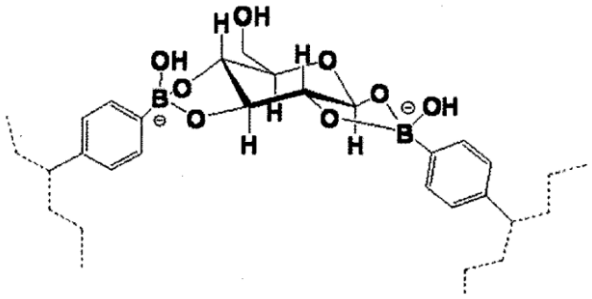

Figure 1.2. Formation of glucose-PBA crosslinks.

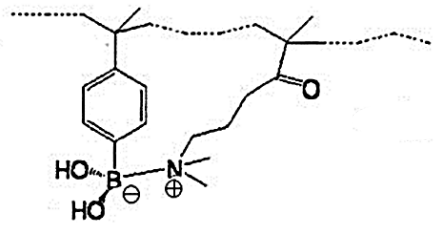

Figure 1.3. Interaction between PBA moiety and amine group at around pH 7.

reaction described previously, associating with unfilled sp^2 orbitals on the boron, lowering pKa to around pH 7, as shown in Figure1. 3. This strategy improves glucose sensitivity at physiological pH and the specificity to glucose molecules against other sugar molecules.

Several groups have exploited this property of PBA for hydrogel based glucose sensing. PBA containing hydrogels swell or shrink depending on environmental glucose concentrations. In principle, one could determine glucose concentration simply by measuring volume or diameter of the hydrogel. However, such measurements are difficult to carry out *in situ*, so alternative techniques to transduce swelling into a measurable signal have been developed.

One approach is to incorporate crystalline colloidal arrays (CCA) inside the PBA containing hydrogel [31,34,35]. As the crystalline colloidal array is formed, it adopts a face centered cubic structure that diffracts light according to Bragg's diffraction law. As the PBA-containing hydrogel swells or shrinks by reaction with glucose, the spacing between the colloids changes, thus affecting light diffraction through the hydrogel. Shifts in wavelength at maximum diffraction are recorded and correlated with changes in the concentration of glucose. However, this hydrogel system needs to be formed without

disturbing the already organized array, and the array may present a diffusion barrier for glucose molecules if it is densely packed. In order to enhance the diffusion high water content is favored, but this can adversely affect mechanical stability of the array. Another approach is to introduce a holographic diffraction grating into the PBA-containing hydrogel system that senses glucose optically [36-38]. Lines of silver nanoparticles, regularly spaced, are produced *in situ* inside the hydrogel matrix. The grating spacing is dependent on the swelling of the hydrogel, thus acting as a wavelength filter for the light reflected from it. However, fabrication of the nanoparticle array itself is difficult, and requires a special laser.

Our laboratory has also been working with PBA-containing hydrogel based microfabricated devices. In one such device, a PBA-containing hydrogel is coupled with a passive LC micromachined resonator. The hydrogel is confined between a stiff nanoporous membrane and a thin glass diaphragm, as shown in Figure 1.4(a) [39-41]. The diaphragm is coated with a metallic film, and serves as the top plate of a microcapacitor that is integrated with a microinductor to form a solid state microresonator circuit. A change in glucose concentration of the external environment causes a change in swelling pressure of the hydrogel, leading to deflection of the glass diaphragm and change in the capacitance. This change translates into variations of the resonant frequency, $f_{res} = 1/(2\pi\sqrt{LC})$, where L is the inductance, and C is the variable capacitance. Resonance frequency can be detected by a remote interrogating unit, and the device can be used as an implantable sensor.

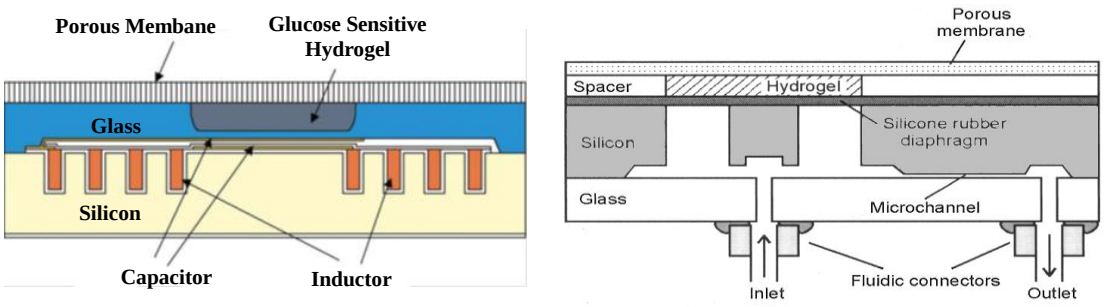

Figure 1.4. Cross-sectional schematics of (a) glucose sensor [39]**,**

(b) glucose sensitive microvalve [42]**.**

With modification of this system, a glucose sensitive microvalve has been constructed for closed loop feedback control of insulin delivery, as shown in Figure 1.4(b) [42,43]. The microvalve consists of a thin PBA-containing hydrogel, sandwiched between a stiff porous membrane and a flexible silicone rubber diaphragm. Diffusion of glucose molecules through the porous membrane results in swelling and shrinking of the hydrogel. This change in volume of the hydrogel is accompanied by deflection of the diaphragm, and closure and opening of the valve intake orifice.

As indicated above, the effect of glucose on swelling of PBA containing hydrogel is due to change in charge on the polymer. To obtain desired performance of the sensor and valve, further understanding of the hydrogel swelling and shrinking, and development of hydrogels for better glucose sensitivity are necessary, along with modification of device designs.

1.3. Structure of Thesis

To obtain desired performance of glucose sensitive hydrogel based sensors and actuators requires further understanding of hydrogel swelling and shrinking, and development of hydrogels with better glucose sensitivity, which is the object of this thesis.

In Chapter 2, synthesis of the glucose- (and fructose-) sensitive hydrogels will be described. In Chapter 3, swelling measurements are performed, and the results are explained in broad physicochemical terms. In Chapter 4, a specific mathematical model is described and fitted to data from one of the sets of swelling experiments. It will be seen that two variants of the model are nearly as successful in fitting the data, suggesting mechanical and osmotic experiments that will be needed to choose the better variant. Chapter 5 discusses progress on such experiments. Chapter 6 proposes future work.

2. Hydrogel Synthesis

2.1. Introduction

In this chapter, synthesis of the PBA-containing monomer, 3-methylacrylamido phenylboronic acid (MPBA), and two kinds of hydrogels, a copolymer hydrogel containing MPBA and acrylamide (AAm) (MPBA-*co*-AAm hydrogel), and a terpolymer hydrogel containing MPBA, AAm, and N-[3-(dimethylamino)propyl] methacrylamide) hydrogel (MPBA-*co*-AAm-*co*-DMP hydrogel), are described. Methods for preparing capillary- and disk shaped hydrogels, both of which will be utilized in experiments, are also described.

2.2. 3-Methacrylamidophenylboronic Acid (MPBA)

MPBA was synthesized from 3-aminophenylboronic acid hemisulfate (PBA^+) by a procedure adapted with modification from [44]. Typically, 10g of PBA^+ is dissolved in 160mL distilled water placed in an ice bath (0℃). pH of the solution is adjusted to 5.0 by adding NaOH. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) (12.34g, 64.5 mmol) is added under continuous stirring in an Argon atmosphere. The mixture is stirred for about 15 minutes. Meanwhile, 6.63g (64.5 mmol) methacrylic acid (MAA) is dissolved in 50 mL of distilled water and pH is again adjusted to 5.0. The resulting MAA solution is added dropwise to the PBA^+/EDAC solution under argon. The entire mixture is purged with Ar at 0℃ for 90 minutes. The mixture is subsequently removed from ice and stirred overnight at room temperature. Product is extracted with methyl t-butyl ether (MTBE) three times and roto-evaporated. The resulting white paste is dissolved in 380

mL distilled water at 70℃ with continuous stirring. Activated charcoal (500 mg) is then added to the dissolved mixture, which is then stirred for 1 min at 70℃. The mixture is poured into a filter funnel to remove the charcoal. The final white crystal is obtained when the solution is left in the dark overnight under argon. The structure of MPBA is verified by ^{1}H-NMR spectroscopy.

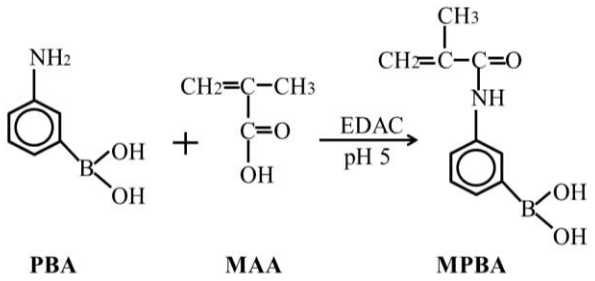

Figure 2.1. Synthesis of glucose sensitive monomer, MPBA.

2.3. MPBA-*co*-AAm Hydrogel

Coplymer hydrogels containing MPBA and AAm are synthesized in an aqueous solution containing 20 mol% 3-methacryl amidophenylboronic acid (MPBA), 80 mol% acrylamide (AAm) by redox copolymerization. The comonomers are dissolved in 1mL of 1N NaOH. 80 µL of N,N,N',N'-tetramethyl ethylenediamine (TEMED: accelerator) and 100 µL of 20mg/mL solution of N,N'-methylene-bisacylamide (Bis: crosslinker) are added to the solution. Five parts of the resulting solution and one part of 10 mg/ml solution of aqueous ammonium persulfate (APS: initiator) are mixed. This pregel solution is loaded into glass capillaries (I.D.=1172µm), and polymerized at 4 ℃ for 12 hours. Hydrogels are separated from the capillaries by acetone and placed in distilled water to extract unreacted materials. Disk shaped hydrogels are prepared by pouring the

pregel solution into a mold, which is made of acrylic polymer with five disks (diameter = 8mm, thickness = 1.8mm). Disk shaped hydrogels are separated from the glass mold after gelation, followed by washing with distilled water. Before swelling experiments the hydrogels are equilibrated in pH 7.4 phosphate buffer at room temperature.

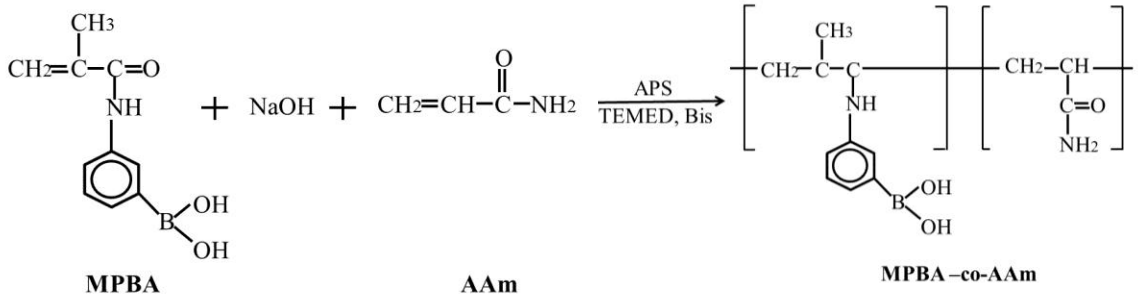

Figure 2.2. Synthesis of MPBA-*co*-AAm hydrogel.

2.4. MPBA-co-AAm-co-DMP Hydrogel

Terpolymer hydrogels based on AAm, MPBA, and N-[3-(dimethylamino)propyl] methacrylamide (DMP) are synthesized in dimethyl sulfoxide (DMSO). The composition is 60 mol% AAm–20 mol% MPBA–20 mol% DMP. The monomers (total 5 mmol) along with 4.24mg 2,2'-azoisobutyronitrile (AIBN: initiator) and 100 µL Bis (20 mg/mL in DMSO) are dissolved in 1mL DMSO. Polymerization is carried out at 60 ℃ in glass capillaries (I.D.=1172µm) or planar molds for 4 hours. Cylindrical and disk hydrogels are prepared for swelling studies in the same way as with AAm-co-MPBA hydrogels.

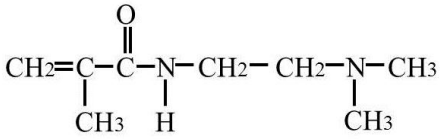

Figure 2.3. Chemical structure of *N*-[3-(dimethylamino)propyl]methacrylamide (DMP).

3. Swelling Studies of Hydrogels

3.1. Introduction

In this chapter, equilibrium free swelling and shrinking properties of MPBA-*co*-AAm hydrogels and MPBA-*co*-AAm-DMP hydrogels, as functions of pH and concentration of fructose and glucose are reported and analyzed.

3.2. Methods

Copolymer (MPBA-*co*-AAm) and terpolymer (MPBA-*co*-DMP-*co*-AAm) hydrogels were synthesized according to methods described in Chapter 2. Equilibrium swelling studies were carried out using cylindrical hydrogels at room temperature. Hydrogels were immersed in buffered 10mL aqueous solutions, that were replaced with fresh solutions every day, with various glucose or fructose concentrations (0, 0.5, 2, 7 and 20mM) at different pH values, spanning a range from pH 4 to pH 10. Swelling media consisted of 0.01M pH buffers, adjusted to 0.154M of ionic strength with NaCl. Buffers were: acetic acid (pKa=4.76) from pH 4 to pH 5.5, sodium phosphate monobasic (pKa=7.2) from pH 6 to pH 8, and ethanolamine (pKa=9.5) from pH 8.5 to pH 10. At the end of the swelling period, final pH of the swelling medium was determined for each condition, and diameter changes of the hydrogel cylinders were measured under an optical microscope.

3.3. Results and Discussion

All results are reported as the ratio of hydrogel cylinder diameter at swelling

equilibrium to the diameter at synthesis, taken to be the inner diameter of the capillary, 1.172 mm. Final pH values were typically very close to the original values.

3.3.1. Swelling of MPBA-*co*-AAm Hydrogels

Results of pH-dependent swelling studies of MPBA-*co*-AAm copolymer hydrogels in the presence of different concentrations of fructose and glucose are shown in Fig. 8a and 8b, respectively. In all cases, an increase of swelling with increasing pH is observed. This behavior is attributed primarily to increased Donnan osmotic pressure and change in hydrophilicity due to ionization. With increasing pH, more hydroxide ions are present, which convert the trigonal PBA moiety to the charged tetrahedral form. The latter is stabilized by condensation with a sugar molecule, as discussed above. Binding of sugar molecules to the charged PBA moiety stabilizes the negative charge, and draws in counterions from the surrounding solution. The excess of counterions inside the hydrogel generates osmotic flow of water, causing the hydrogel to swell. Also, the stabilized charge may alter the polarity and enhance hydrophilicity of MPBA, promoting swelling.

For the experiment with fructose (Figure 3.1a), a simple acid shift is observed in the swelling curves with increasing fructose concentration. The acid shift corresponds to a change in apparent pKa of PBA, which can be expressed [44] as

$$\mathrm{p}K_{app} = \mathrm{p}K_a - \log\left(1 + \frac{C_s}{K_s}\right) \tag{3.1}$$

The estimated K_s value based on Eq. (3.1) and the sugar-free swelling results in Fig. 8a is 0.094mM on average. This value is different from the literature value [45] for binding of

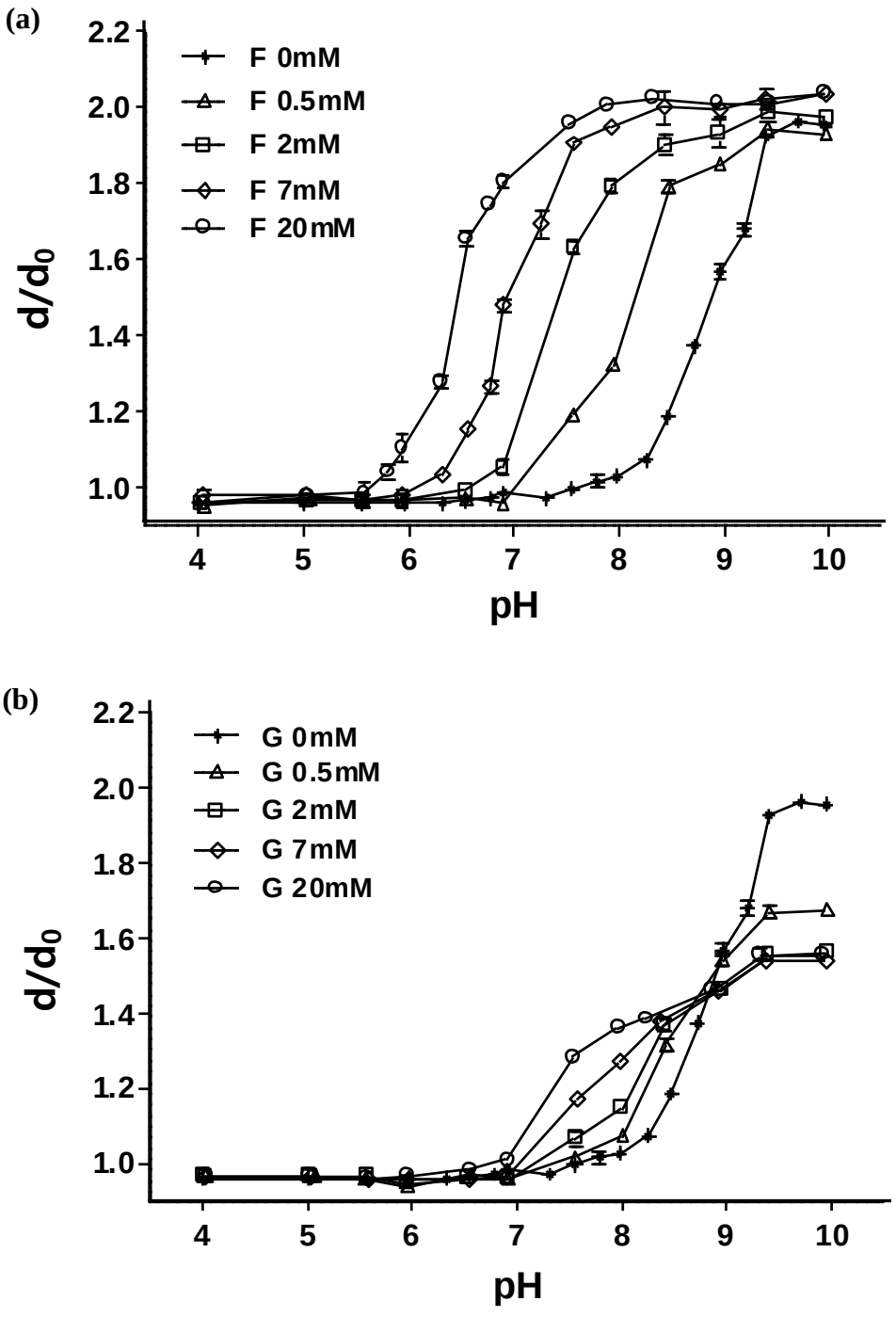

Figure 3.1. Equilibrium swelling of MPBA-*co*-AAm hydrogels in buffer solution at different (a) fructose, or (b) glucose concentrations (n=2).

fructose to free ionized PBA, 0.27mM, but it is very close to an estimated K_s value determined by model fitting in Chapter 4. A slight increase in the plateau swelling at high pH values in the presence of fructose suggests that fructose-bound PBA is more hydrophilic than bare ionized PBA. A quantitative model for pH- and fructose dependent swelling will be presented in Chapter 4.

The response to glucose, detailed in Figure 3.1b, is more complicated. Below pH 8.8, glucose effects an acid shift in swelling as with fructose, but the degree of swelling of hydrogels in glucose solutions is much less than that in fructose solutions at the same pH. This observation is explained by less avid binding of glucose to PBA, compared to fructose [46]. Moreover, while increasing fructose concentration always leads to increased swelling, increased glucose concentration lead to shrinkage of the hydrogel above pH 8.8. Binding of glucose molecule to PBA moiety affects the swelling of hydrogels in two ways. First, binding of glucose increases the charge density of the hydrogel, increasing Donnan osmotic pressure and hydrophilicity, causing the hydrogel to swell, in the same way as with fructose. Second, formation of 1:2 complexes, which occurs with glucose (see Fig. 2) but not with fructose, increases the effective number of crosslinks in the hydrogel, causing it to shrink. Below pH 8.8, the first effect dominates, while the second effect competes more effectively above pH 8.8. This is the reason for the differing behaviors of the swelling between fructose and glucose.

3.3.2. Swelling of MPBA-*co*-AAm-*co*-DMP Hydrogels

Results of pH-dependent swelling studies of MPBA-*co*-AAm-*co*-DMP terpolymer hydrogels in the presence of different concentrations of fructose and glucose are presented in Figures 3.2a and 3.2b, respectively. Whereas swelling of the MPBA-*co*-AAm copolymer hydrogels always increases with pH (Fig. 8), the swelling curves for the terpolymer hydrogels exhibit definite nonmonotonic behavior, characteristic of polyampholyte hydrogels, with maximum swelling at the extreme pH values and a swelling minimum near neutral pH. Above pH 6, addition of fructose (Fig. 9a) increases swelling, and there is an acid shift in the swelling minimum. In contrast, addition of glucose (Fig. 9b) causes shrinkage. There is virtually no effect of sugar below pH 6. For sugar free hydrogels, and with addition of fructose, swelling is higher in the alkaline extreme, while the opposite is the case when glucose is added.

We can explain the different swelling behavior of the DMP containing hydrogels based on the change in the interaction between the PBA moiety and the neighboring amine group, as shown in Figure 3.3 [16]. We first consider the swelling behavior in the absence of sugar. At low pH, where there are plenty of hydrogen ions available in solution, the nitrogen on the amine group is protonated (pKa~9), changing it to the positively charged state, while the PBA moiety remains uncharged. The net charge is therefore positive, leading to a Donnan osmotic pressure, and swelling of the hydrogel. At high pH, the PBA moiety becomes negatively charged by Lewis acid/base reaction with hydroxide ion, while the amine group loses its charge, so the charge on the hydrogel becomes negative, leading to Donnan osmotic pressure driven swelling. The asymmetry

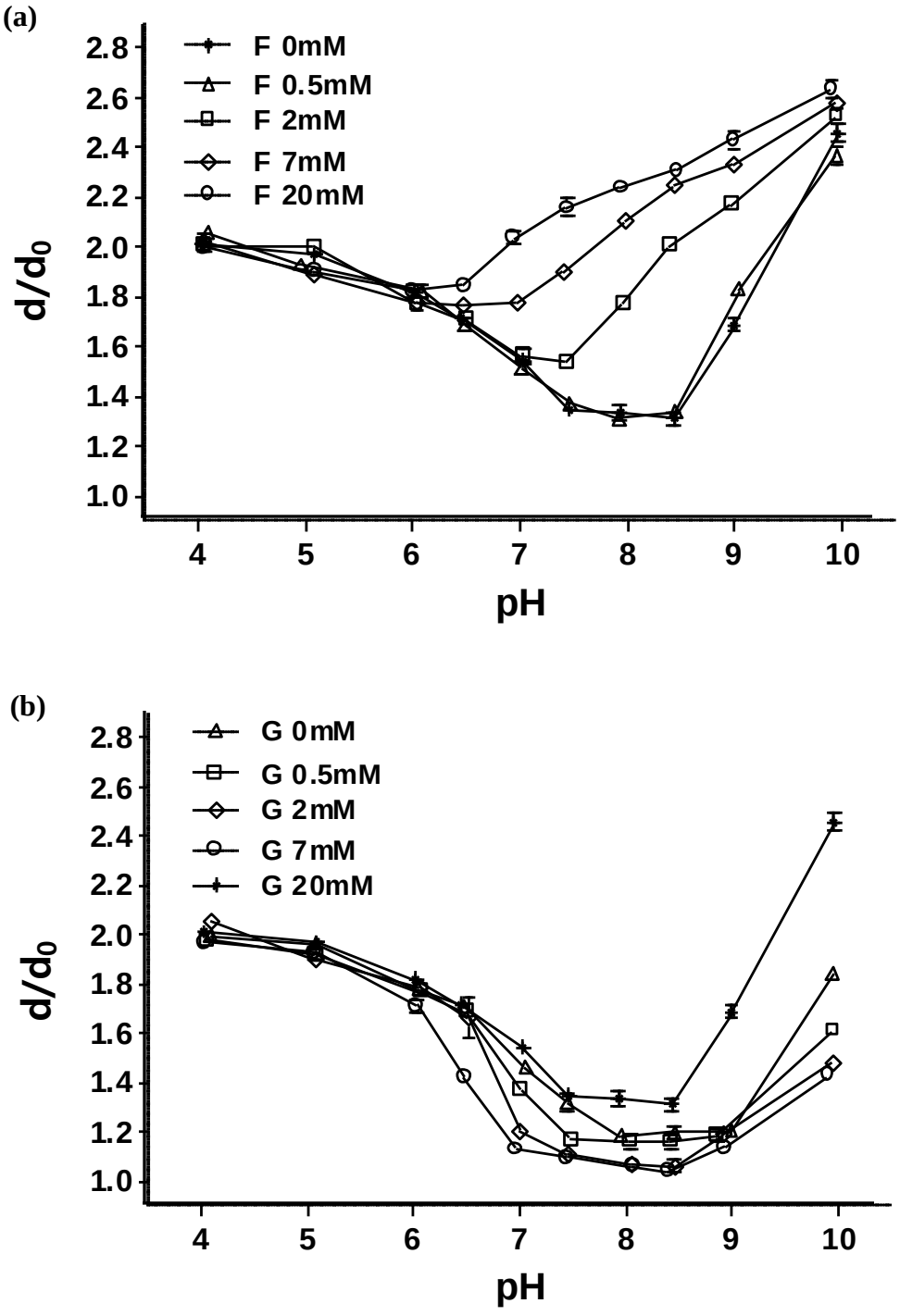

Figure 3.2. Equilibrium swelling of DMP-co-MPBA-co-AAm hydrogels in buffer solution at different fructose (a), or glucose (b) concentrations (n=2).

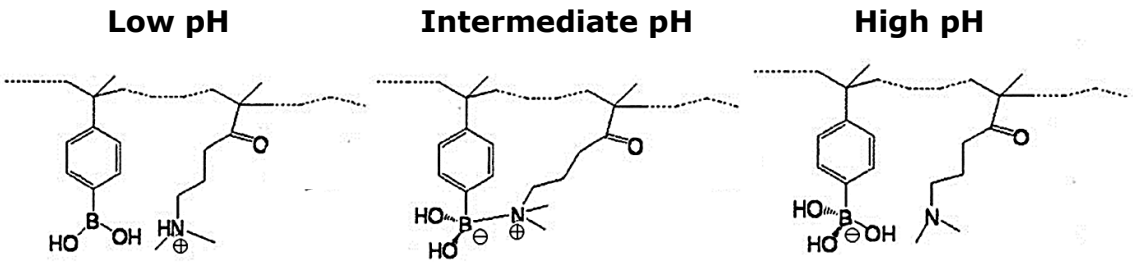

Figure 3.3. Acid-base equilibrium of PBA moiety in DMP-*co*-MPBA-*co*-AAm hydrogel. The B-N bond is shown to be zwitterionic, though it may only be dipolar.

in swelling between high and low pH conditions might be due either to a) a small but net excess of MPBA over DMP in the hydrogel, or b) greater hydrophilicity of the negatively charged MPBA compared to positively charged DMP. Such swelling asymmetries have been noted in other polyampholyte hydrogels [47, 48]

At intermediate pH values, two things can happen, both promoting shrinkage of the hydrogel. First, in this range the charges on PBA and DMP moieties may cancel each other out. Second, the lone pair of electrons on the nitrogen in the tertiary amine group may associate with the electron deficient boron in PBA moiety, forming a dative bond, which will have dipolar character and may even be regarded as zwitterionic. As the negative charge of boronate and the positive charge of nitrogen are canceled out, the overall charge in the system is neutral, so relative shrinkage compared to the extreme pH cases is expected. It is also well known that the presence of near equal amounts of positively and negatively charged groups, along with zwitterions, may promote extra

shrinkage in hydrogels via the so-called polyampholyte effect, which may be present here [47, 48].

On the other hand, it is noteworthy that swelling of the terpolymer gels in the net neutral state is greater than for the copolymer hydrogels not containing DMP. This is somewhat puzzling, since DMP is expected to be more hydrophobic than AAm. One possible explanation is that there is some variation of local content of MPBA and DMP monomers, such that at many places in the hydrogel one or the other species dominates. Such domains would contribute positively to swelling. This explanation is highly speculative at this point, but is consistent with data gathered previously by S. Mujumdar in our laboratory [49], which also showed increased swelling of MPBA/DMP matched terpolymer hydrogels with increasing incorporation of MPBA/DMP.

We now turn to the effects of added sugar molecules, Note first that in the terpolymer hydrogels, PBA can undergo a Lewis acid/base reaction with either OH^- in solution or with neighboring DMP groups. In either case the resulting tetrahedral PBA moiety can complex with sugar molecules. It should be noted however that the MPBA-DMP complex does not carry a net charge, so binding of a sugar molecule to such a complex, while potentially stabilizing it, does not in itself promote charging of the network. On the other hand, binding of sugar to a PBA-OH^- complex will stabilize that latter's charge.

At any particular pH, addition of fructose either causes swelling to occur or there is no significant effect. Roughly, the swelling curves in Fig. 9a resemble a superposition of the fructose effect observed in Fig. 8a for MPBA-AAm copolymer hydrogels, with the

polyampholyte behavior of the terpolymer hydrogels observed with no sugar added. This resemblance is not quantitive, and there are some small but perhaps significant discrepancies. For example, the fructose effect is stronger at pH 10 in the terpolymer hydrogels than in the copolymer hydrogels, and the effect of 20mM fructose on swelling of the copolymer hydrogel at pH 6 is not seen in the terpolymer hydrogel. Nevertheless, we note that these rough observations may be consistent with speculations regarding heterogeneity of composition in the hydrogel. Fructose-enhanced swelling would be due to effects of fructose in the PBA-rich domains, with other domains much less affected.

Conversely, the effect of glucose is to shrink the terpolymer hydrogels at all pH values. In this case note that a glucose molecule can act as a crosslinker for Lewis base-form PBA groups, regardless of whether this configuration arises from interaction of MPBA with OH^- or DMP. At high pH, it is the charged MPBA-OH^- groups that are crosslinked by glucose, while at intermediate pH values, it is the PBA-DMP groups that are involved. Below pH 6, neither of these groups are present, so no crosslinking occurs.

3.5 Conclusions

In this chapter we conducted swelling studies of pH- and sugar sensitive polyacid copolymer and polyampholyte terpolymer hydrogels at different pH values, in the presence or absence of sugar molecules. In the swelling studies of the polyacidic MPBA-*co*-AAm hydrogel with no sugar added, a typical swelling transition was observed near the pKa for MPBA. The swelling curve exhibited an acid shift upon addition of fructose, which could be explained by stabilization of the charged form of MPBA upon

complexation with fructose. Glucose acts on the hydrogel in two ways. First, it stabilizes charged MPBA, thus enhancing swelling as does fructose, although with less avidity. Second, it can complex with two MPBA units simultaneously, leading to extra crosslinks, which cause the hydrogel to shrink. Whereas glucose promotes swelling below the pKa of MPBA, it leads to shrinkage above the pKa. The amine containing MPBA-*co*-AAm-*co*-DMP hydrogels exhibited expected polyampholyte behavior. At pH 6 and above, addition of fructose always led to increased swelling, while glucose always caused shrinking. Importantly, the shrinking responses occurred at physiological pH, suggesting that the shrinking mechanism can be used in biomedical sensing and actuation.

4. Modeling of pH and fructose effects on swelling of MPBA-*co*-AAm copolymer hydrogels.

4.1 Introduction

In the previous section, swelling response of MBPA-*co*-AAm copolymer and MBPA-*co*-DPM-*co*-AAm terpolymer hydrogels was measured as a function of pH, without and with addition of fructose and glucose. In the absence of added sugar, the MBPA-*co*-AAm copolymer hydrogel behaved as a polyacid hydrogel, exhibiting a sharp volume transition in the vicinity of the pKa (8.86) of the MBPA Lewis acid group. The effect of added fructose was a straightforward acid shift in swelling response, which could be explained by stabilization of the charged, Lewis base form of MBPA by complexation with fructose. Such complexation was estimated to occur with dissocation constant K_{F} = 0.010mM, such that the effective pKa of MPBA in the presence of fructose at concentration c_{F} is given by Eq. (3.1), $\mathrm{p}K_{app} = \mathrm{p}K_a - \log(1 + c_{\mathrm{F}}/K_{\mathrm{F}})$. The effect of glucose is more complicated, since single glucose molecules can simultaneously complex with two MPBA units on different chains, leading to reversible crosslinks and shrinkage of the hydrogel. Swelling of the MBPA-*co*-DPM-*co*-AAm terpolymer hydrogels exhibits even more complex behaviors due to the presence of both acid and basic groups, which may have the ability to form Lewis acid-base complexes and also interact by polyampholyte effects.

In this chapter, a well known model of swelling of ionizable polyacid hydrogels is applied to the pH-fructose experiments with the MBPA-*co*-AAm copolymer. It will be shown that this model fits the data quite well, provided auxiliary assumptions are made

regarding one of the model's parameters. Two such assumptions lead to similar fitting fidelity, suggesting that extra experiments are needed to distinguish between the models.

The present modeling only covers fructose with the MBPA-*co*-AAm copolymer hydrogels. Added modeling, with more complex parameter estimations, is needed to model the response of the copolymer hydrogel to glucose, and of the MBPA-*co*-DPM-*co*-AAm terpolymer hydrogel to either sugar. The success of modeling this simplest case is a prerequisite to modeling the more complex cases.

4.2. Flory-Rehner-Donnan-Langmuir model

PBA-containing hydrogels can be categorized as polyelectrolyte hydrogels, which are crosslinked networks of polymer chains containing ionizable groups. Three factors are most significant in determining polyelectrolyte hydrogel swelling; compatibility of water and polymer, the elasticity of polymer chains, and Donnan osmotic pressure. The influence of these three factors is modeled using Flory-Rehner-Donnan theory [50]. According to this theory, the equilibrium free swelling of a hydrogel network is based on the balance of three pressures, i.e. $\Delta\Pi = \Delta\Pi_{mixing} + \Delta\Pi_{elastic} + \Delta\Pi_{ionic} = 0$. Inserting expressions for each of these pressure contributions, the equilibrium condition becomes

$$[\ln(1-\varphi) + \varphi + \chi\varphi^2] + \bar{v}_w \left[\rho_0 \left(\frac{\varphi}{\varphi_0}\right)^{\frac{1}{3}} - \frac{\varphi}{2\varphi_0}\right] - \bar{v}_w \sum_i (C_i^{int} - C_i^{ext}) = 0 \qquad (4.1)$$

where

φ = polymer volume fraction

φ_0 = polymer volume fraction in the unperturbed (as prepared) state

χ = polymer-solvent interaction parameter

$\bar{V}_w$ = molar volume of water

ρ_0 = crosslinking density or number of active network chains per unit volume

C_i^{int} = concentration of the soluble ion 'i' inside the hydrogel

C_i^{ext} = concentration of the soluble ion 'i' outside the hydrogel

The first term in Eq. (4.1), $\Delta\pi_{mixing}$, represents the tendency of the polymer to mix with the solvent. This term can be separated into two parts; one is the mixing entropy contribution and the other is the polymer/solvent contact contribution. Mixing of polymer chains and solvent increases the configurational entropy of the whole system, favoring swelling of the hydrogel, while the polymer/solvent contribution may favor swelling or shrinking, depending on the value of the χ parameter. The second term, $\Delta\pi_{elastic}$, reflects the elastic restorative force due to the crosslinks. Hydrogels become more resistant to swelling and shrinking with increasing crosslink density. The third term, $\Delta\pi_{ionic}$, is due to the excess of counterions in the hydrogel compared to the external solution which balance the charge on the hydrogel chains. This excess promotes osmotic flow of water into the hydrogel.

To calculate the mobile ion excess, Donnan equilibrium theory is used, wherein internal and external ion concentrations are linked via a single Donnan ratio, λ, according to $C_i^{int} = \lambda^{z_i} C_i^{ext}$, where z_i is the valence of the i'th mobile ion. In the present studies, the dominant mobile ions are univalent, and it is possible to write the third term in Eq. (4.1) as $\bar{V}_w C_s(\lambda + \frac{1}{\lambda} - 2)$, where C_s is the external ionic strength.

The Donnan ratio is determined by assuming electroneutrality inside the hydrogel. In the univalent case, this condition is represented, in the univalent mobile ion case, as

$$(1-\varphi)C_s\left(\lambda-\frac{1}{\lambda}\right)-f\left(\frac{\varphi}{\varphi_0}\right)\sigma_0=0 \tag{4.2}$$

where σ_0 is the mole density of acidic groups per unit per 1000 cm^3 of hydrogel and *f* is the fraction of these acidic groups that are ionized. . The first term accounts for mobile ion concentrations in the aqueous part of the hydrogel [more generally it will take the form $(1-\varphi)\sum_i z_i C_i^{int}=(1-\varphi)\sum_i z_i\lambda^{z_i}C_i^{ext}$], while the second term accounts for the ionized PBA groups. The ionization fraction, *f*, is calculated assuming mass action (Langmuir binding equilibrium) in accordance with the scheme in Figure 1.1, according to

$$f=\frac{1}{1+\lambda\,10^{-(pH-pKa)/(1+C_F/K_F)}} \tag{4.3}$$

where *pH* refers to the external solution, C_F is the external fructose concentration, pK_a is the acidity constant of the PBA groups, and K_F is the dissociation constant of fructose with the ionized PBA.

Together, Eqs. (4.1)-(4.3), which we call the Flory-Rehner-Donnan-Langmuir (FRDL) model, can be used to predict swelling behavior of PBA hydrogels in univalent salt solutions with sugars that bind only one PBA unit (e.g. fructose). For the case of glucose, which adds crosslinks to the hydrogel, Alexeev et al.[51] propose incorporation of an extra term corresponding to these crosslinks, to the left hand side of Eq. (4.1), along with mass action-like equilibrium conditions for 2:1 PBA:glucose binding. For the polyampholyte hydrogels, a much more complicated accounting is required for

complexes among sugar, PBA, and amine groups to determine net hydrogel charge and density of glucose crosslinks; such modeling will not be pursued here.

4.3 Fitting the model to the data

4.3.1. Initial volume fraction, and binding constant of fructose

In order to fit the model, which makes predictions in terms of volume fraction φ, against data taken from swelling cylinders, which is represented by the diameter *d*, we assume that swelling is isotropic, so $\varphi = \varphi_0(d/d_0)^3$ where d_0=1.172 mm is the inner diameter of the capillary in which the hydrogels were synthesized, and φ_0=0.24 based on the recipe for forming the hydrogels presented in Ch. 2.3.

We also fix pKa of PBA at 8.86, its literature value. We treat the crosslink density at preparation, ρ_0, the interaction parameter χ, and the binding constant of fructose, K_F, as free parameters to be determined by fitting. First, ρ_0 and χ are fit to the fructose-free swelling data, with C_F =0. A nonlinear least squares program written in Matlab [52] is used to carry out these fits. With these parameters determined, the value of K_F providing the best fit for all the fructose concentrations is determined.

4.3.2. Fitting of different variants of the model

Fits of different variants of the model to be discussed below, to swelling equilibrium data taken for hydrogels in sugar (fructose) free solutions are shown in Figure 4.1a. The simplest variant of the model assumes a constant χ parameter. In order to predict the low pH (collapsed) and high pH (swollen) limits, the values χ=0.595 and

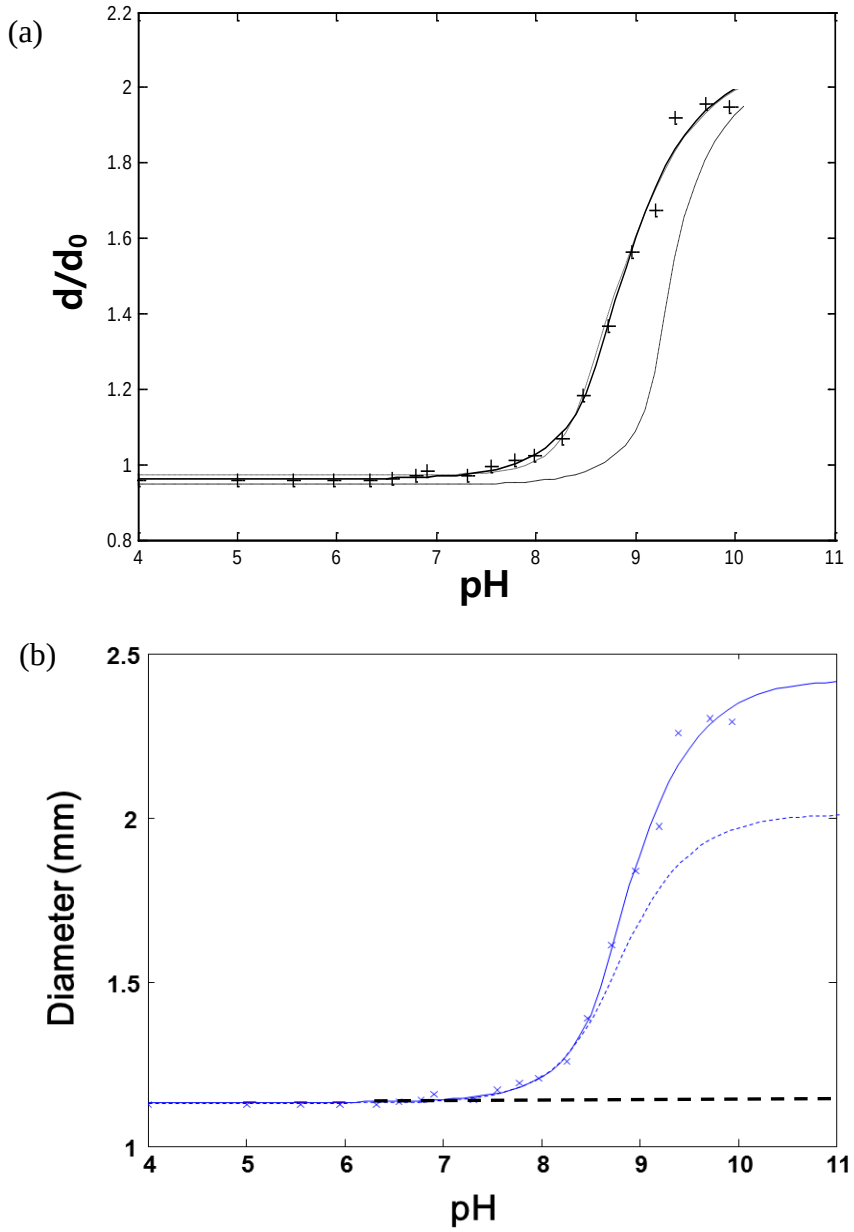

Figure 4.1 a) Comparison of fits of constant χ variant (- - -), φ-variant (. . .), and *f*-variant (—) of FRDL model with sugar-free swelling equilibrium data (+). b) Predictions of *f*-variant with Donnan osmotic pressure suppressed (. . .), *f*-variant with Donnan osmotic pressure term included (—), and φ-variant with Donnan osmotic pressure suppressed (- - -).

ρ_0=0.008 mol/L were determined. The dashed curve in Fig. 4.1a indicates that the constant χ assumption fails to account for the location of the swelling transition. This behavior is explained by noting that such a high value of χ is indicative of a strong hydrophobic force that is resistant to other influences such as charging of the hydrogel. Only at very high pH values, where most of the MPBA units are charged, does the ion osmotic pressure have influence, but once it does it becomes dominant. Ultimately, however, swelling is limited by the elastic retraction of the network, as determined by ρ_0.

A common variant of Flory-Rehner (FR) theory, which predicts the mixing and elastic parts of the swelling pressure, is to assume that χ depends on polymer volume fraction. In general, a quasi-virial series, $\chi = \sum_{i=1}^{\infty} \chi_i \, \varphi^{i-1}$ can be used, but it is customary and usually prudent (especially when $\varphi \ll 1$ as in the present case) to limit the series to two terms, i.e.

$$\chi = \chi_1 + \chi_2 \varphi \tag{4.4}$$

Incorporating this assumption into the full FRDL model, we arrive at the "φ-variant." The best fit of this variant to the data occurs with the parameter values (±s.e.m.) χ_1 = 0.500±0.001, χ_2 = 0.408±0.001, ρ_0 = 0.0159±0.0011 mol/L. This fit captures the location and general shape of the volume transition quite well,although there is a slight underestimation of swelling just above physiological pH.

In a second variant, we assume that χ depends on the degree of ionization of the MPBA units. It can be argued that complexation of OH- redistributes electron density on the PBA moiety, making it more polar, and might also prevent hydrophobic associations based on, for example, stacking of the phenyl rings. Regardless of the molecular

mechanism, in this variant it is assumed that χ is a convex linear combination of values it takes when PBA is uncharged ($\chi = \chi_u$) and when it is charged ($\chi = \chi_c$), i.e.

$$\chi = (1 - f)\chi_u + f\chi_c \tag{4.5}$$

where *f* is calculated according to Eq. (4.3). This is the "*f*- variant" of the FRDL model. The best fit of this variant to the sugar free data is the solid line in Fig. 4.1. Parameter estimates and errors are χ_u=0.609±0.001, χ_c=0.374±0.023, ρ_0=0.030±0.035 mol/L. It should be noted that the estimate of ρ_0 is somewhat higher in the *f*-variant than in the φ-variant.

Comparing the two variants, it appears that the *f*-variant is slightly superior near physiologic pH. This range corresponds to the "early stages" of ionization, where a small decrease in χ may have a greater effect on swelling than the Donnan osmotic pressure. To emphasize this point, Figure 4.1b shows the effect of completely suppressing the Donnan osmotic pressure contribution. For the *f*-variant, the Donnan contribution is negligible up to pH 8.5, above which its contribution is comparable to that of the change in χ. For the φ-variant, suppression of the Donnan term completely eliminates pH-dependent swelling.

In principle, if the Donnan effect could be eliminated experimentally, say by introducing an excess of salt, which would screen the electrostatic interactions, then a clear choice between the model variants would be possible. Unfortunately, given the relatively high density of ionizable groups in the hydrogel, salt concentrations needed to carry out this test would be extremely high, calling into question assumptions regarding the nature of interactions among solvent, polymer, and solutes. Measurement of swelling

pressure of a hydrogel in a confined structure could be another way to check validity of the two models. Volume of the hydrogel is constant in the confined structure, and its swelling pressure would not change with pH if the φ-variant model is valid, according to Eq. (4.1). Because pH affects the ionization of PBA, the *f*-variant model leads different swelling pressure with pH. However, the measurement of swelling pressure is difficult at current stage.

In the following chapter, mechanical measurements will be used to estimate ρ_0. As will be seen, the estimates will favor the *f*-variant, although we have already noted that estimates of this parameter are somewhat loose for both variants. We will also discuss osmotic deswelling experiments as one of methods to suppress the Donnan term, which may also permit selection between the two variants. For the present, we can only state that both variants remain good candidates, but a couple of lines of evidence presently favor the *f*-variant.

Finally, Figure 4.2 shows the fit of the *f*-variant to the full set of data taken at the different fructose concentrations. This data is well represented if the dissociation constant of fructose with PBA-OH^- is taken to be K_F=0.1 mM, which is essentially the same as that estimated "by hand" (K_F=0.094 mM) with Eq. (3.1). Importantly, this estimate significantly less that the literature value for binding of fructose to free PBA-OH^-, indicating some kind of effect of the PBA moiety being immobilized on a polymer. Similarly good fits would be seen with the φ-variant, since the acid shift does not depend on other aspects of the model.

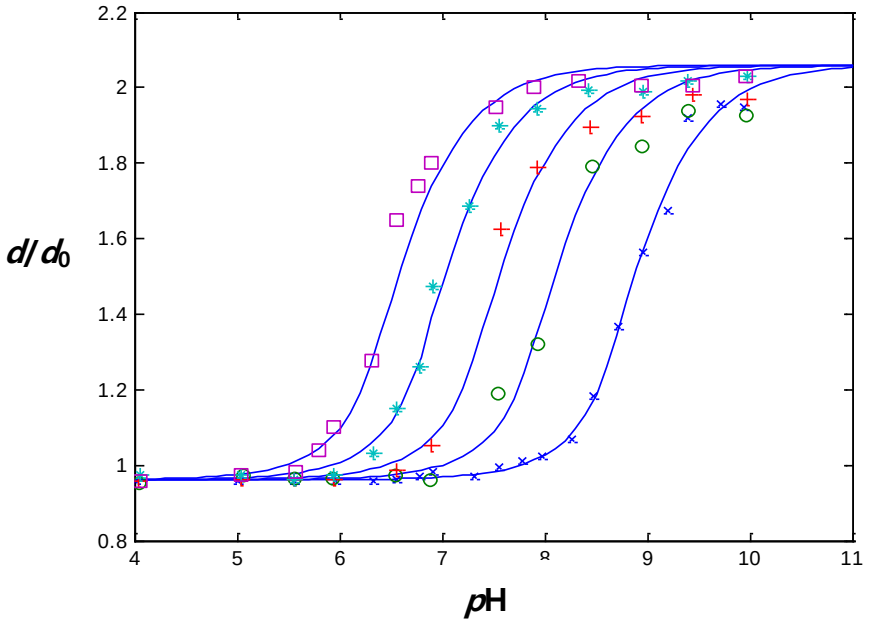

Figure 4.2 Swelling data and predictions of *f*-variant of FRDL model. Fructose concentrations: (x) 0 mM; (○) 0.5 mM; (+) 2 mM; (*) 7 mM; (□) 20 mM.

4.4 Conclusions

The FRDL model with constant χ was shown to be unable to fit the equilibrium swelling data for MPBA-*co*-AAM copolymer hydrogels. Very good fits were found however, by assuming that χ depends either on polymer volume fraction, or on degree of ionization of the MBPA units. Although some of the data favors the latter variant, independent experiments are needed to provide an unambiguous selection. It was also found that binding of fructose to ionized MPBA-OH^- when the latter is fixed in the hydrogel than when MPBA is in free alkaline solution.

As noted in Chapter 3, there is some evidence that fructose bound to MPBA-OH^- leads to a slight increase of swelling compared to the sugar-free case. Because this effect is relatively small, and would require addition of extra parameters with little gain in fitting fidelity, this aspect was not pursued in the present study.

Chapter 5. Auxiliary Experiments

5.1 Introduction

In the previous chapter a model was developed to describe swelling of MPBA-*co*-AAm hydrogels in solutions of varying fructose concentration. Flory-Rehner-Donnan-Langmuir (FRDL), augmented to include the effects of binding of fructose to ionized MPBA-OH- units was tested. It was found the assumption of a constant interaction parameter, χ, could not enable good fits. However, two other variants worked quite well. In the first variant (φ-variant), χ was taken to be a linear function of the volume fraction of polymer in the hydrogel, i.e. $\chi = \chi_1 + \chi_2\varphi$. In the second variant, χ was taken to be a linear function of the degree of ionization, i.e. $\chi = (1-f)\chi_u + f\chi_c$.

Based on the swelling data presented in Chapter 4 alone, there is no definitive means to favor one model variant over the other. However, the two models required different values of the initial crosslink density, ρ_0, to provide adequate fits. Thus, it seems worthwhile to determine ρ_0 experimentally. The first "auxiliary" experiment is therefore to perform mechanical compression experiments to make this determination.

The two models also make different predictions regarding shrinking of the hydrogels as a joint function of pH and external osmotic pressure in Fig. 4.1b. Experiments were therefore carried out to measure shrinkage of hydrogels in solutions of poly(N-vinylpyrrolidone) (PVP) at different pH values.

5.2. Experiments

5.2.1. Compression Studies

Individual disks, prepared as described in Ch. 2.3, were equilibrated in sugar free, 9mM fructose or 9mM glucose solutions at pH 10 and pH 7.4. For measurement of compression modulus, disk shaped hydrogels were mounted between parallel plates of a rheometer (Rheometric Scientific Inc., ARES). The top plate was lowered further at constant velocity until the hydrogel was compressed to 90% of its original thickness. The hydrogel was held at fixed strain for 5 min, and then decompressed by raising the top plate at the constant velocity. Compression force was continuously measured during the compression-hold-decompression process.

5.2.2. Osmotic Pressure Measurement with Fiber Optic Pressure Sensor

Polyvinylpyrrolidone (PVP) has been widely used to investigate the effect of osmotic pressure on hydrogels [53,54]. We dialyzed 40 KDa PVP with a semipermeable membrane (10 KDa cutoff) for three days, followed by freeze drying.

A home made osmometer, illustrated in Figure 5.1, was used for to calibrate osmotic pressures of PVP solutions of varying concentrations. The osmometer consists of a side-by-side diffusion cell, with the two chambers separated by dialysis membrane, which is impermeable to PVP but permeable to water. A fiber optic pressure sensor was introduced into one chamber though the access port, and tightly sealed in the port using silicone adhesive. The chamber containing the fiber optic pressure sensor tip was filled with different PVP solutions, and DI water was placed in the other chamber. The

semipermeable membrane was clamped between the chambers and secured with and O-ring to prevent leakage. The PVP induced osmotic flow and buildup of pressure in the PVP chamber, which was measured by the fiber optic pressure sensor, and recorded every 8 min for 24 hours.

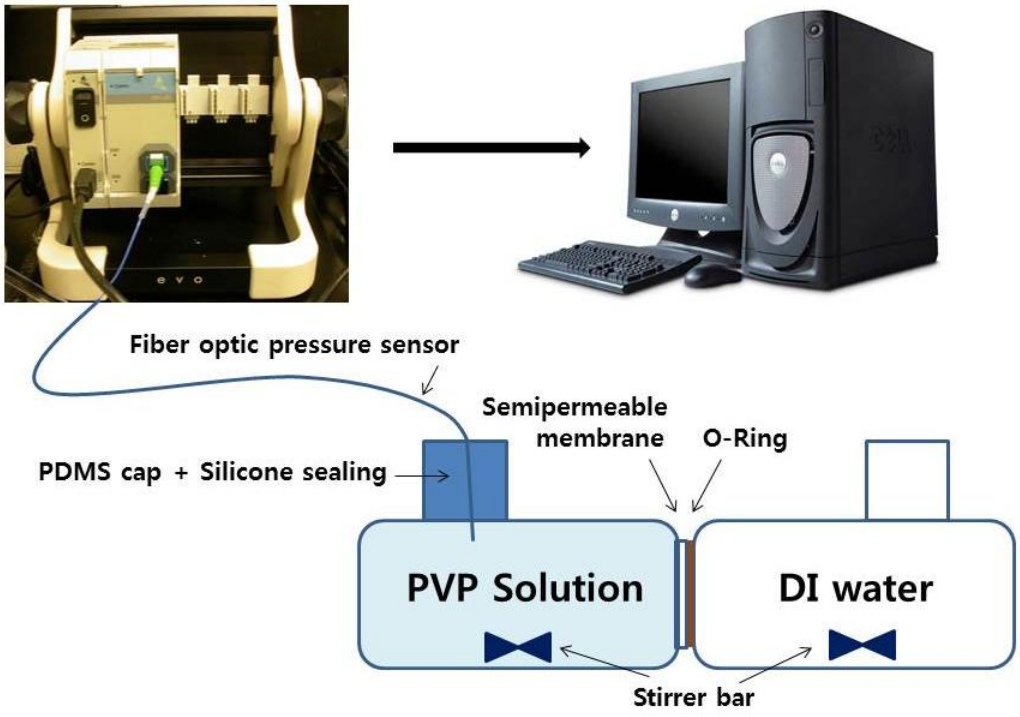

Figure 5.1. Diagram of Experimental Setting of Osmotic Pressure Measurement.

5.2.3. Measurement of Diameter Changes of Hydrogel in PVP Solutions

Capillary shaped hydrogels, prepared as in in Ch. 2.3, were placed inside semipermeable dialysis membrane tubes, which were tied off and immersed in PVP solutions with concentrations ranging between 0g/mL and 0.15g/mL, at pH 8, 9, and 10. For better contact of hydrogels to external solution, 1mL of buffer solution was

transferred into the tube before tying the two ends of the tube together with a rubber band. As the internal buffer solution became equilibrated with the external buffer solution, the internal buffer solution was drawn out, and the tube and hydrogel were squeezed depending on the osmotic pressure. We waited for two weeks for complete equilibrium, regularly adjusting pH, and then extracted the hydrogels from the tubes and measured their diameter changes under a microscope.

5.3. Results and Discussion

5.3.1. Compression Modulus and Crosslink Densities

The compression modulus of a hydrogel reflects its crosslink density. The compression modulus, *G*, is calculated by [55] [56]

$$G = \frac{F}{A_0(\alpha - \alpha^{-2})} = \rho_0 R T \frac{d_0}{d} \quad (5.1)$$

where

F = normal force of compression

A_0 = cross sectional area of the hydrogel disk before compression

α = compression ratio at constant volume (α=0.9), defined as the ratio of the thicknesses of the hydrogel after and before compression

ρ_0 = initial crosslinking density

R = gas constant

T = temperature

$\frac{d_0}{d}$ = ratio of an hydrogel cylinder's diameter at formation (1.176 mm) to its diameter following swelling

The permanent crosslink density at formation, ρ_0 can be determined by the plateau stress, measured following a period of relaxation during the "hold" phase of the compression measurement. This relaxation phase is most significant in glucose solutions at high pH, since there will be an initial stress transient due to the reversible crosslinks formed by complexation of glucose with PBA units attached to different hydrogel chains, as discussed in Chapter 3. However, these crosslinks are transient and should not contribute to the final plateau stress..

Fig. 5.2 displays stress changes (F/A_0) during the compress-hold-decompress experiments, for hydrogels equilibrated in sugar free solutions, 9mM fructose solutions, and 9mM glucose solutions at pH 10 and pH 7.4. Also, we confirmed reversible crosslinks between glucose molecules and charged PBA moieties from a high peak and a substantial relaxation at pH 10 in 9mM glucose, while there are no relaxations at pH 7.4 in glucose because of lack of charged PBA moieties. Similarly, sugar-free and fructose solutions do not show large relaxations, since there are no transient crosslinks formed.

Calculated values of ρ_0 based on the plateau stress values are tabulated in Table. 5.1. These values cluster around an average 7.43mM, which is substantially lower than the values of ρ_0 estimated in Chapter 4 under the *f*- and φ-variants of the extended FRDL model. Both of these models fit the data quite well, and it was hoped that the present experiments could be a basis for model selection. Unfortunately, neither model provides

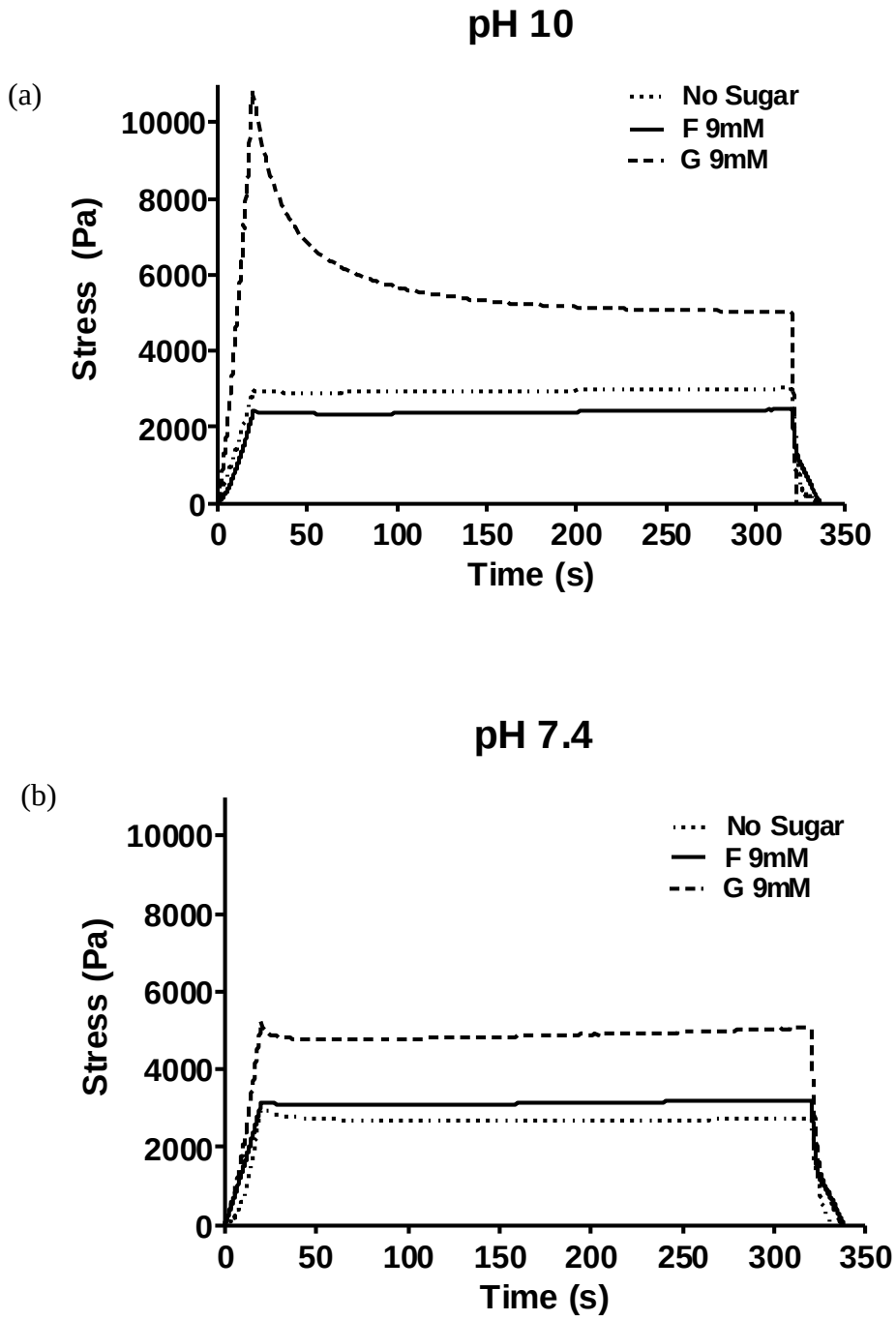

Figure 5.2. Compression stress during the compression for MPBA-co-AAm hydrogels equilibrated in sugar free solutions (small dotted line), 9mM fructose solutions (solid line), and 9mM glucose solutions (large dotted line) at pH 10 (a) and pH 7.4(b).

		Stress (Pa)	d/d_0	Crosslink density (mM)
pH 10	**0mM**	3012	2.05	7.45
	F9mM	2460	2.02	5.99
	G9mM	5042	1.54	9.35
pH 7.4	**0mM**	5131	1.13	6.96
	F9mM	3200	1.86	7.19
	G9mM	5031	1.26	7.62

Table 5.1. Crosslink density values of MPBA-*co*-AAm hydrogels calculated by Eq. (5.1).

good fits to the data when ρ_0 is fixed at any of the measured values in Table 5.1 (fits not shown) . Interestingly, the value of ρ_0 estimated in the "constant χ" model, 8 mM, lies exactly in the range of measurements, However, that model is otherwise unable to fit the swelling data.

5.3.2. Measurements of Osmotic Pressures and Hydrogel Diameter Changes in PVP Solutions

Fig. 5.3. shows changes in the osmotic pressure measured by the fiber optic pressure sensor in time for 24hrs, at different PVP concentrations. Following an initial lag phase, osmotic pressures approached their equilibrium values as decaying exponentials, and numerical extrapolations were performed on that basis;. The highest PVP concentration generated the largest osmotic pressure. The osmotic pressure values at equilibrium are listed in Table 5.2. The osmotic pressures determined in the present apparatus are, in some cases, different from literature values [57]. At a PVP concentration of 0.15g/mL, osmotic pressure values listed in the literature are 1.006 atm for 28K Da

PVP and 0.9454 atm for 117 K Da, while we obtained only the half of those values, 0.5 atm. We are not certain of the origin of these discrepancies, but one possibility is a leak in the silicone plug surrounding the fiber optic pressure sensor.

Osmotic effects on swelling are represented by the linear swelling ratios, $d_\pi/d_{\pi=0}$, where d_π is the diameter of the hydrogel measured after immersion in constant pH PVP solutions for two weeks, and $d_{\pi=0}$is the diameter of hydrogel in PVP free solution at the same pH value. Values of $d_\pi/d_{\pi=0}$ at all three pH values are listed in Table 5.2 and plotted in Figure 5.4.

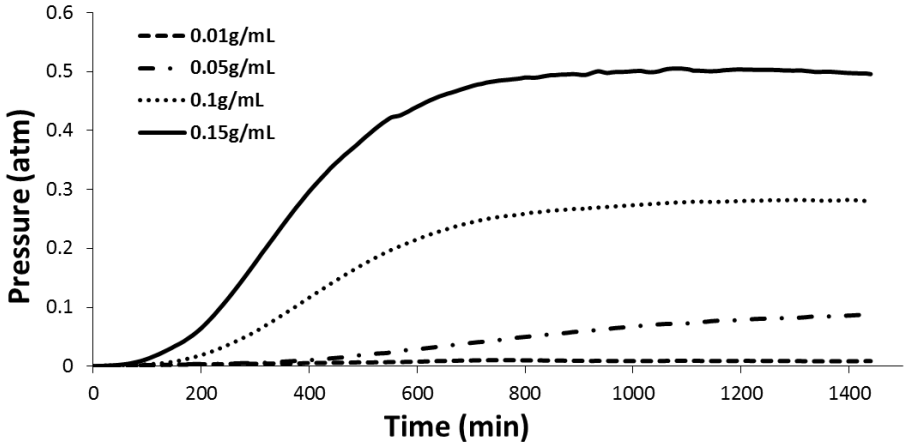

Figure 5.3. Osmotic pressure measurements by fiber optic pressure sensor. Osmotic pressure was caused by PVP concentration difference.

		$d_\pi/d_{\pi=0}$,		
Concentration (g/mL)	**Osmotic Pressure (atm)**	**pH 8**	**pH 9**	**pH 10**
0.01	0.0095	0.994	0.991	0.989
0.05	0.1175	0.988	0.977	0.975
0.10	0.2811	0.982	0.968	0.951
0.15	0.5025	0.977	0.950	0.908

Table 5.2. Osmotic pressure values at equilibrium in Fig. 5.2., and d/d_0 when d is the diameter of the hydrogel in PVP solution, and d0 is the diameter of hydrogel in PVP free solution at the same pH value.

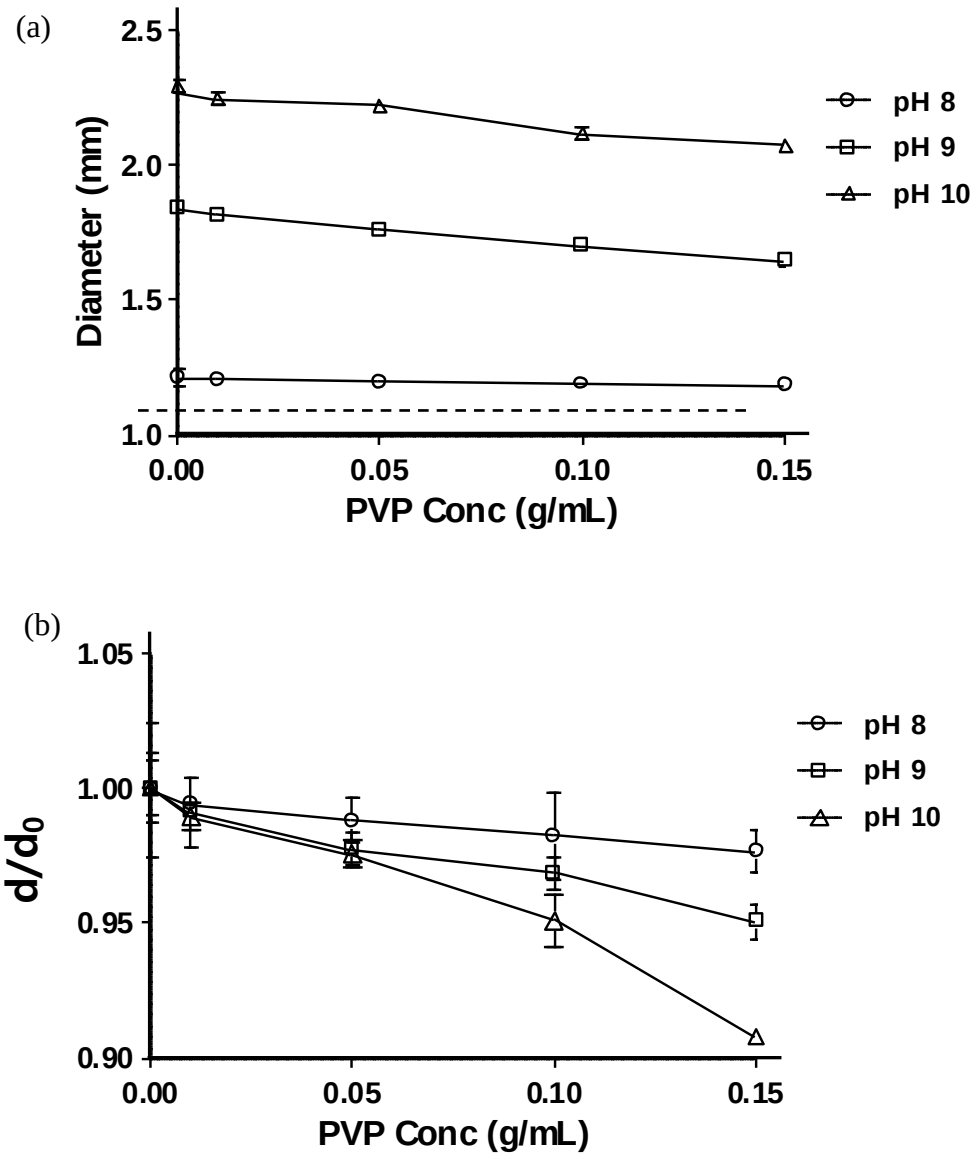

Figure 5.4. Diameter changes with initial diameter at synthesis (dotted line, 1172mm) (a) and d/d_0 b) of MPBA-co-AAm hydrogles after immersion for two weeks in 0–0.15g/mL PVP solution at pH 8, 9, and 10 (n=2).

Even though 0.15g/mL PVP solution generated high osmotic pressures, up to 0.50 atm, only small changes of diameters of the hydrogels, less than 10% were observed. The largest changes in diameters occurred at the higher pH value, indicating a higher susceptibility of swollen hydrogels to osmotic deswelling. Because of the relatively small degree of deswelling, it is apparent that the present experiment cannot provide the desired information to distinguish between variants of the FRDL swelling model.

5.4 Conclusions

Crosslink density values of the copolymer MPBA-*co*-AAm copolymer hydrogels at different conditions were calculated based on the stress values measured by the compression tests. These values were less than expected based on both variants of the FRDL model, suggesting that the models are not complete descriptions. Also, the stress relaxation measurements provided evidence for reversible crosslinks between glucose molecules and PBA moieties. Osmotic pressures of PVP solutions and diameter changes of the hydrogels in those PVP solutions were measured. At a fixed PVP concentration, higher pH led to larger changes in hydrogel diameter, but swelling changes were rather small.

6. Conclusions and Future Directions

6.1. Conclusions

In this thesis, we reported synthesis and characterization of two kinds of hydrogel systems for glucose sensing applications, and mathematical modeling of hydrogel swelling.

In chapter 2, MPBA monomer and two kinds of hydrogels were synthesized for swelling studies and mechanical tests. Chapter 3 covered equilibrium swelling properties in different fructose and glucose solutions at varying pH values. The MPBA-*co*-AAm hydrogel always swells with increasing pH because the degree of ionization of PBA moieties increases. Also, the hydrogel swells in the presence of fructose due to stabilization of the ionized groups on the hydrogel. There are two modes of swelling in the presence of glucose. At pH values below pKa of MPBA, swelling of the hydrogel increase monotonically with glucose concentration, while the hydrogel shrinks with increasing glucose concentration at pH values above pKa due to formation of reversible crosslinks between a glucose molecule and two PBA moieties on separate polymer chains. MPBA-*co*-AAm-*co*-DMP hydrogel has lower pKa due to an interaction between PBA and the amine group in DMP, allowing shrinking with increasing glucose concentration at pH 7.4. The absence of shrinking in the fructose solutions confirms that the reversible crosslinking mechanism holds for glucose in the DMP containing gels.

The FRDL model was applied to fit the MPBA-*co*-AAm hydrogel swelling in Chapter. 4. The fitting was good, but we could not distinguish between two variants of the model for the polymer-solvent interaction parameter without extra information that

could be obtained by mechanical or osmotic deswelling experiments. In Chapter 5 such measurements were carried out, first by using mechanical compression measurements to determine the crosslink density at formation, a critical parameter in the FRDL model whose estimate differed for the two variants, and second by measuring osmotic deswelling using PVP solutions with calibrated osmotic pressures. Unfortunately, the mechanical measurements yielded crosslink density estimates that were not consistent with either model variant, and the osmotic measurements produced unexpectedly weak results.

The compression tests confirmed the formation of glucose mediate crosslinks at pH 10 in 9mM glucose solutions. The initial high stress due to these transient crosslinks relaxed however, such that the final calculated crosslink density was similar to those computed with sugar-free and fructose solutions, and pH 7,4 solutions containing 9 mM glucose. Thus, the permanent and transient crosslinks can be seen to contribute differently to hydrogel behavior.

6.2. Future Directions

6.2.1 Modification of Chemical Structure of Hydrogel

We have studied PBA-containing hydrogels containing 3-methacryl amidophenylboronic (*m*-PBA), which is glucose sensitive. We showed, however, that swelling of *m*-PBA containing hydrogels is dependent on pH as well as glucose concentration. This cross-sensitivity is problematic since diabetics are prone to swings in blood pH.

In order to remove the pH dependence of hydrogel swelling, we can use 2-acrylamidophenylboronic acid (*o*-PBA) instead of *m*-PBA. The chemical structures of *o*-PBA and *m*-PBA are shown in Fig. 6.1a. According to [33,36], this structure undergoes an internal condensation in acidic and neutral pH environments, producing a quaternized boron center without the need for OH^- or separately provided amine groups, as described in Fig. 6.1b. This configuration binds glucose without alteration of charge, and a single glucose molecule can complex with two of these moieties, forming crosslinks. Yang *et al.* [58] have shown that hydrogels containing *o*-PBA are much less cross-sensitive to pH

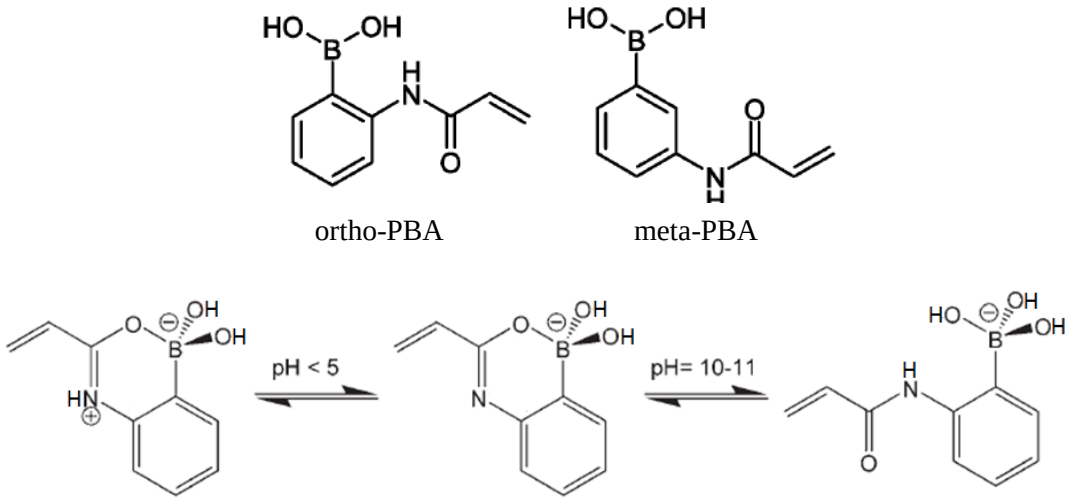

Figure 6.1. (a) Chemical structure of ortho-PBA and meta-PBA,

(b) Chemical equilibrium in water.

than those containing *m*-PBA. Future studies should involve preparing hydrogels containing *o*-PBA.

The monomer, 2-acrylamidophenylboronic acid (*o*-PBA), can be synthesized by the method described in [36]. 2-aminophenylboronic acid (0.75g) and HCl (4.4 mmol)

are mixed with a sodium hydroxide solution (5N in water, 5 mL) in an ice bath. Acryloyl chloride (0.5mL) is added into the resulting solution dropwisely for 10 min. After 30min of stirring in the ice bath, it is stirred for 2 hours at room temperature, and then adjusted to pH 8 by using dilute HCl (0.1M). The resulting precipitate is filtered off and washed with water and acetone. A fine, white powder is obtained after drying.

o-PBA-containing hydrogel is synthesized by photopolymerization. Appropriate quantities of the monomers (*o*-PBA, and acrylamide) and N,N'-methylene-bisacylamide (Bis: crosslinker) are dissolved in dimethyl sulfoxide (DMSO) containing 2,2'-dimethoxy-2-phenyl acetophenone (DMPA, photoinitiator). Glucose is also added to the solution, which is then heated for 30 min at 50 °C to encourage dissolution of *o*-PBA [59]. After completing dissolution of monomers and initiator, the solution is poured into appropriate site such as silianized glass plates, capillaries, or a designed mold, and polymerized by exposure to UV light for 60-90 min. After polymerization the hydrogel is washed by distilled water at 40 °C for 12 hours to remove DMSO and glucose, and stored in pH 7.4 buffer.

6.2.2. Mathematical Modeling of Swelling Behavior

Although we have established a direct correlation between the swelling and mechanical properties of PBA containing hydrogels, it is still difficult to predict swelling of hydrogel swelling quantitatively based on the experimental results and the current mathematical modeling. The current mathematical model, even with its limitations as just discussed, only handles hydrogels in sugar-free or fructose solutions. Extensions of the

model to include reversible glucose crosslinks, perhaps along the lines of Alexeev [51] are needed to properly account for hydrogel behavior in glucose sensors.

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