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Development and Investigation of Unique Organic Materials to Detect Disease Biomarkers

George Kiplimo Samoei
Portland State University

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DEVELOPMENT AND INVESTIGATION OF UNIQUE ORGANIC MATERIALS TO DETECT DISEASE BIOMARKERS

by

GEORGE KIPLIMO SAMOEI

A dissertation submitted in partial fulfillment of the
requirements for the degree of

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CHEMISTRY

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DISSERTATION APPROVAL

The abstract and dissertation of George Kiplimo Samoei for the Doctor of Philosophy in Chemistry were presented June 6, 2008, and accepted by the dissertation committee and the doctoral program.

COMMITTEE APPROVALS:

Robert M. Strongin, Chair

Mingdi Yan

Reuben H. Simoyi

David H. Peyton

Andres H. La Rosa
Representative of the Office of Graduate Studies

DOCTORAL PROGRAM APPROVAL:

Kevin Reynold, Director
Chemistry Ph.D. Program

ABSTRACT

An abstract of the dissertation of George Kiplimo Samoei for the Doctor of Philosophy in Chemistry presented June 6, 2008.

Title: Development and Investigation of Organic Materials to Detect Disease Biomarkers

Chemomechanical polymer gels with the capability of recognizing specific biological molecules are promising materials for many biomedical applications. Glucose-selective hydrogels have potential utility in the continuous monitoring of glucose and glucose-triggered insulin delivery for the management of diabetes. Herein, the development and study of the first material which detects glucose via significant volume changes in the challenging matrix human blood plasma is described. The material was created via the modification of PMMA with supramolecular binding sites. The polymer exhibits excellent selectivity and reversibility including continuous expansion-contraction cycles.

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Chapter 1: Review of Chemomechanical Polymers

1.1 Introduction

Chemomechanical polymers which exhibit both hydrophilic and hydrophobic characteristics have promising potential because of their reversible swelling and contracting character. [1-4] Such polymers are the basic building blocks for many devices which function in response to physical or chemical stimuli. [5-8] They are used in constructing sensors, [9, 10] drug delivery agents, [11-13] and actuators [14] which function autonomously, thus eliminating the need for instrumentation, complicated procedures, and an external power source. [15] Others recent applications include filtration/separation [16] and artificial biological tissues. [17, 18]

Hydrogels cancontain covalent and/or non-covalent receptor sites for effectors to bind. Such receptor sites can be modified to specifically bind a target molecule and/or respond to selectively to other specific conditions. In synthesizing hydrogels, they are either polymerized from functionalized respective monomers, or receptors are introduced into the backbone of an already existing polymer. [19]

An interesting feature chemomechanical polymers add to traditional sensors is that they facilitate operation either with or without additional external devices such as transducers or transmitters, or any power supply. The binding sites may either be on the surface or within the polymer. [1, 20] Binding receptors interacting covalently or non-covalently with a guest molecule are illustrated in Figure **1**. This reaction is largely dependent on the number of exposed binding sites, which could be enhanced

by downsizing or by functionalizing with more binding sites, as demonstrated in Figure **2.**

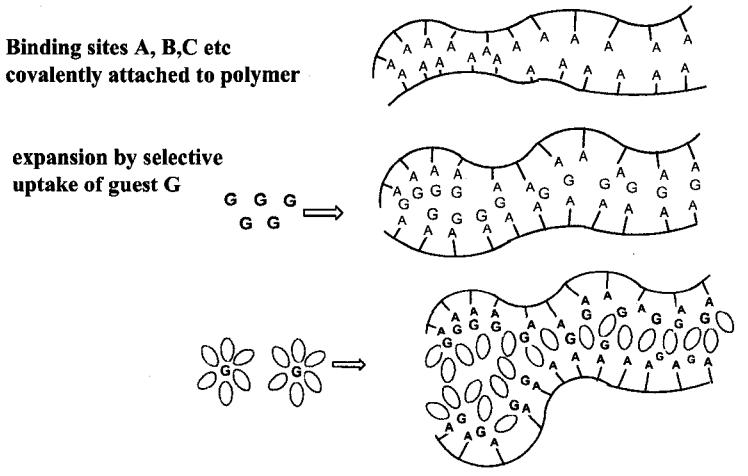


Figure 1: Expansion of hydrogels by uptake of guest molecules G and water during solvation.

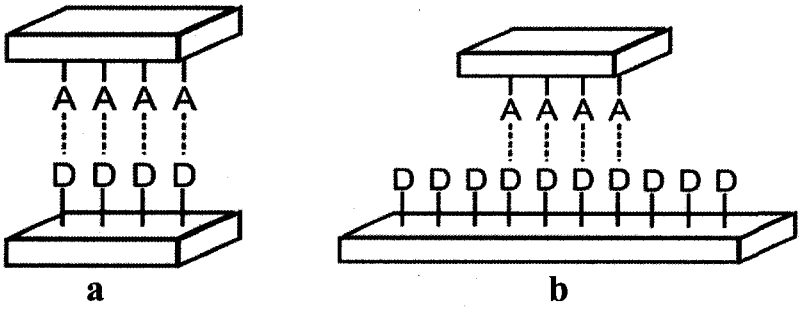


Figure 2: Complexes with matching number of binding sites (chelate effect, cases a) and with a surplus of binding sites in the receptor (case b). (see H-J. Schneider, L. Tianjun, N. Lomadze. *Chemistry Communication,* **2004**, 2436-2437)

In order to improve performance and drive applications, an understanding of the underlying physical and chemical properties of such polymers is desired. The function of certain chemomechanical polymers can be activated by voltage, Electrically Activated Polymers (EAP's), in the form of ionic polymer-metal composites. The electric stimulus will transform the chemical energy into mechanical energy, and trigger an electric signal, analogous to an artificial muscle. The reversible change in volume of other chemomechanical polymers can also be triggered by other stimuli such as light, temperature and pH, and the like.

In the swollen state, a guest molecule, metal ions, or drugs can be adsorbed into the polymer network. The receptors of the polymer network can bind the target molecule and either collapse the gel or expand it further, depending on details of the underlying mechanism. The kinetics of swelling and the water transport mechanism which effect this transformation are a function of the composition of the hydrogels and the pH of the swelling medium. [21] Herein, I will focus on how chemomechanical polymers can be designed and developed, and how their resulting size changes can be utilized in a number of applications.

1.2 Chemomechanical hydrogels triggered by pH changes

pH-responsive chemomechanical polymers have been studied extensively because of their potential applications in many fields. For instance, some controlled drug release devices have been developed based on the pH sensitive hydrogels. [22] These chemomechanical polymers form complexes which exhibit a pH-responsivive swelling and shrinking mechanism to release drugs. Swelling and deswelling

properties are also influenced by some reaction parameters such as monomer concentration, co-monomer molar ratios, the amount of cross-linking agent, polymerization time, particle size and size distribution. The chemomechanical responses can also depend strongly on the ionic strength of the medium. [23, 24]

Some of the hydrogels reported show asymmetric behavior with changes in pH. Scheneider *et al.* synthesized a polymer derived from modifying poly(methyl methacrylate) (PMMA) with diethylenetriamine and dodecylamine. The polymer was pH-responsive with liphophilic binding sites that displayed symmetrical pH profiles as shown in Figure **3**. [1]

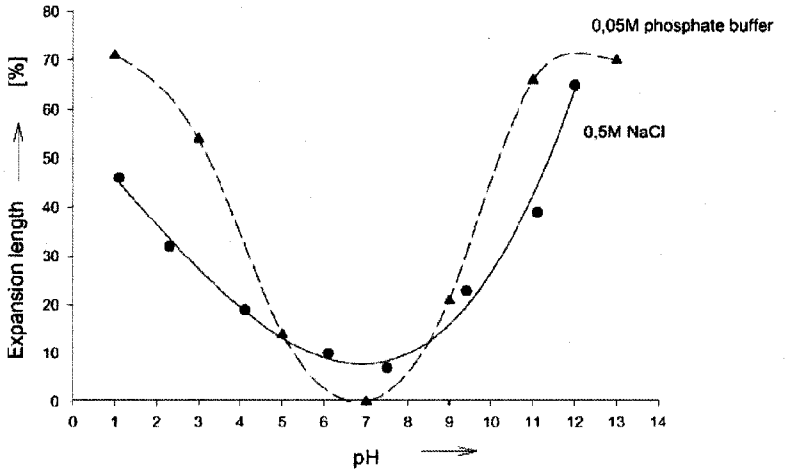


Figure **3**: Expansion vs. pH; with polymer **A** in 0.05 m phosphate buffer (circles); with Polymer **B** in 0.5 m sodium chloride (triangles). (see © 2006 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. *European Journal of Organic Chemistry,* **2006**, 677–692)

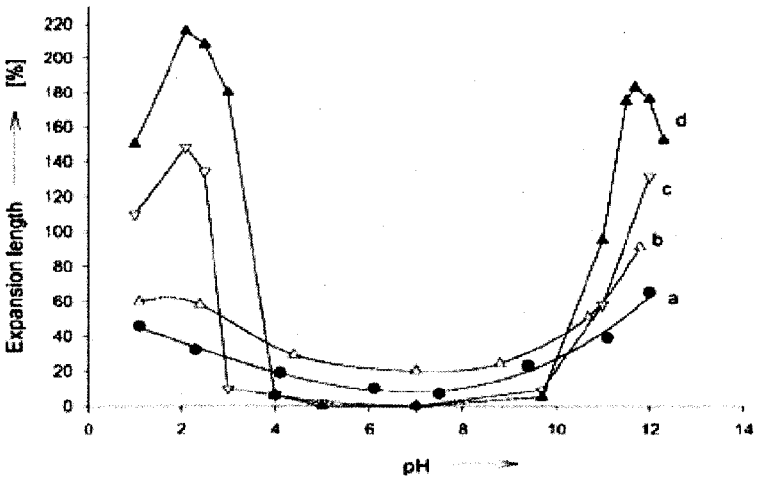


Figure **4**. pH expansion profiles at different salt concentrations (with polymer **B**): in 0.5M (● - a), 0.05 M (Δ - b), and 0.025 M (∇ - c), sodium chloride, and in water in the presence of very dilute HCl or NaOH (▼ - d). (see © 2006 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. *European Journal of Organic Chemistry,* **2006**, 677–692)

The performance and response of a pH-sensitive polymer are determined by a number of factors such as particle size, as well as type and composition of the polymer. As early as the 1950's, it was reported that a chemomechanical polymer changed viscosity when the pH was raised, as shown in Figure **5**. Kuhn, Hargtay, Katchalsky and Eisenberg demonstrated a size increase when sodium hydroxide was added into their polyanion. [25]

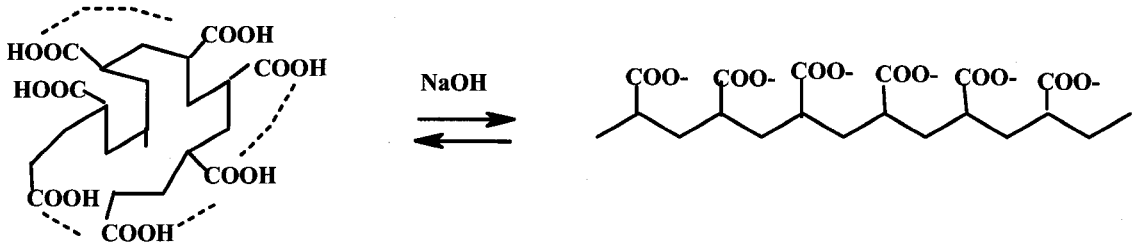


Figure **5**. *Viscocity change a polyanion solution by pH (see* W. Kuhn, B. Hargtay, A. Katchalsky, H. Eisenberg. *Nature,* **1950,** *165,* 514-516)

In the application of chemomechanical polymers as drug delivery vehicles, Peppas and coworkers reported that the particle size and particle size distribution affects the kinetics, degradation rate of the particles, biodistribution, delivery options, and interaction of the polymer with the biological membrane. [26] Small particles are important in allowing penetration through the walls of membranes e.g. the gastrointestinal walls.

Some pH-sensitive chemomechanical polymers were microfabricated to increase their surface area. Peppas *et al.* have studied pH-responsive poly(methacrylic acid-*g*-ethylene glycol) hydrogels. These hydrogels were synthesized by a suspension polymerization method to obtain microspheres for the oral delivery of protein drugs. In their work, they used dimethacrylate as the crosslinking agent. They were able to control the size of the polymer particle by controlling the concentration of the monomer in water during the synthesis. The results showed that P(MAA-*g*-EG) nanospheres size decreased when the ratio of the crosslinker was increased i.e. from 1:2 of methacrylic acid/ethyl glycol molar ratio. Further, Peppas *et al.* investigated the equilibrium swelling behavior of P(MAA-*g*-EG) nanospheres with buffer solutions with the pH range 3.3-7.5, showing a pH-responsive size change. They found that the equilibrium swelling ratio of P(MAA-*g*-EG) nanospheres also decreased when the crosslinking agent was increased during polymerization.

Zhang and Seitz synthesized a hydrogel based on 2-methacryloyloxyethyl phosphorylcholine (MPC) which showed good biocompatibility. [27] They established that the hydrogel swells under acidic pH and dissociates under neutral and alkaline

conditions. In their studies, they investigated how the polymer network affects the deformation. They reported that it showed a low swelling and low elasticity when the PMA molar ratio increases during the preparation. The polymer showed a slow dissociation behavior which was attributed to the formation of intramolecular crosslinks caused by hydrogen bonds provided by carboxyl groups inside the hydrogel. This did not contribute to the swelling or elastic properties of the polymer.

The crosslinking of the polymer affected the ionization of the carboxyl groups. The average crosslink molecular weight decreased with a higher PMA feed ratio, indicating that the network is actually denser. PMA contributed to intramolecular crosslink networks and fewer entanglements for high mechanical strength. Further investigation showed that denser networks were formed when a higher PMA feed ratio was used. The carboxylic groups acted as pH responsive side groups as well as cross-link junctions through the hydrogen bonding.

This hydrogel remained stable at low pH values because of hydrogen bonding, while it dissociated at higher pH values as a results of repulsive electrostatic forces. In their investigations, Zhang and Seitz found that this polymer was eroded on the surface under neutral pH conditions, but swelled under acidic conditions. This phenomenon could be used in the application of drug delivery. The release mechanism of the drug under this approach would be suppressed at low pH values and promoted at higher pH values. At low pH the polymer is in its swollen state where the binding sites are free to bind the drug more tightly than at neutral or higher pH values. This

behavior can be altered by controlling the hydrophobicity of the loaded drugs, polymer concentration, or polymer composition.

Park and Bae copolymerized novel pH-sensitive polymers from a monomer derivatized from 4-amino-*N*-[4,6-dimethyl-2-pyrimidinyl]benzene sulfonamide with *N*,*N*-dimethylacrylamide. [28] They synthesized and studied the properties of both linear and crosslinked copolymers as to how they responded to varying pH. The linear copolymer dissolved when the pH was raised while the chemically crosslinked gels exhibited a sharp transition in swelling around physiological pH. In both cases, changes due to pH were reported to be reversible. Park and coworkers through varying the type of sulfonamide and the copolymer composition to come up with a new class of pH-sensitive polymers with a broad range of transition pHs.

In their investigation they showed the interfaced pH-responsive polymers to shrink and swell repeatedly in cycles between pH 2.0 and 8.2. This novel polymer contracted at lower pH and expanded at higher pH. This behavior was attributed to the fact that at lower pH the carboxylate groups of the polymer are hardly ionized, while at high pH carboxylate functional groups are fully ionized. Thus the ionized carboxylate group (COO^{-2}) will exert the ionic repulsive electrostatic forces which expanded the polymer chain to form a rigid structure with more negative charge on the surface. [16] This phenomenon of the interfacial charge density and the ion permeability concept can be utilized by tuning the pH of the environment to suit many applications. For instance in their study Park and coworkers copolymerized NIPAm and AAc to give a P(NIPAm-g-AAc) polymers which are responsive to temperature,

ionic strength, and pH values. This copolymerized P(NIPAm-g-AAc), multi-responsive to temperature, salt concentration and pH, was immobilized on an Au electrode surface. This was studied to determine the polymer's behavior by switching the temperature between 20 and 50 ^{0}C (at NaCl = 0 M; pH = 5.6), concentration of NaCl between 0 M and 2.5 M (at T =20 ^{0}C, pH 5.6), and pH between 2.0 and 8.2 (at T =20 ^{0}C, NaCl = 0 M). The investigations showed that the states which present different interfacial properties were switchable. This behavior were attributed to the reversible change in hydrogen bonding between the two components P(NIPAM-co-AAc) and water, and the ionization of carboxylate groups in different environmental conditions.

Another noticeable feature of P(NIPAm-co-AAc) pH-sensitive interface copolymer as reported by Park *et al.* was its reversibility, quick to transform a single cycle in few minutes and the stability attributed to the crosslinking. The qualities of such interfaced polymers which are temperature, ionic strength and pH responsive when microfabricated could be a potential novel material for many useful applications. Such applications may include uses as multi-functional components, biological actuators, controlled permeation membranes, separations, immobilized biocatalysts, interfacial engineering, responsive filters, and so on.

1.3 Water content changes

The mechanism of swelling and contracting of chemomechanical polymers can be due to a large association of water molecules with charged receptors of the hydrogel e.g. $-NH_3^+$ in the case of chitosan. [20] The water swollen polymers with large

amounts of water or biological fluids can be used to simulate the function of tissues of living organisms. These gels can trap solutes to form a solid-like gel structure. Some hydrogels have been reported to be biocompatible with minimal mechanical friction to the surrounding cells and tissues, and therefore could be attractive as artificial tissues and organic implants.

When the polymer is in the swollen state by water, it will helps to govern the interactions of the polymer's receptors and the effector molecules and also affects its biocompatibility as an implant material.

Kim *et al.* reported in their work that water uptake and release mechanism in chemomechanical hydrogels is significantly dependent on the pH of the swelling medium. At a high pH, more than the p*Ka* of the gel, the uptake or release of water is controlled by polymer relaxation than by penetrant diffusion. [14] When the pH is raised above the *pKa* value of the gel, it increases the degree of ionization which causes the electrostatic repulsion between adjacent ionized groups such as $-NH_2$, -COOH etc and thus leads to the chain expansion of the polymer.

There is a need to design and study chemomechanical polymers to improve their capability to trap large amounts of water and solutes within their structures. The high water content, soft texture and elastic nature may potentially afford polymers more resemblance to the activities of the living tissues. If biocompatible, such materials may be suitable for artificial tissues and organic implants. [30]

The uptake and release of water by chemomechanical polymers determines the swelling and contracting properties of the gel. Wang and coworkers reported a pH-

sensitive biodegradable chitosan polymer grafted with polyvinyl alcocohol (PVA) crosslinked with glutaraldehyde (GA). [29] In their investigation they used differential scanning calorimetry to determine the water bound in the polymer as a function of the chitosan/PVA molar ratio, GA concentration (*C*GA), and ionization state. These investigations were done on the dry hydrogel and equilibrated gel at pH 3 and pH 7 which included the determination of the swelling and contracting of the polymer. Wang *et al.* reported the increase of the bound water fraction (XBW) at the initial state when the PVA concentration was increased without any effect from the concentration of crosslinker. In the case of the buffer-equilibrated polymer, they found that the bound water fraction decreased when the concentration of PVA was increased and the crosslinker decreased. Also they reported that the amount of water bound based on the dry polymer mass was higher in the ionized (swollen) state compared than with unionized state. These results might be attributed to the association of large amounts of water molecules which solvate the protonated $-NH_3^+$ groups of chitosan in acidic medium, and/or probably the repulsing protonated amines of chitosan, which creates a wide compartment for a large volume of water to enter.

1.4 Electric Activated Chemomechanical Polymers (EAPs)

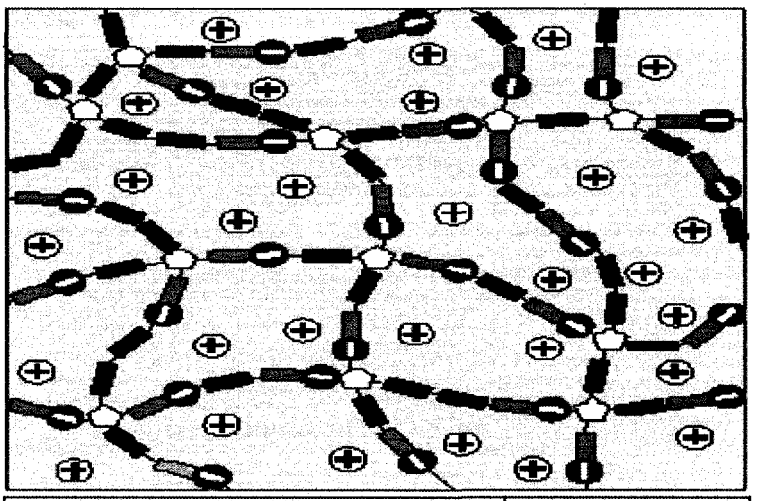
Chemomechanical polymers which change volume and shape in response to a stimulus can transform chemical energy into mechanical energy. Electric current sensitive hydrogels having material activated by electric stimulus seem are very interesting because of the fact that mechanical energy is triggered by an electric signal. These polymers which are activated by voltage (EAPs) have are interesting in the

development of actuators because of their reversible properties. They undergo phase transformations which result in a macroscopic volume change due to external stumuli.

Effector molecules would bind the charged receptors of the polymers through the through the electrostatic interactions, thus causing volume change. [31, 32] Okuzaki and Osada reported a synthetic polymer driven by electricity with a worm-like swing which have motility (swings) in water. [33]

Electrically driven polymers can be improved by varying the various operating parameters and network structure of the hydrogel. Shamsudeen *et al.* reported hydrogels with semi-interpenetrating networks from acrylamide and polyvinyl alcohol (PVA) with and without polyethyleneglycol dimethacrylate (PEGDMA) crosslinker. [34] In their study, they reported the bending degrees of the gel by varying the concentration of electrolyte solution, gel size and electric current. The response and performance of their polymers was improved when the size of the gel was reduced.

They suggested that the material's architecture must contain both positively and negatively charged moieties in order to be electrically active. Such polymers are achieved by polymerizing ionizable monomers or introducing ionizable monomers into existing polymer network as in Figure **6** with a polymer having fixed anionic groups and mobile counter ions.



Chemical Composition	Example
backbone monomer	acrylamide
electrolyte comonomer	acrylate$^-$
mobile counterion	Na^+
crosslinking comonomer	bisacrylamide
solvent	H_2O

Figure 6: Schematic of a typical polyelectrolyte hydrogel. (*See* Proceeding of ISSS 2005 International Conference on Smart Materials Strucures and Systems, July 28-30, Bangalore, India)

1.5 Kinetics, concentration effects and sensitivity

The swelling and de-swelling properties of chemomechanical polymers have been utilized in a number of useful applications. These range from drug delivery systems [35, 36] to on-off control valves in microfluidic systems, [37] filtration/separation of chiral compounds, [38] muscle-like actuators [39, 40] and sensors for different internal and external chemical, biological and electrical stimuli. To optimize these polymers for their better applications, controlled physical parameters have been exploited by many researchers. To achieve these goals, enhanced speed of swelling/deswelling (rate) and the degree of swelling/deswelling process for the same stimuli have

proposed. These physical parameter includes ionic strength, diffusivity, etc. The rate of change in volume (swelling and/or deswelling) of a chemomechanical polymer is a function of several factors:

i) Diffusion of effector molecule/ion into the hydrogel.

ii) The pH factor

iii) The size of the polymer

Hydrogels contain acidic, basic or both moieties within the network of their structures. The acidic moiety deprotonates at higher pH to release a H^+ which combines with OH^- to form water. The result is a charge compensation from the effector ions which diffuses with OH^-. There is an underlying mechanism to maintain charge neutrality in the polymer at all times. When the concentration of ions (cations and/or anions) is increased, it gives rise to an osmotic pressure which causes the polymer to either swell or deswell in the process of compensating pressure. The diffusion of these effector ions and the dissociation of the polymer's ionic group could affect the swelling rate. [41-46] Chu, Kumagai, Sakiyama, Ikeda and Nakamura developed a model that describes the swelling rate of an ionic gel based on the mobile ions using a xanthan-chitosan hydrogel. [47] They confirmed in their model that the swelling rate of a xanthan-chitosan gel at higher pH values is dominated by diffusion of the effector ions rather than the cooperative diffusion of the polymer network. Changes in the external environment enhanced deformation of hydrogels. [48] Chu, Varanasi and McGlade reported gels that swell faster in buffer solution. They concluded that the speed of swelling/deswelling to be a function of effector ions

diffusing into the gel as described by Fick's Laws. [49] Moore and coworkers demonstrated that conjugate base of the buffer solution binds H^+ ions at a higher concentrations and releases it after diffusing into the lower region of H^+ concentration. The phenomenon depends on the concentration of the buffer; higher concentration implies more H^+ would be bound, transported and released in a region of lower H^+ ion concentration. [50]

Ruckenstein and Sasidhar measured apparently higher diffusion rate coefficients for H^+ in buffered solution than without buffer. [51] In their analysis of swelling and deswelling, Moore and coworkers developed a simulation in which results were compared to the experimental data. This simulation was based on Nernst-Plank equation [52] coupled with the Poisson and the mechanical equilibrium equation [53, 54] to calculate the ionic concentration, electric potential and hydrogel swelling. The swelling and/deswelling was found to match with the experimental values, and they concluded that hydrogel swelling was basically a diffusion limited process.

1.6 Chiral discrimination

The characteristic properties of natural occurring polymers which reversibly contract and expand have inspired many scientists to create smart biocompatible supramolecular polymers for a wide range of applications. [55-57] The synthesis of these materials has evolved from traditional supramaolecular materials that utilize few hydrogen bonding sites. [58-66]

Meijer *et al.*, in an attempt to advance the design of noncovalent polymers based on quadruple hydrogen bonding, created a graft copolymer with a quadruple

hydrogen bond motif in the main chain by acyclic diene metathesis (ADMET) polymerization from a polymerzible monomer with a 2,7-diamido-1,8-naphthyridine protected by 2-ureido-4[1*H*]-pyrimidinone (UPy).[13] The derivatives of UPy were then grafted onto the free poly-Napy. This grafted polymer selectively formed strong heterodimers in one of its tautomeric forms via a hydrogen bonding acceptor donor-donor-acceptor (ADDA) array with the DAAD array of 2,7-diamido-1,8-naphthyridines. [68, 69] Meijer *et al.* reported a copolymer which favored UPy-Napy heterocomplexation over UPy dimerization by a factor of >20:1 in concentrations above 0.1 M in 1:1 mixtures (*K*a(UPy-Napy) > 106 M-1). The selectivity for hetorodimerization of UPy-Napy-based copolymers [16,17] renders it suitable to explore the possibility of obtaining supramolecular graft copolymers with Napy units in the main-chain and UPy molecules as side chains.

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Chapter 2: Selective Detection of Glucose Using a Functionalized Chemomechanical Polymer

2.1 Introduction

Recently, chemomechanical polymer gels have been the subject of extensive research. They exhibited potential utility in diverse fields such as artificial muscles, [1] separations science, [2-3] drug delivery [4] and sensing. [5,6] The first generation of these so-called "smart/intelligent" polymers was activated by heat, [7-9] light, [10] pH [11-13] or voltage [14-16] to induce readily monitored changes in their physical and chemical properties. Many of the current stimuli-responsive gels are capable of recognizing chemical changes in their environment. [8] In our research we were interested in developing polymers in which the recognitive nature of their binding sites allows the detection of glucose. This is an extension of the Strongin group's work on the spectroscopic detection of specific biological molecules.

There has been a major research effort towards non-invasive and/or minimally-invasive monitoring of glucose in human subjects. The unmet need for glucose sensors has motivated the investigations of numerous approaches. Success would be a milestone in the management and treatment of diabetes by delaying the onset of medical complications associated with the disease. Progress has been hindered by the selectivity and sensitivity of the current materials. In human subjects, there are many components which constitute major interferents to the sensitive and selective detection of glucose. These may include water, carbohydrates, proteins, amino acids, urea, fatty acids, triglycerides, lipid membranes and other classes of biomolecules in the

detection matrix. [17] Also to be considered is the fact that glucose is distributed heterogeneously throughout human tissues which raises the question of which of the tissues and/or body fluids will afford the optimal and/or relevant measurements. Continuous monitoring of glucose is thus also needed to understand the uneven concentrations and distributions in the human body. [18]

Current methods have been reported to have drawbacks such as:

- Current meters are accurate to only 15 %.
- Most require blood fingersticking.
- Device must be carried by patient

Chemomechanical polymers which swell and shrink reversibly can be functionalized with receptor sites to bind molecules and ions. Related systems for monitoring glucose have been reported such as pH-sensitive hydrogels immobilized with glucose oxidase, [19] electrodes coated with functionalized hydrogels, [20] a polymer network embedded with a crystalline colloidal array, [21] materials activated with fluorescence labeled insulin, and magnetic activated hydrogels [20].

In a review, Arnold *et al.* suggested that there is a need to significantly improve minimally- and non-invasive systems for the effective monitoring of blood glucose. This would improve the accuracy and reproducibility of results as well as eliminating pain encountered using current methods. However, the main issue discussed is overcoming the many interfering analytes in complex biological matrices such as blood. [17] My work described herein addresses this problem.

In their first work, Asher *et al.,* developed a sensor system based on PCCA (polycrystalline colloidal arrays) which was embedded with glucose oxidase (GOx) as the molecular recognition agent. This enzyme converts glucose to gluconic acid and creates an osmotic pressure which causes the polymer to swell. The swollen polymer as results of glucose oxidation to gluconic acid, was reported to red shifted the Bragg diffraction devices.

Over the years, Asher and coworkers demonstrated a colorimetric glucose recognition material which contained a crystalline colloidal array (CCA) embedded within a polyacrylamide-poly(ethylene glycol) (PEG) hydrogel, or a polyacrylamide-15-crown-5 hydrogel functionalized with phenylboronic acid receptor. They prepositioned boronic acid moiety and PEG (or crown ether) functional groups into a photonic crystal hydrogel, in which glucose binds boronic acid and self-assembled to form a supramolecular complex. The swelling and contracting motion of the polymer induced by glucose altered the angle of diffraction of light. The degree of light diffracted was calibrated with respect to the concentration of glucose. [20] The supramolecular complex formed from glucose binding boronic acid functional group increased the hydrogel's cross-linking which affected the swelling. Asher *et al.* reported that physiological concentration of glucose blue-shifted the diffraction of the photonic crystal across the visible spectral region over physiologically important glucose concentration ranges (5-8 mmol/L). The system was specific to glucose over galactose, mannose and fructose-common sugars in the human body. This material has shown a higher prospect in continuous glucose monitoring. This improved system of a

glucose sensing agent (PCCA) showed decreased pKa of phenylboronic acid upon binding glucose to form boronate anions. Boronate anions increased the osmotic pressure, and so swelled the PCCA thus red-shifted the diffraction. They proposed the glucose-binding mechanism to form a bisdentate complex stabilized by PEGs which cross-linked the hydrogel. The crosslinking caused the polymer to shrink and blue-shifted the photonic crystal diffraction from the embedded CCA.

Many other related hydrogels for monitoring levels of glucose have been suggested. In the work of Kataoka *et al.* a synthetic glucose-responsive hydrogel was reported which contained both phenylboronic acid and tertiary amine moieties as the recognition sites. [22, 23, 24] The boronic acid of the moiety of the polymer formed a crosslink with the hydroxyls group of poly (vinyl alcohol) (PVA). The complex formation between the two polymers bound by boronate induced a swelling when the D-glucose was introduced in Dulbecco's phosphate buffered saline solution (PBS) at pH 7.4. This was as a result of the formation of a higher complex between cis-diols of D-glucose by displacing the PVA-polymer complex. The glucose-responsive swelling of the polymer complex was utilized to control the current changes with a membrane-coated platinum electrode. They reported that only glucose added to PBS induced swelling of the cast gel membrane, which allowed the diffusion of ion species and increased measurable current changes. This was not the case when methyl-D-glucoside was added, which induced little or no current change. This was an indicative of the higher selectivity of this system for glucose and its cis-hydroxyls. They also observed that the current change was proportional to glucose concentration in the

range of physiological conditions (0-300 mg/dL). This reproducible signal was demonstrated by repetitive, stepwise glucose concentration changes. This supported the application of platinum electrodes coated with functionalized phnylboronic acid gel and PVA as a novel glucose-sensing material. [25]

In the following years, Katoaka and coworkers did an extensive study of their polymer material. They found that poly (3-acrylamidophenylboronic acid-co-N-2-vinyl-pyrrolidone) formed stable complexes with PVA at pH 8.5, and this complex dissociated with the addition of glucose to form new complexes between boronate and glucose due to the higher association of glucose hydroxyls toward the tetrahedral boronate anion over PVA hydroxyl groups. This complex was stabilized by the introduction of amino group into the polymer by transferring the charge from nitrogen atom to boron atom under physiological conditions pH 7.4.

In their studies they were able to utilize this phenomenon to design a novel glucose-responsive insulin delivery device. The gluconated insulin was immobilized on boronate-containing hydrogels via complexation of boronate with hydroxyls of gluconate residues in the modified insulin, and amino groups introduced into the boronate-containing hydrogels. The modified insulin was successfully released from the hydrogels in a pulsatile fashion by applying stepwise glucose changes under physiological pH. They utilized boronate-containing polymer complexes with PVA to measure changes in glucose-responsive cyclic voltammography, and found that the peak current increased with increasing glucose concentration. This was explained to be as results of the competitive dissociation of complexes between boronate and

hydroxyls in PVA and association of boronate and glucose. This increased the diffusion of ferrocyanate anion within the complex gel membrane into the working electrode in cyclic voltammograms.

Grimes and coworkers developed a glucose-responsive material based on the glucose oxidase (GOx) which enzymatically oxidizes glucose to gluconic acid. [26] In their design, Grimes *et al.* developed a polymer which was functionalized with glucose oxidase enzyme. The polymer was embedded with a magnetoelastic film. The pH-sensitive polymer was obtained by slightly crosslinking polyelectrolyte gel synthesized from acrylic acid and isooctyl acrylate monomers. The polymer functioned in a similar manner to what was earlier reported by Kataoka *et al.,* in which the glucose was oxidized by GOx to gluconic acid thus lowers pH. The volume change of the polymer induced by glucose oxidation caused magnetoelastic coupling in the sensor resulting into a measurable voltage. The measured magneto-elastic vibration frequency was reported to be inversely dependent on the sensor volume and/or mass loading. They also found that there was no physical connection between the sensor and the monitoring instrument, a characteristic feature which can be utilized *in vivo* and *in situ*. The mechanism of the enzymes in the process is illustrated in the equation below:

$$\beta\text{-D-glucose} + O_2 + H_2O \xrightarrow{GOx} \beta\text{-D-gluconic acid} + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{catalase}} \tfrac{1}{2}O_2 + H_2O_2$$

$$\beta\text{-D-glucose} + \tfrac{1}{2}O_2 \xrightarrow{GOx + \text{Catalase}} \beta\text{-D-gluconic acid}$$

This approach would solve the challenges faced by glucose electrode sensors, where the GOx on the electrodes are deactivated by hydrogen peroxide produced in the process of glucose oxidation. When glucose was introduced, the reaction of the sensor converts glucose to gluconic acid, producing H^+. In the response of pH change, acrylic acid moiety of the polymer dissociated causing the polymer to swell. The detection of glucose was then based on the signal of the transducer between the shrunken (acidic) and the swollen (basic) states of the polymer. The selectivity of the sensor was based on the high specificity of the enzymatic reaction. This sensor material could presumably be inserted into the skin, while the detecting system could be packaged in a wristwatch-like if found to be biocompatible.

In another contribution to the development of non-invasive and/or minimally-invasive systems for monitoring glucose, Lowe and coworkers developed a holographic system for detecting changes in glucose concentrations. [27] Their system was constructed by incorporating a holographic device unto a polymer that has phenyl boronic acid binding site for glucose. Laser light was exposed to the complex formed by glucose-polymer bound and the hologram. The optical response of holograms (hologram diffraction wavelength) was tracked, and this was attributed to swelling of the polymer in the presence of glucose. The color of the hologram red-shifted when polymer swelled as result of glucose binding the boronic acid moiety. To improve on the selectivity of the polymer towards glucose over lactate interference, Lowe *et al.* designed two polymers based on different phenyl boronic acid moiety. They created a polymer containing a higher concentration of 3-acryamidophenylboronic acid, the

other having 2-acrylamido-5-fluorophenylboronic acid. Both showed increased selectivity over lactate at physiological conditions.

For the control of hyperglycemia and prevention of the resulting complications in diabetic patients, it would be highly desirable to develop a simple, continuous non- and/or minimally-invasive glucose sensor/insulin delivery system. Our group reported a unique hydrogel based on the Saarbrüken systems, which exhibit high glucose selectivity over other common blood sugars such as fructose and galactose. This PMMA-based polymer affords readily-seen glucose-promoted size changes in human blood plasma. [28] At that time (2006), there were no other examples reported of chemomechanical polymers which exhibited rapid and reversible response to glucose in human blood plasma. Polymers containing methyl methacrylate, methacrylic acid, or acrylic acid have been reported to recognize, protect, and stabilize biologically significant compounds such as sugars, proteins, and peptides and could be biocompatible as human implant sensor. [29] Important considerations in developing the recognitive polymer/actuator are based on:

- the generation of well-defined binding sites during/after polymerization by functionalizing with the relevant interaction sites and;
- making sure there is ease of diffusion of solutes into and out of the network by having enough crosslinker incorporated into the polymer.

The major issue in designing these polymers is based on the choice of the functionalizing monomers and the approach of introducing such binding sites. There are ways of polymerizing related polymers which include traditional polymerization,

molecular imprinting techniques and others. [27] In our relatively simple approach (originating in the Saarbruken methodology for related systems), we start with the already existing homopolymer (PMMA) as a substrate, and incorporate modifiers with desired binding sites. This approach allows ready tunability and ready optimization of the binding sites.

The polymers I have created swell and contract reversibly like human muscles, but depending on the local concentration of glucose. In medical applications, these polymers might someday be used as sensors as well as actuators for drug (e.g. insulin) delivery. Indeed, long-term goals of this project are to design a simple methodology to synthesize recognitive hydrogels that can be used to monitor glucose and/or deliver insulin. Boronic acids have been shown to interact covalently with cis-diols of glucose and so elicit a response which can be utilized in sensors. [30-35] However, the nature of such receptors can limit the ability of selective recognition towards glucose in the presence of other body sugars such as fructose and galactose which have cis-diols. Our research group has, for over a decade, extensively studied the properties of boronic acids and their ability to bind and detect sugars. [36]

2.2 Synthesis of a Chemomechanical Polymer

There is great interest in the development of new materials that function selectively for a specific intended purpose, such as glucose monitoring and/or drug release. Particularly promising has been the use of gels, in which swelling or shrinking can be induced by external stimuli. Such gels have been investigated as potential components with respect to many applications ranging from sensors to medical

devices. Due to interest in these gels, a methodology to easily design and introduce desired binding sites in these new materials is attractive.

In this work, I explored an approach in which already existing polymers are further modified by introducing new binding sites. In this approach, one can easily control the number and type of binding sites needed for each guest molecule to be introduced. I established that the chemical and mechanical properties of the polymer such as softness and hardness may vary with mode of preparation. Factors such as temperature, introduction of the modifiers into the parent polymer, and stirring speed during preparation greatly determine the properties of the polymer formed. The main result we obtained was control of parameters so as to obtain a reproducible polymer capable of selectively binding glucose in human plasma, which results in a macroscopic size change. This material is potentially applicable for developing an actuator for glucose-triggered insulin release.

2.2.1 Materials and Method:

All reagents were purchased from Sigma-Aldrich and used without further purification. The dimensions of cut polymers **A, B,** and **C,** and contraction factors were measured with a Leica MZ75 modular high-performance stereomicroscope (Leica Microsystems) or with a standard desktop ruler. Fourier-transform infrared spectra (FTIR) were acquired on a Tensor 27 Infrared Spectrophotometer (Bruker Optics Inc.). Solid state ^{13}C NMR spectra were acquired on a Bruker 100 MHz 3-channel solid-state instrument. The chemical reagents used were poly (methyl

methacrylate) PMMA: MW 350000, diethyline triamine, dodecyl amine, butylamine, 3aminophenylboronic acid, spermidine, and dimethyl sulfoxide (DMSO) as a solvent.

2.2.2 Experimental Procedure

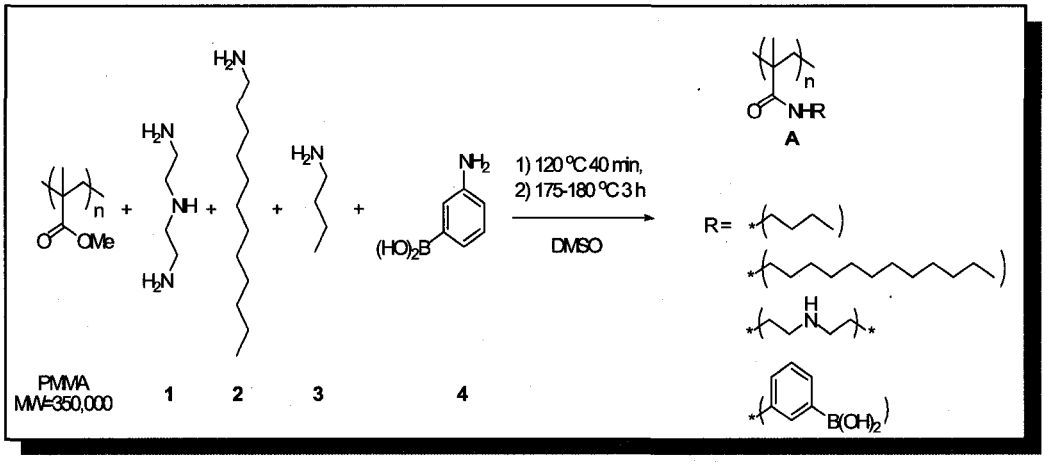
The chemomechanical polymer **A–G** (see Table **7**, pg 59) was synthesized via a method analogous to that of. Schneider but modified significantly to allow for glucose-induced macroscopic motions. [37] The polymer was prepared by crosslinking a homopolymer (PMMA: MW 350000) with diethylenetriamine (**2**), and the functional monomers dodecylamine (**3**), butylamine (**4**) and 3-aminophenylboronic acid (**5**) as shown in Scheme **1** below. Different modifiers were introduced into PMMA and in varying ratios to optimize properties. For instance, various amounts of the crosslinker, diethylenetriamine and spermadine, and side branch monomers dodecylamine, butylamine and the 3-aminophenylboronic acid receptor were used.

The well-mixed pregel solution was heated to a temperature of 120 °C for 2 h, then 175-180 ^{0}C for 1 h while stirring. PMMA (MW 350,000) 1.38 g, 3-aminophenylboronic acid monohydrate 0.7 g (4.5 mmol) and 20 mL anhydrous DMSO were placed into a 100 mL round-bottom flask. The reaction mixture was stirred and heated at 120 °C for 40 min. A mixture of 2 mL (1.9 g, 18.4 mmol) diethylenetriamine, 0.2 mL (0.1612 g, 0.87 mmol) dodecylamine and 0.4 mL (0.296 g, 4.04 mmol) *n*-butylamine was slowly added to the reaction mixture. The temperature was increased and maintained at 175-180 °C for 3 h until a thick gel formed. The gel was cooled and transferred into a 250 mL beaker and heated 12 h at 60 °C, followed by heating at 80 °C for 1h until a viscous solution is obtained. The viscous gel was

cooled to room temperature and transferred to a flat-bottomed flask. The flask and its contents are put in an oven at 60 °C at 10 mbar pressure overnight. The oven temperature was raised to 80 °C for 2 h. After cooling to room temperature, the solid polymer was washed several times with distilled H_2O.

The hydrogel is measured and cut into smaller sizes to be studied with several analytes using a microscope fitted with a digital camera.

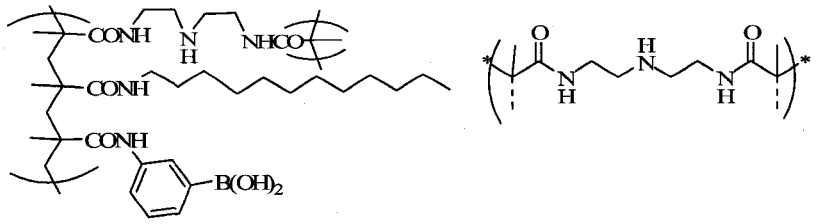
Scheme 1: Synthesis of Polymer **A**



An alternative preparation involved using higher temperatures (170 ^{0}C for 3 h). The polymer obtained from these conditions was harder while one prepared at a temperature of 120 ^{0}C for 2 h followed by 170 ^{0}C for 1 h was softer. The two different temperature conditions produced polymers which exhibited similar properties in our studies; however the soft polymer was relatively difficult to handle (e.g., cut).

The resulting polymers could have a structure which contained the following

Figure 7: Possible structure of the polymer

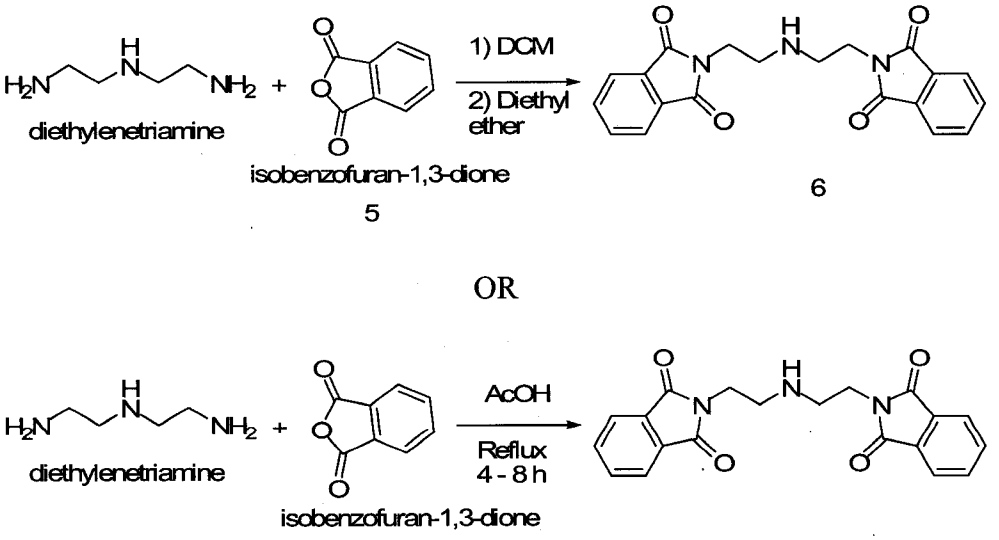


Other polymers (**B-G**) were synthesized using similar procedure as in polymer **A**, except the use of different modifiers. Polymer's **G** crosslinker and/or phenylboronic acid moiety was synthesized as outlined below by Delcros [38]

(i) Diethylenetriamine (2 ml; 46 mmol) was dissolved in 12.5 ml water and 6 N HCl added to raise the pH to 1.5. This was stirred to give a homogeneous solution and cooled on an ice bath. Phthalic anhydride (13.78 g; 93 mmol) was added followed by $NaHCO_3$ slowly until the effervescence stopped (at about pH 5.5). The precipitate formed was filtered and washed with water (2 x 4 ml), then dried.

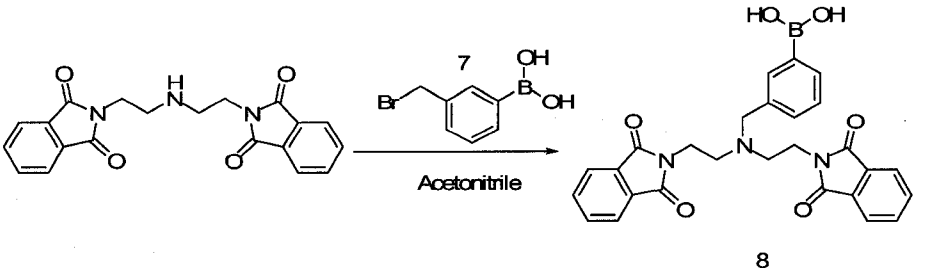
(ii) Aternatively, diethylenetriamine was dissolved in DCM and suspended in an ice bath while stirring for 15 minutes. A solution of isobenzofuran-1,3-dione (gm) in 40 ml DCM was added dropwise for 1 hour. The resulting solution was rotovaped and extracted into diethyl ether. The resulting solution was washed with dil. NaOH (4 x 10 ml), dried with anhydrous Na_2SO_4, and rotovaped to remove the solvent. Further purification was done with column chromatography with 0.5% conc. NH_4OH/Methanol as the eluting solvent. All fractions were combined and solvent removed.

Scheme 2: Protection of diethylenetriamine



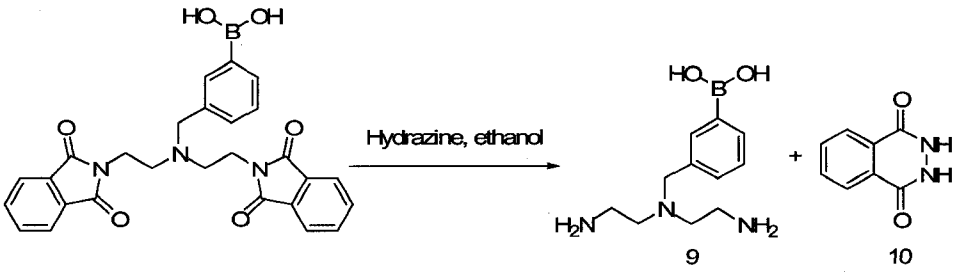
(iii) Reaction of a protected diethylenetriamine with 3-bromomethylphenyl boronic acid. Phthamide (1.008 g; 2.77 mmol) was added to 3-bromophenylboronic acid (0.524 g; 2.44 mmol) in acetonitrile (20 ml) and refluxed 15 h. the resulting solution was concentrated in a rotovap. The residue was dispersed in cold water (100 ml) and filtered. The water insoluble solid was extracted into diethyl ether and made alkaline by adding dil. NaOH while stirred for 1 hour at room temperature. The crystals were washed with EtOH and dried to give a yield of 79%.

Scheme 3: Reaction of a protected diethylenetriamine with 3-bromomethylphenyl boronic acid



(iv) Deprotection of phthalamide: A hydrazine solution (0.4 ml; 12.6 mmol) was added to phthalamide (0.94 g; 1.89 mmol) in refluxing ethanol (7 ml). The solution was refluxed for 3 h, and allowed to cool at room temperature. The precipitate formed was collected and recrystallized with ethanol to form a pure product. It was then dried in a vacuum pump overnight. Alternatively this was done using conc. HCl (12 M) and ethanol instead of hydrazine. Each gave yields of 76% and 62% respectively.

Scheme 4: Deprotection of crosslinker, **8**.



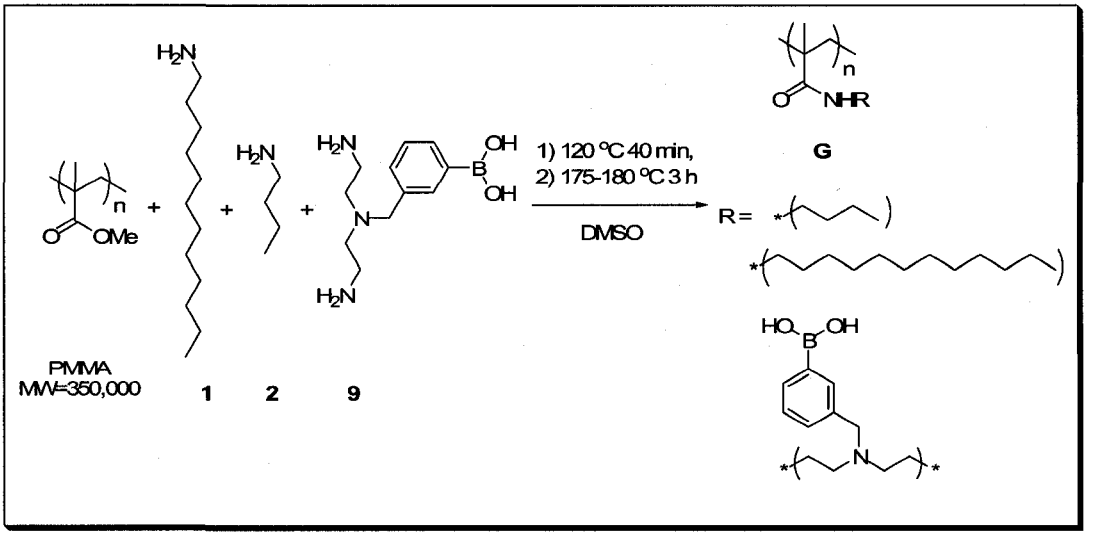
Synthesis of the polymer G

The polymer was prepared by crosslinking a homopolymer (PMMA: MW 350,000) with 3-((bis(2-aminoethyl)amino)methyl)phenylboronic acid (**9**), and the functional monomers dodecylamine (**3**), and butylamine (**4**) as shown in the Scheme **I** below. Different modifiers were introduced into PMMA with varied ratios as described in *Schneider's et al.* [29] Various amounts of the crosslinker, diethylenetriamine and spermadine, and side branch monomers dodecylamine, butylamine and the 3-aminophenylboronic acid receptor were used.

The well-mixed pregel solution was heated to a temperature of 120 for 2 h, then 175-180 ^{0}C for another 1 h while stirring. PMMA (MW 350,000) 1.38 g, 3-

aminophenylboronic acid monohydrate 0.7 g (4.5 mmol) and 20 mL anhydrous DMSO was placed into a 100 mL round-bottom flask. The reaction mixture was stirred and heated at 120 °C for 40 min. A mixture of 2 mL (1.9 g, 18.4 mmol) diethylenetriamine, 0.2 mL (0.1612 g, 0.87 mmol) dodecylamine and 0.4 mL (0.296 g, 4.04 mmol) *n*-butylamine was slowly added to the reaction mixture. The temperature was increased and maintained at 175-180 °C for another 3 h until a thick gel formed. The gel was cooled and transferred into a 250 mL beaker and heated 12 h at 60 °C, followed by heating at 80 °C for 1 h until a viscous solution was obtained. The viscous gel was cooled to room temperature and transferred to a flat-bottomed flask. The flask and its contents were put in an oven at 60 °C at 10 mbar pressure for overnight. The oven temperature was next raised to 80 °C for 2 h. After cooling to room temperature, the solid polymer was washed several times with distilled H_2O.

Scheme 5: Synthesis of Polymer **G**



Using the same procedure in modifying poly methyl methacrylate (PMMA), a number of polymers was formed from different modifiers. These different polymer structures are summarized in the Table **7**, pg 59.

2.3 Results and Discussion

2.3.1 Sample preparation

In the process of preparing the sample for contraction and expansion studies, the polymer was allowed to swell completely for several hours in water, buffer, or plasma before the studies. The completely swollen polymer was cut into pieces of 8 × 4 × 1 mm. These pieces were immersed in a solution of freshly prepared glucose, galactose, or fructose at room temperature. The measurement of the polymer was taken immediately, as the polymer started to contract, and recorded at time intervals of 5 min. This was carried out using a microscope fitted with a digital camera.

To study water uptake by the polymer, a dry polymer was immersed in pure distilled water and allowed to swell completely. The weight and size of the completely swollen polymer was determined and recorded as in Tables **1-3**.

Samples of polymer **A, B,** and **C** were placed in liquid media (distilled water, buffer or reconstituted human blood plasma) and allowed to swell for > 5 h. The completely swollen hydrogels were cut into rectangular pieces of 8 × 4 × 1.5 mm. The polymer was immersed in a fresh solution containing 0.005 M of sugar (glucose, galactose, or fructose). We used a range of glucose concentrations, but focused on 0.005 M of glucose, which corresponds to a normal level of glucose concentration in blood plasma of a normal person (4 - 8 mM). The change in size of the polymer in the sugar solutions was recorded at different times via pictures taken with the stereomicroscope.

The polymer was allowed to return to its original size by immersing in a 0.05 M NaOH solution for ≤ 5 min. The polymer was then washed thoroughly with water and put into the respective liquid media (distilled water or plasma) for another run. The lengths registered were used to calculate the contraction factors.

$$\text{Contraction Factor} = \frac{\text{Length after sugar addition}}{\text{Initial Length}}$$

Polymers **A** and **C** were allowed to swell completely for several hours in water, and then cut into pieces of 8 × 4 × 1 mm. The cut polymer was immersed in a solution of glucose (0.005 M, approximating actual human physiological levels) and began to contract immediately. A significant size change was observed over time (see Figure

10, pg 48). Polymer **B** was prepared under the conditions similar to those described for **C**, but in the absence of 3-aminophenylboronic acid (5). No size change was observed for polymer **B** after immersion in glucose solution for 50 min (see Figure **11**, pg 48). This indicated that the glucose-responsive behavior was due to the boronic acid moiety.

2.3.2 Characterization of the Polymer A

Polymer **A** was characterized by elemental analysis, solid-state ^{13}C CP/MAS NMR and FTIR. The IR spectrum of polymer **A** shows an N-H stretch in the range 3400-3000 cm^{-1}, which is not present in the IR spectrum of PMMA. Along with the decrease of the signal corresponding to the PMMA ester carbonyl, new carbonyl absorption appears at 1655 cm^{-1}, along with small peaks at 1695 and 1537 cm^{-1} which is probably corresponding to N-H bend. The 100 MHz solid state 13C CP/MAS spectrum of polymer **A** exhibits a resonance at 177.5 ppm, revealing the presence of the amide carbonyl carbon (the PMMA ester carbonyl carbon resonates at 184.5 ppm).

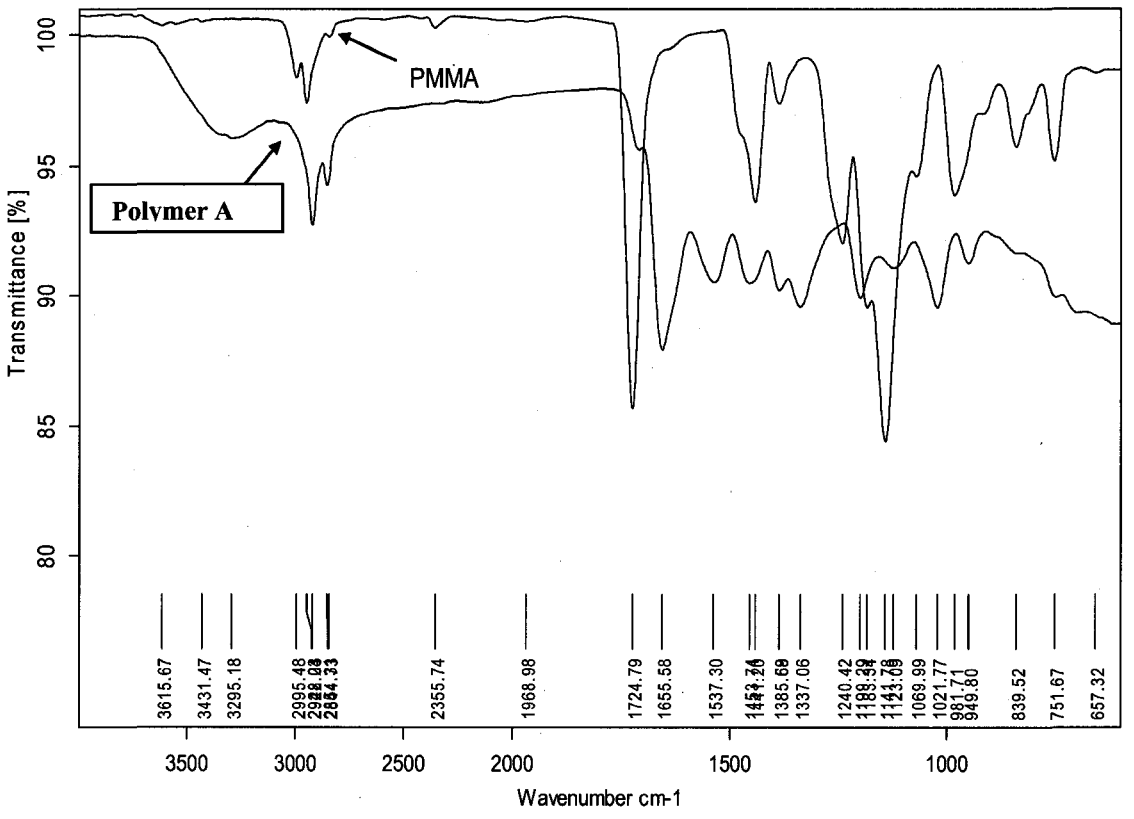
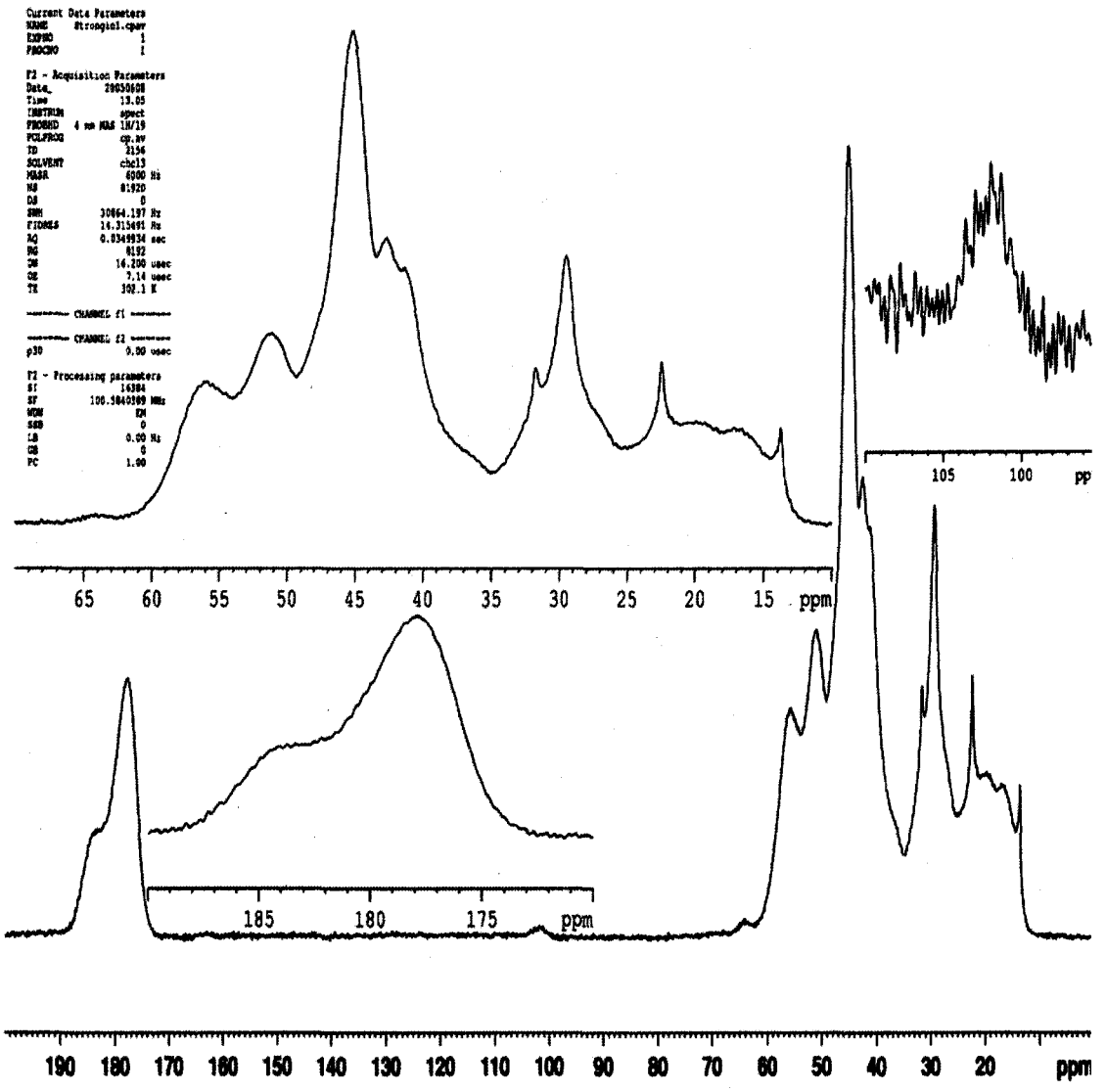


Figure 8: *FT-IR spectra of PMMA and Polymer **A**. N-H stretch is observed in the range 3400-3000 cm^{-1}. Also there was a decrease in the PMMA ester carbonyl and new absorption band at 1655 cm-1 which corresponds to an amide (C=O, amide stretch).* (*see Angewandte Chemie International Edition,* **2006**, *45,* 5319)



Solid State -13C CP/MAS Spectrum of Polymer A

Figure 9: *Polymer A exhibits a resonance at 177.5 ppm, confirming the presence of the amide carbonyl carbon (the PMMA ester carbonyl carbon resonates at 184.5 ppm). see Angewandte Chemie International Edition,* **2006**, *45,* 5319

c) Elemental Analysis of Polymer A.

A sample of polymer **A** was pulverized by using a mortar. The pulverized polymer was dried under vacuum for 3 days. The dry sample was sent to Baron Consulting Co. (Milford, Connecticut) for elemental analysis of C, H, N and B. The results are shown in Table **1**.

Table 1: Elemental Analysis of Polymer **A**

Parameter	Estimated	Result
% C	55.20	52.23
% H	11.00	8.13
% N	20.00.	9.19
% B	1.50 – 2.00	0.20

From these results, the presence of the boronic acid moiety is confirmed. The estimated values were calculated based on 100% insertion of the modifiers. 0.2% of boron indicates that at least 13% of the boronic acid moiety was introduced into the polymer network.

2.3.3 Sample Studies

Polymer **C** was allowed to swell completely for several hours in water, then cut into pieces of 8 × 4 × 1 mm. The cut polymer was then immersed in a solution of glucose (0.005 M, approximating human physiological levels) and began to contract immediately. A significant size change was observed over time (Figure **10**). Polymer **B** was prepared under conditions identical to those described for **C**, but in the absence of 3-aminophenylboronic acid (5). No size change was observed for polymer **B** after immersion in glucose solution for 50 min (Figure **11**). This indicated that the glucose-responsive behavior was due to the boronic acid moiety.

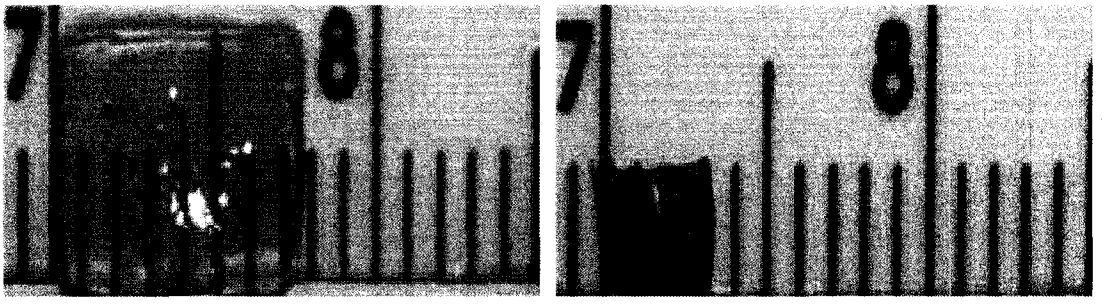


Figure 10: *Polymer **C** containing boronic acid. Left: after 50 min in distilled water. Right: after 50 min in 0.005M glucose*

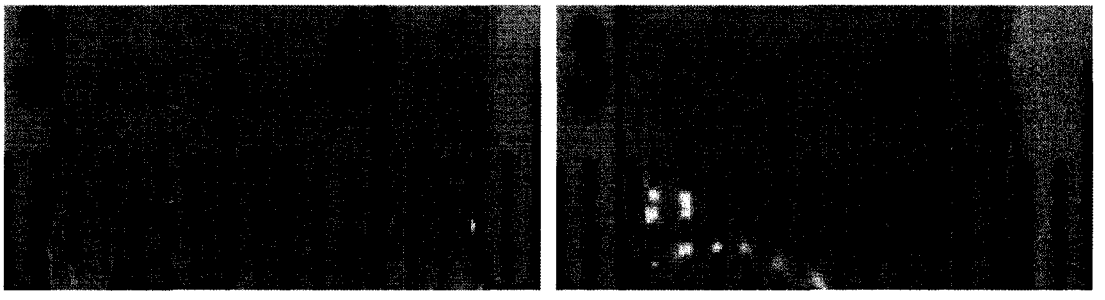


Figure 11: *Similar Polymer **B** without boronic acid. Left: after several hours in distilled water. Right: after 50 min in 0.005M glucose*

Polymer **C** also exhibited reversibility. It swelled to its original size within two minutes after immersion in a 0.05 M NaOH solution. After removal from the NaOH solution and washing with distilled water, the swollen polymer contracted once more when immersed again in a 0.005 M glucose solution (Figure **12**).

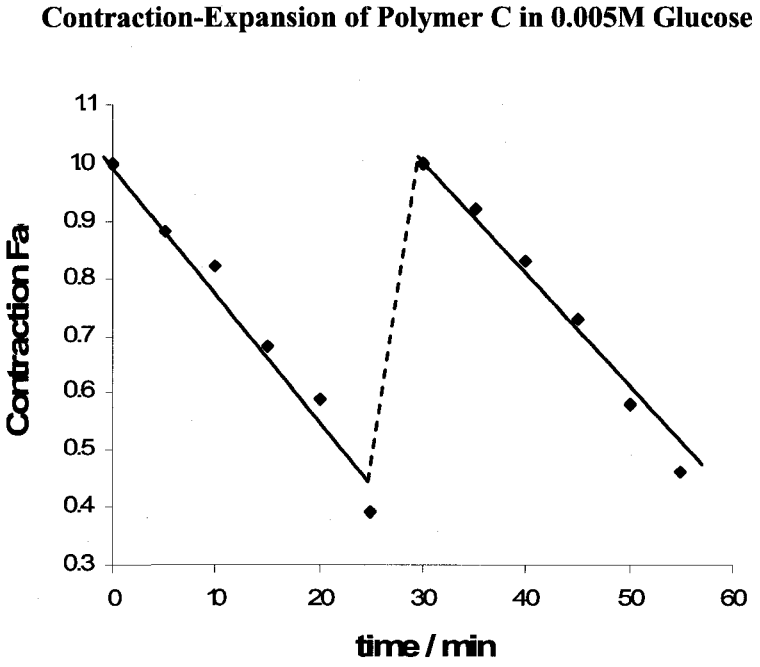


Figure 12: *Contraction-expansion of polymer **C** in response to glucose (0.005 M). The polymer was expanded to its initial size between runs by rinsing with 0.05 M NaOH (dashed line). The size of the cut polymer after swelling in water in this experiment is 5 × 5 × 1 mm.*

Further studies also show that polymer **C** was selective for glucose over the other two common blood sugars, fructose and galactose (Figure **13**, pg 51). The error bars in Figure **14** indicate the reproducibility of the glucose-responsive contraction of polymer **C**. Three different batches of polymer **C** were tested on three different days.

Table 2: *Contraction-expansion of Polymer **C** in response to glucose (0.005 M). The polymer was expanded to its initial size between runs by rinsing with 0.05M NaOH*

Time (min)	Contraction Factor
0	1.00
5	0.88
10	0.82
15	0.68
20	0.59
25	0.39
30	1.00
35	0.92
40	0.83
45	0.73
50	0.58
55	0.46

Table 3: *Selective response of Polymer **C** to glucose over fructose and galactose. The three sugars are at the same concentration (0.005 M)*

Time (min)	Contraction Factor		
	Fructose	Glucose	Galactose
0	1.00	1.00	1.00
5	1.01	0.96	1.00
10	1.04	0.94	0.99
15	1.01	0.91	0.98
20	1.02	0.88	0.97
25	0.99	0.83	0.97
30	1.00	0.79	0.96
35	0.99	0.74	0.95
40	0.99	0.67	0.94

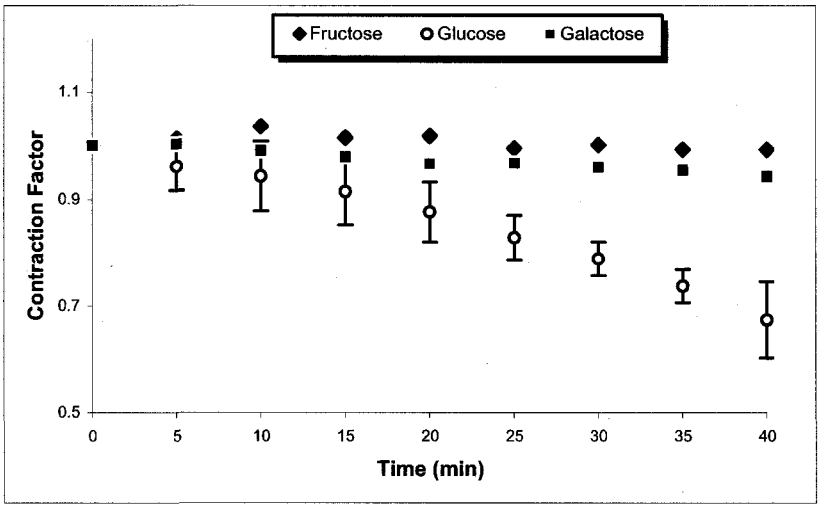


Figure 13: *Selective response of polymer* ***C*** *to glucose over fructose and galactose. All the three sugars are at the same concentration (0.005 M). The size of the cut polymer after swelling in water in this experiment is 8 × 4 × 1 mm.*

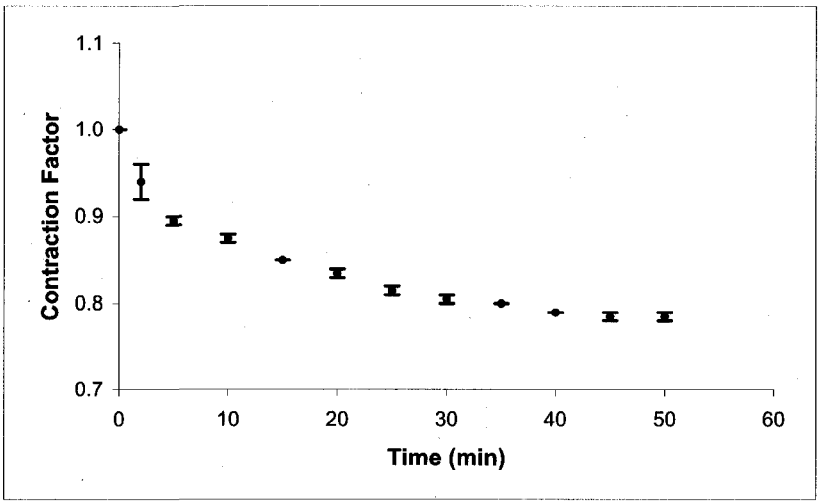


Figure 14: *Contraction of polymer* ***C*** *in the presence of reconstituted human plasma containing 0.005 M glucose. The size of the cut polymer after swelling in water in this experiment was 8 × 7 × 1 mm.*

Using a commercial sample of human blood plasma, similar glucose responsiveness and reproducibility were observed. A piece of swollen polymer **C** was

immersed in a solution of human blood plasma which contained 0.005 M added glucose. The polymer contracted sharply within 5-10 min (Figures **7** and **8**, pg 49). Boronic acid–glucose binding in aqeous and non-aqeous media has been reported that it is: promoted by strong and reversible covalent interactions, and accompanied by negative charge accumulation on boron (Figure **15**). The charge accumulation, in the case of a polymeric gel, will render it more hydrophilic and thus induce some swelling. Thus, the boronic acid moiety, during the synthesis of the polymer network, if controlled could promote selective volume change towards particular sugar. In the cyclic complex (boronate ester), sp^2 hybridized boronic acid are easily converted into a charged sp^3 hybridized anion during the binding.

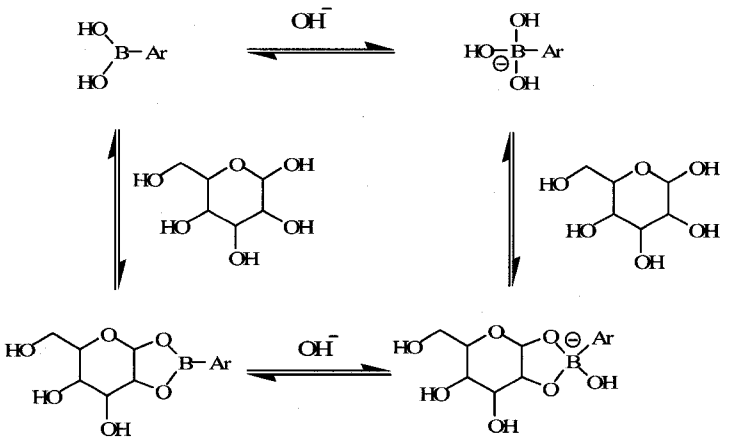


Figure 15. *Saccharide-induced hybridization. (See M. Takeuchi, S. Yoda, T. Imada, S. Shinkai. Tetrahedron 1997, 53:8335)*

Boronic acid binds preferentially with furanose form of sugars rather than the pyranose form to form cyclic boronate ester. Glucofuranose has been reported to bind boronic acid in a bindentate manner, while galctofuranose and fructofuranose forms a tridentate bind as shown in the Figure **16.**

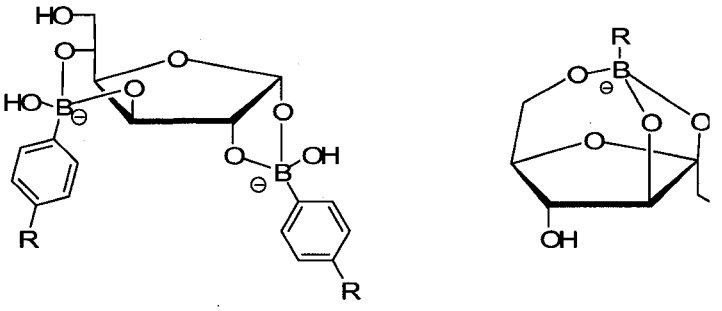


Figure 16: *Schematic diagram of glucofuranose and fructofuranose bidentate and tridentate binding to boronic acid respectively. (see J. C. Norrild, H. Eggert. J. Am. Chem. Soc. 1995, 117:1479)*

Bidentate binding of glucose by two boronic acid moieties is known to occur in certain cases selectively over galactose and fructose.[39] The binding of boronic acid to glucose, galactose, and fructose are predicted from their furanose structures (Figure **17)**

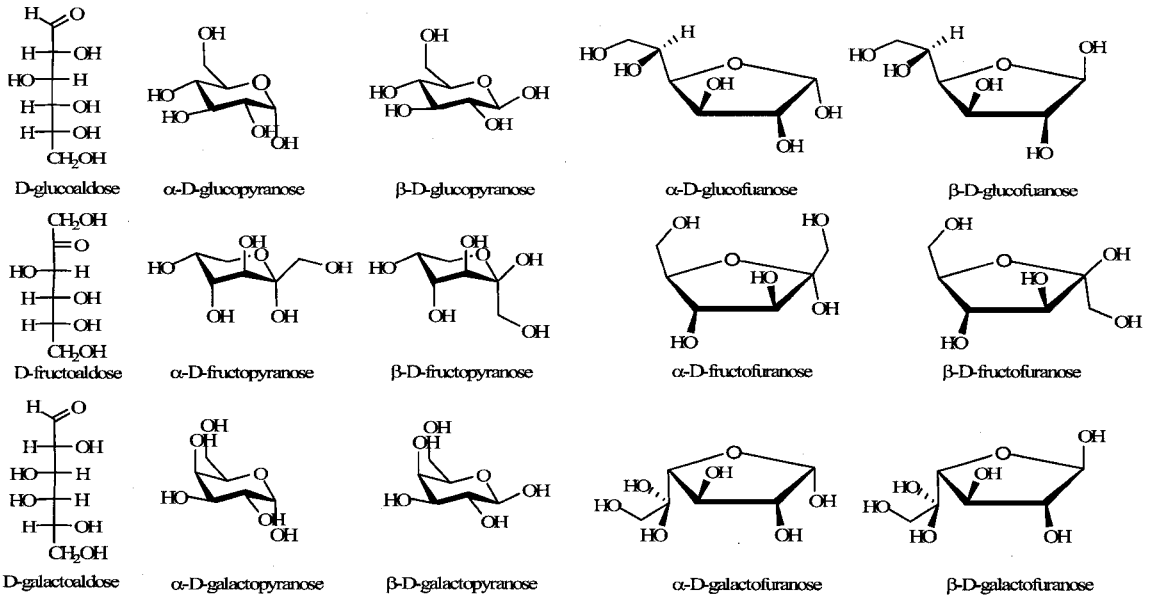


Figure **17:** *Structures of glucose, galactose and fructose in their pyranose and furanose forms*

Shrinkage has been observed previously in another example of glucose-induced cross-linkage of boronic acid-containing polymers. [40] In our system, the glucose molecule may also act as an additional crosslink (besides the triamine). This results in the expulsion of water and gel shrinkage (Scheme **6** and Figure **18**).

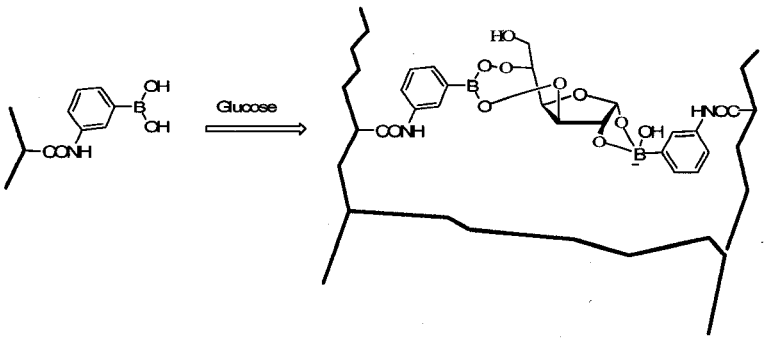
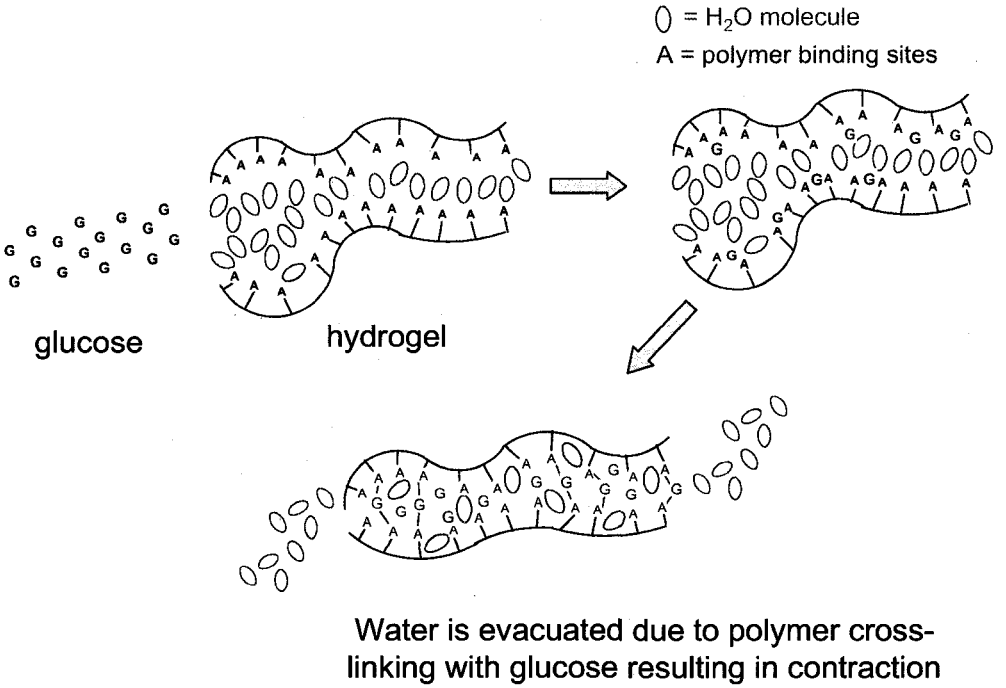


Figure 18. Schematic of a chemical cross-linker when glucose binds two boronic acids. The binding will results in selective shrinkage for glucose over galactose and fructose.



Scheme 6. Mechanism for the contraction of polymer **C** hydrogel in response to a glucose solution.

Additionally, sugar binding-induced H^+ release has been used previously by Arnold *et al.* (for signal transduction) in a boronic acid-containing hydrogel sensor for glucose. [41] In an analogous manner, we propose that the presence of emerging cationic charge from water and glucose bound to boron should stabilize the anionic nature of glucose-bound boron, thereby reducing the electrostatic repulsion that would lead to swelling. This may serve as an additional and/or alternative mechanism to glucose cross-linking via bidentate binding to boron.

Conversely, treatment with hydroxide may deprotonate the amino groups on the polymer and could also add to boron. The concomitant increase in anionic charges would enhance electrostatic repulsion in the hydrogel, causing it to swell back very nearly to its initial size. [42]

Kataoka and co-workers have reported innovative strategies towards synthetic hydrogels, proposed as glucose detection/self-regulated insulin delivery systems. [43-47] Polymer sensitivity was heightened via rational design of the co-polymer structure. In their material, as well as in most other boronic acid-containing hydrogels reported to date, the hydrogels were prepared via the polymerization of boronic acid-containing monomers. In contrast, our method for the preparation of glucose-responsive hydrogels involves simple modification of commercial PMMA to achieve glucose selectivity as well as a more rapid equilibration time. In addition, the type of macroscopic response (shrinkage or expansion) and the selectivity to a certain stimulus of the hydrogels can be controlled simply by varying modifiers (Table **4**, pg 56).

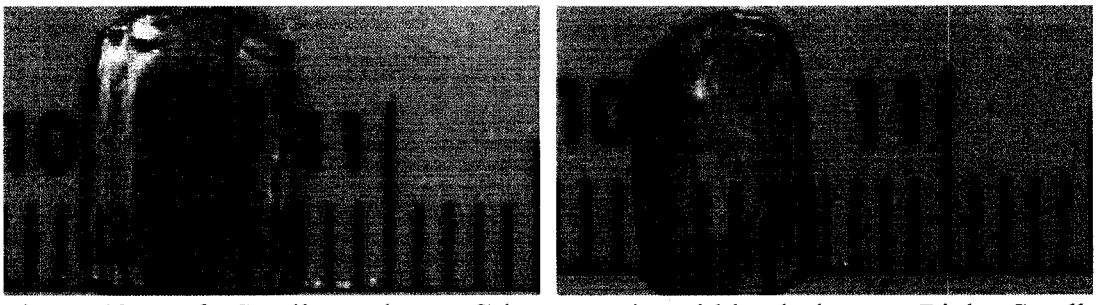
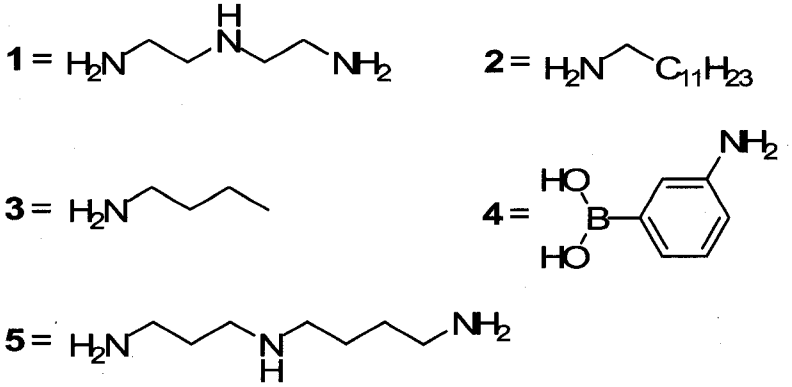


Figure **19**. Left: Swollen polymer **C** in reconstituted blood plasma. Right: Swollen polymer **C** in reconstituted blood plasma 5 min after addition of 0.005 M glucose.

Table 4: Polymer Response and Selectivity with different modifiers used

Entry	Polymer Modifiers	Contraction Factor		
		0.005M Glucose	0.005M Galactose	0.005M Fructose
1	5 + 2 + 4	1.7	1.61	1.5
2	1 + 2 + 4	1.22	1.25	1.31
3	1 + 3 + 4	0.63	0.96	0.93
4	1 + 2 + 3 + 4	0.83	0.99	0.97



[a] The size of cut polymers was 8 × 4 × 1 mm. Contraction factors were obtained after 25 min

Length of Side Chain

When the polymer was modified with the long alkyl chain amine, dodecylamine (modifier compound 2, Scheme **1**, pg 37), it exhibited expansion in all three sugar solutions (Table **4**, entries 1 and 2). This may be attributed to steric hindrance of the alkyl chain towards bidentate binding of glucose by two boronic acid moieties. Thus, the binding of sugar molecules enhanced the formation of charge on boron atoms and increased the polarity and water-solubility of the polymer, resulting in polymer expansion rather than contraction, as cross-linking was suppressed.

In the case of butylamine (modifier compound **3**, Scheme **1**), the smaller butyl chain apparently did not significantly hinder bidentate binding of glucose. The crosslinking of polymer chains by glucose molecules could thereby overcome the effect of charge accumulation (*vide supra*), resulting in gel contraction (Table **4**, entry **3**). Additionally, if a mixture of dodecylamine and butylamine used (in half the original amounts each), as in polymer **A** (Table **4**, entry 4), the gel contracted, but to a lesser extent than when incorporating butylamine alone as expected. This afforded additional evidence that alkyl chain length can control crosslinking and contraction.

Length of Crosslinker

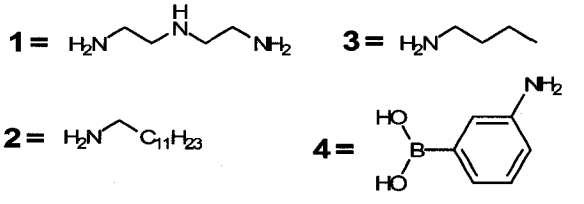
A significant difference in size change was observed when the length of the cross-linker was varied (Table **4**, entries 1 and 2, pg 56). In the presence of the same alkyl amine (dodecyl), the hydrogel with the longer carbon chain cross-linker (modifier 5, Scheme **1**, pg 37) exhibited a greater degree of expansion as compared to the case

wherein the shorter carbon chain cross-linker (1) was used. This is attributed to the fact that longer chain cross-linkers provide wider cavities for the uptake of water.

Table 5: Optimized Polymer **C** vs Polymer **A**

Entry	Modification Reagent	Glucose	Galactose	Fructose
3	1 + 3 + 4	0.63	0.96	0.93
4	1 + 2 + 3 + 4	0.83	0.99	0.97

Polymer **C** Polymer **A**



An optimized polymer **C** (Table **5**, entry 3) exhibited the highest selective contraction in the presence of glucose. The polymer was studied with a varied concentration of the glucose. The polymer contracted faster and to a higher degree with higher concentration of glucose. Importantly, we found that the contraction factor of polymer **C** exhibits a linear decrease proportional to the increasing glucose as shown (Figure **20**, pg **59** and Table **5**).

Table 6: Contraction of polymer **C** in the presence of reconstituted human plasma containing 0.005M glucose

Time (min)	Contraction Factor	Error
0	1.00	0.000
2	0.94	0.020
5	0.90	0.005
10	0.88	0.005
15	0.85	0.000
20	0.84	0.005
25	0.82	0.005
30	0.81	0.005
35	0.80	0.000
40	0.79	0.000
45	0.79	0.005
50	0.79	0.005

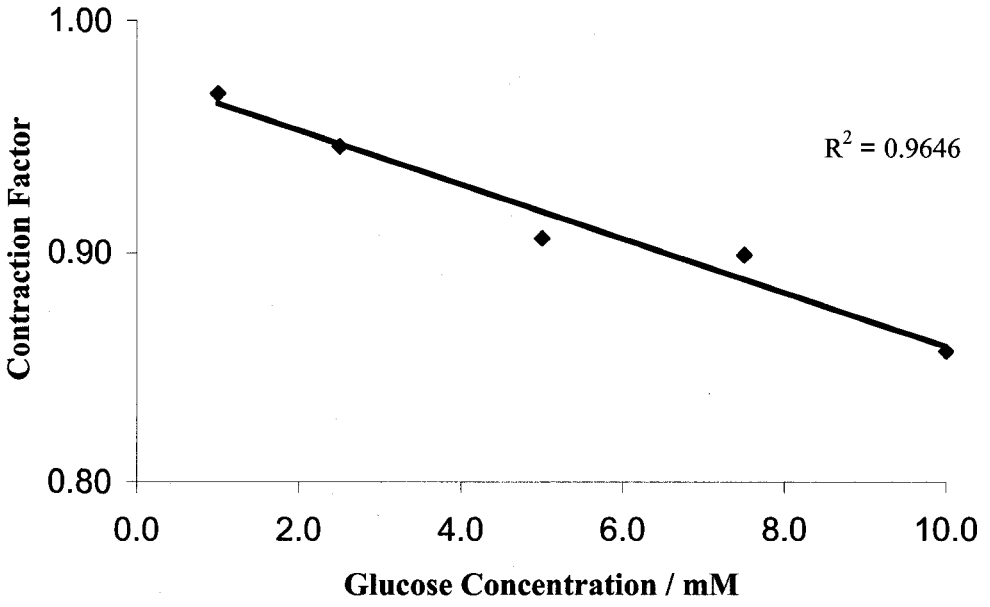
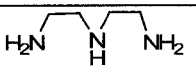
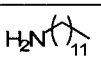
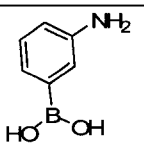
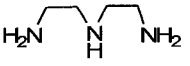
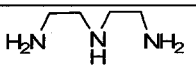
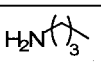
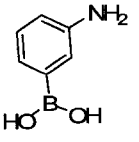
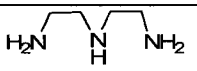
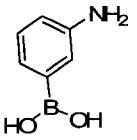
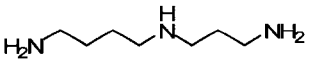
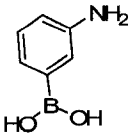
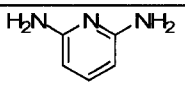
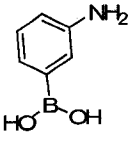
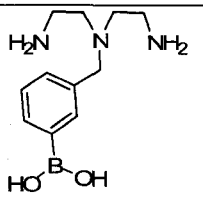
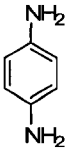


Figure **20:** Contraction factor of polymer **C** in the presence of various concentrations of glucose at 25 ^{0}C. The values were the average of three consecutive determinations after 20 min. The size of the cut polymer after swelling in water in this experiment was 14×7×1 mm.

Table 7. Summary of polymers from different modifiers (monomers)

Polymer type	Modifiers	Remarks
Polymer A		Swollen polymer continues to swell when the three sugars was introduced (see figure **19**)
		
		
		
Polymer B		No contraction or swelling when glucose, fructose and galactose are introduced to swollen polymer B (see Fig **5**.)
		
Polymer C		The swollen polymer contracted greatly when glucose was introduced, but no or slight contraction with fructose and galactose.
		
		

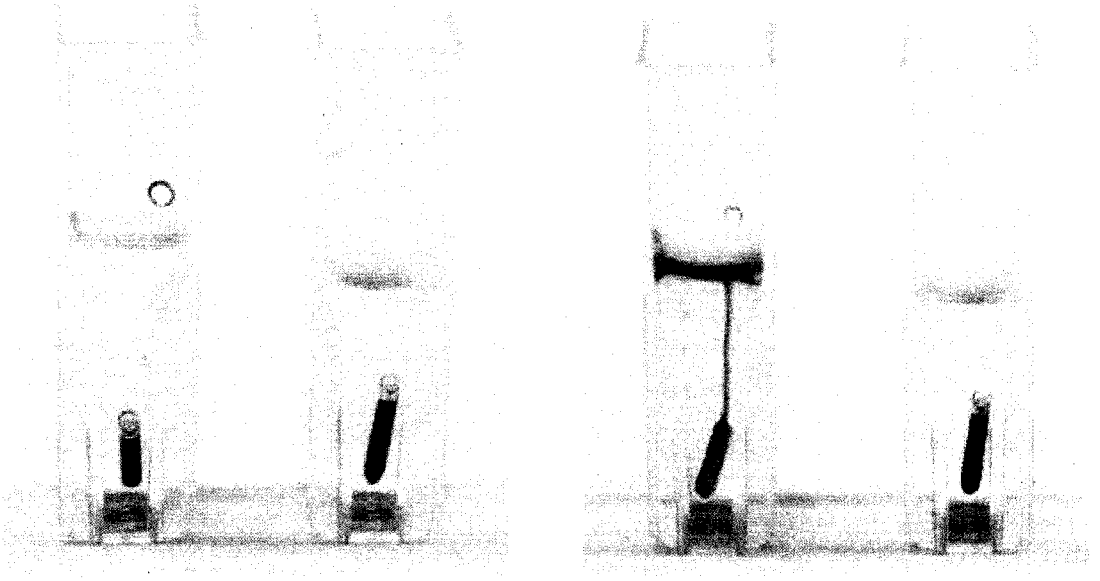
Polymer D	  	Swollen polymer D further expanded when glucose, galactose and fructose was introduced.
Polymer E	  	Swollen polymer C expand more when glucose, fructose and galactose are introduced
Polymer F	  	Formed a hard solid material which could neither swell nor contract

Polymer G		No polymer was formed
		
Polymer H		No polymer formed
		

2.4 Future Plans

Introduction. Chemomechanical polymers analogous to the ones described herein might be used in applications such as: i) glucose sensors, ii) drug delivery devices and iii) self-regulating valves in micro-fluidic systems, etc. They could thus be used as artificial valves to control the flow of fluids, as in both Figure **21** and illustrated in Figure **22** (see pg 63 and 64). They can thus embody "chemical corkscrews". In the swollen state, the polymer would close the valve and stop the flow of the fluid, and after contraction triggered (in the current case) by glucose, allow fluid flow. In the present example, polymer **C**, for instance, changed size in a glucose concentration-dependent, reversible, and reproducible manner. Such features are a start in designing a useful material for the continuous monitoring of glucose level in

blood plasma. As demonstrated in Figure **21**, using polymer **C** as a seal for a capillary containing a dye solution, the dye was released upon contact with glucose solution. This type of system (after a great deal of further research, *vide infra*) may someday lead to an artificial pancreas (insulin delivery system). Insulin in such a scenario would diffuse from an insulin reservoir into the blood system in response to the glucose level. This methodology may thus promote the eventual optimization of self-regulating actuators that can work without interfacing to external devices.[27]



0 Minute **25 Minutes**

Figure 21: *A demonstration using polymer C in glucose-regulated chemical delivery. A capillary tube is filled with ink and sealed with swollen polymer C. The capillary is immersed in H_2O (control, cuvette on the left in each picture) and in* 0.005 M *glucose solution. After 25 min, the ink is released due to the contraction of the polymer, only in the glucose solution (far right).*

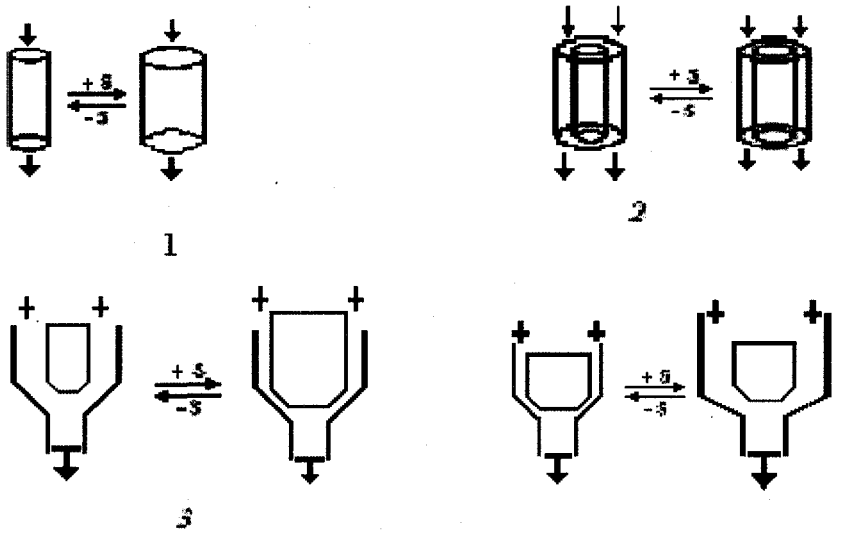


Figure 22: *Schematic representation of the microfluidic system with the polymer to control the flow of fluids by controlling the closing and opening of the valve*

Future Research Plans

Many challenges besides simply achieving glucose selectivity remain before self-regulated insulin delivery systems can be realized. These include:

In vitro biocompatibility testing

There has not been a single test of *in vitro* testing that has accurately predicted the *in vivo* performance of a glucose sensor, although the International Organization

for Standar (ISO) and American Society for Testing and Materials (ASTM) require it. *In vitro* biocompatibility studies encompass: i) cytotoxicity testing; ii) sensor performance demonstartion; iii) low molecular weight and interfering substance screen; iv) temperature; v) stability; vi) oxygen partial pressure; and vii) hydrodynamics.

Cytotoxicity testing

For our future plans, we will first design a protocol for testing the toxicity of our polymer, as needed for any biocompatibility test. We will use tissue culture as it is reported to be more sensitive to toxins than animal models. [48-50] The biological matrices (for the test e.g. tissue cells) will be exposed to our polymer for several days and studies will be done to establish if there are leachable toxic substances in the material and the residue from polymer's starting material.

Sensor performance testing

When characterizing the performance of the sensor, the following parameters could be the focus.

i) *sensitivity of the polymer.* Here we will concentrate on improving the receptor/binding sites. The ratio of the modifiers has to be optimized, and further downsizing of the polymer particles to increase the surface area to volume ratio;

ii) *response time. In vitro* respond time should be extremely rapid to as to compensate for the loss of sensitivity when the material is implanted. It has been reported that response times in *in vitro* testing

are quite higher than *in vivo*. The testing of various sensors both *in vitro* and *in vivo* using different acceptable methods must be done on biological matrices and in animal models. The results were compared as shown in the Table **8** below, and we will apply such a protocol to our materials. [51]

Table 8: Comparison of in vitro and in vivo performance of glucose oxidase-based glucose sensors using different test species and different in vivo testing periods

Species	Implantation site	Sensitivity (nA/mmol		Background current (nA)		Responsive Time	
		In vitro	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*
Rats after 3 days [66]	Interscapular subcutaneous tissue	1.7±0.2	0.5±0.1	1.9±0.4	5.8±1.4	190±0.4 s (T90%)[a]	< 300 s
Dogs after 36 h [41]	Neck subcutaneous tissue	1.7±0.7	1.6±0.8	1.4±0.4	1.3±0.5	< 300 s (T95%)[b]	Not provided
Humans after 7 h [43]	Abdominal subcutaneous tissue	0.7±0.3	0.2±0.1	0.3±0.1	1.1±0.1	24 s (T90%)[a]	Not provided
Dogs after 10 days [74]	Interscapular subcutaneous tissue	~6	~3	Not provided	Not provided	Not provided	Not provided
Humans after 2 h [44]	Abdominal subcutaneous tissue	0.4±0.1	0.1±0.02	0.47±0.2	0.9±0.3	290±110 s	580±290 s

[a]T90% refers to the time required to reach 90% of the in vitro steady-state response after a step change in bulk glucose concentration. [b]T95% refers to the time required to reach 95% of the in vitro steady-state response after a step change in bulk glucose concentration. (see H. E. Koschwanez, W. M. Reichert. *Biomaterials*, **2007,** *28*, 3687-3703

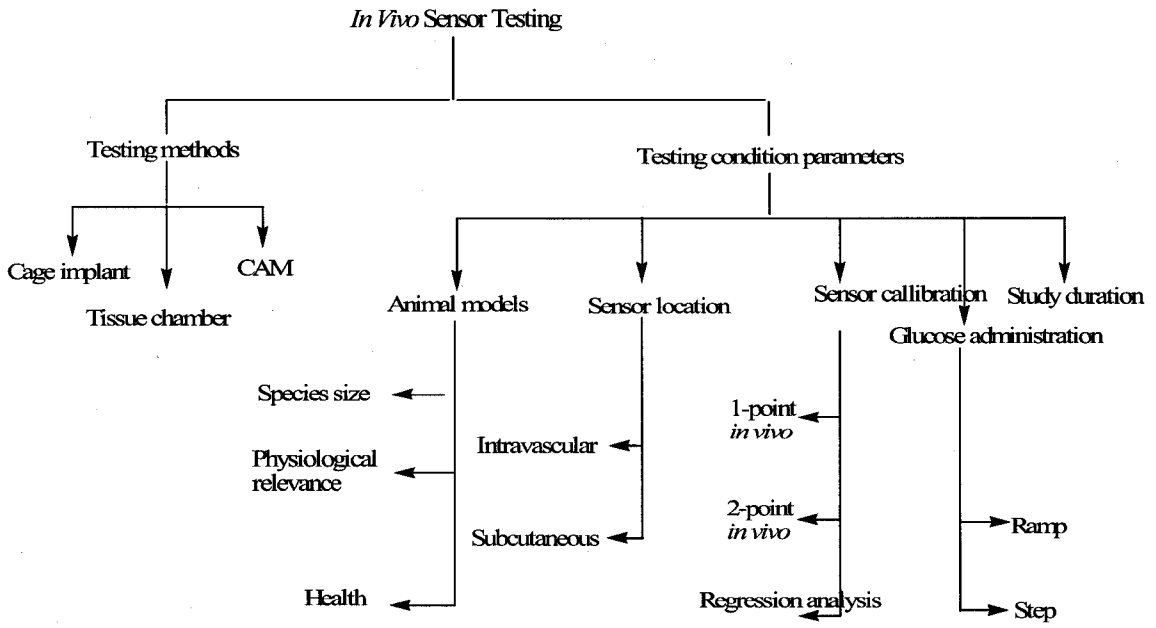
iii) *temperature.* The calibration of the sensor would be done at the physiological temperature of 37 ^{0}C. The temperature effects on the

sensor should be investigated because in subcutaneous media there would be some degree of temperature fluctuations daily. In our temperature studies, we will try to account for *in vivo* inflammatory, fever, exercise, etc. which are parameters that can affect *in vivo* temperature;

iv) *stability.* While testing the stability of our polymer material, we will focus mainly the stability of the polymer associated with storage. We will used the accepted methods which have been reported by Brindra *et al.,* [52] and Schemidtke *et al.* [53] The polymer should be stored with a glucose concentration 4-6 mM (normal blood glucose level) for many days, and each day the sensor should be calibrated to determine the sensitivity, response time, detection limit and the linearity with respect to glucose concentration.

***In vivo* biocompatibility testing**

In testing the biocompatibility *in vivo* of our polymer, we will base our plan on an accepted, reported technique:



Biomaterials, **2007**, *28*, 3687

Scheme 7. Basic organization of *in vivo* sensor testing

i) cage implant method. This testing will be used in our initial stage before advancing to animal models. *In vivo,* a stainless steel cage having the polymer material inserted inside would be immersed into the biological matrices (cells, enzymes, glucose etc). This method will be used to test the inflammatory response to the polymer material. [54,55] The contents which diffuse to the mesh (cells, secreted enzymes, fibrous encapsulation, glucose etc) from the biological subjects would be removed by a syringe and examined for inflammatory cells, enzyme secretion, cell-material interactions such as fibrous encapsulation and cell infection [56,57] and glucose diffusion. [58] These activities, which include antibody immune responses against the polymer material would affect its sensitivity. [59] Many suspected interferents from biological matrices would need to be investigated.

ii) animal models. This *in vivo* method could be done after the material (sensor) has been implanted subcutaneously. This method is used to establish the safety, efficacy, and biocompatibility of the material to the body. [60] Standard animal tests have been done on variety of animals such as dogs, [61-63] rabbits, rats, [64] and human beings. [61] While performing the analogous tests, our focus will be on the animal size, health, cost and availability etc.

In our future work to tackle the biocompatibility issue, we will investigate and confirm the already existing facts about biocompatibility of poly(methyl methacrylate) and other related methacrylates. This will entail investigation of reproducibility and toxicity of PMMA when impregnate with some proteins such as collagen and compare them with reported information. These results will be analyzed to establish if it is reproducible and the methodology used is acceptable.

Methacrylate containining monomers (e.g. methyl methacrylate, ethyl methacrylate, n-butyl methacrylate, isobutyl methacrylate, 2-ethylhexyl methacrylate, cyclohexyl methacrylate, benzyl methacrylate, morpholinoethyl methacrylate, tetrahydrofurfuryl methacrylate etc.) have been reported to form a biocompatible composite material with proteins, and used as implants in many applications. In our approach in determining biocompatibility, we will follow the procedure in a previous patent where the polymerized material is impregnated with collagen allowing the fine fiber of the collagen to be on the surface of the composite. We will investigate the properties, optimize to obtain reproducible data. All of this has to be done in collaboration with individuals in the respective fields (bioengineering and medicine)

- *Sensor calibration in biological matrices.*

In the future, we will focus on quantifying the measurements of the swelling in correlation with the concentration of glucose. In our prior work we have managed to establish the relationship as concentration dependent as shown in Figure **20,** pg 59. This will be done in biological matrices. The results from these measurements will be used to design a calibration model of an artificial pancreas etc.

In calibrating our polymer material/sensor, first *in vitro* testing will be performed before calibrating it *in vivo (see above).* The differences would be investigated.

- *Optimization of response time*

In collaboration with other researchers we will design a method of measuring and quantifying this hydrogel's swelling and contracting. The method will optimize and improve the response time of our material towards glucose concentration as low as 2 mmol. This will be done by downsizing the polymer to increase its surface are to volume ratio, and expose more binding sites to surface for more interaction. We will achieve this by:

Spin coating approach. This will create thin films of the polymer on a flat surfarce e.g. a thin film of thickness of 9 nm. This will be controlled to achieve a film of desired thickness. Each film thickness will be studied and results used to correlated with the glucose concentration, which will be eventually transposed to biological subjects

Grind dried polymer 3 to a nancomposite material. This will be investigated to establish if this enhances efficiency and sensitivity of the polymer. A model will be

designed and developed in which ground polymer **3** will be packed into a tubule and several samples used to illustrate the delivery phenomenon. Finally real insulin could be used to see if it is delivered when triggered by glucose of varied concentration. [65]

Direct nanoimprinting lithography (NIL). This technique allows the fabrication of materials into two or three dimensional structures. [66] This patterns or modifies a functional material, especially materials with low viscosities. Low viscosity materials can be either thermoplastics e.g. PMMA or thermostat polymers. [67] UV-curable polymers. [68, 69] The actual method involves the pressing of a mold with nanostructures to deform and shape materials into thin films which are deposited onto a substrate material. [70, 71] The imprinted materials are heated until they are liquid or viscous. Other methods are also available to render the material micro- or nanoscale.

- and the material's lifetime

In order to optimize the performance of our new material, we will perform periodic testing to predict its functional lifetime. The laboratory testing will include reproducibility, reversibility and selectivity of the material over a period of time. All the data obtained will be subjected to detailed statistical analysis to determine the significance of the variation and consistency of performance. These data will guide us in improving the lifetime of material via structural changes, in collaboration with bioengineers and materials scientists.

2.5 Conclusion

In summary, the general synthetic procedure developed by Schneider for synthesizeing chemomechanical polymers is highly useful as the recognition groups can be readily introduced and the properties of the polymer can be easily optimized. [23a] Current efforts involve further optimizing hydrogel properties via side-chain modification and miniaturization, which is known to enhance the performance of related materials. [23b] Many challenges besides achieving glucose selectivity remain before a self-regulated insulin delivery system can be implemented. [17] These include response time, biocompatibility, calibration and the material's lifetime.

Table 9. Reversible swelling and contraction of polymer C versus glucose concentration in water (PMMA, 3-aminophenylboronic acid, diethylenetriamine, n-butylamine)

Concentration (mmol)	Cyce 1				Cycle 2 (after first contraction)		
	Time	0	30		Time	0	30
10 mmol/L	Length	4.0	4.0		Length	9	4.0
	Width	2.0	2.0	Wash with NaOH and rinse with water	Width	5	2.5
15 mmol/L	Length	6.0	3.8		Length	6.2	4.0
	Width	2.9	1.5		Width	2.8	1.5
20 mmol/L	Length	5.0	3.8		Length	5.0	3.5
	Width	1.9	1.2		Width	2.9	1.9
30 mmol/L	Length	6.1	4.0		Length	7.0	3.0
	Width	4.0	2.8		Width	4.5	2.1

Time (minutes); length (mm); concentration (mmol)

Table 10. Contraction of polymer **C** in a carbonate buffer pH 7.4 (PMMA, 3-aminophenylboronic acid, diethylenetriamine, n-butylamine)

Glucose (mmol)	Time (min)	0	60	240	Contraction factor
10 mmol	Length (mm)	13.20	12.90	7.20	0.45
	Width (mm)	6.70	6.0	4.0	0.40
	Average (mm)	9.95	9.45	5.60	0.43

Table 11. Mass of polymer **C** in different media before and after glucose is added for 30 minutes. Negative (-) indicates the polymer contracted (when glucose is introduced)

Water				Buffer pH 8				$NaHCO_3$ Solution			
Water		10 mmol Glu.		Buffer		10 mmol Glu		$NaHCO_3$		10 mmol Glu	
W_O	0.0138	W_S	0.0725	W_O	0.0081	W_S	0.0305	W_O	0.0015	W_O	0.0581
W_S	0.0725	W_C	0.0388	W_S	0.0305	W_C	0.0252	W_I	0.0581	W_I	0.0115
ΔW	0.0587	ΔW	-0.0337	ΔW	0.0224	ΔW	-0.0053	ΔW	0.0566	ΔW	-0.0466
%	425.4	%	- 46.4	%	276.6	%	- 17.4	%	3777.3	%	- 80.2
W_O	0.0120	W_O	0.0520	W_O	0.0132	W_O	0.0545	W_O	0.0098	W_O	0.0434
W_I	0.0520	W_I	0.0340	W_I	0.0545	W_I	0.0408	W_I	0.0434	W_I	0.0232
ΔW	0.040	ΔW	-0.018	ΔW	0.0413	ΔW	-0.0137	ΔW	0.0336	ΔW	-0.0202
%	333.3	%	-34.6	%	312.9	%	-25.13	%	342.9	%	-46.5

W_O = weight of dry polymer **C**; W_S = weight of swollen polymer **C**; W_C = weight of contracted polymer **C**; $\Delta W = W_S - W_O$ (swelling) or $W_S - W_C$ (contracting) ; % = percentage of polymer **C** swell or contracted.

Table 12. Comparison swelling and contraction of polymer C in presence of 10mmol of glucose, fructose and galactose are introduced

Time	**0**	**5**	**10**	**15**	**20**	**25**	**30**	**35**
Length (mm)	4.748	4.808	4.915	4.815	4.830	4.723	4.753	4.713
Fructose 10mmol	1	1.013	1.035	1.014	1.017	0.995	1.001	0.993
Galactose 10mmol	1	0.996	0.991	0.973	0.966	0.967	0.965	0.949
Glucose 10mmol	1	0.998	0.965	0.920	0.848	0.795	0.707	0.591

Table 13. Swelling/contraction of polymer **C** in the presence of galactose. There is a slight contraction compared with polymer **C** in presence of glucose, because there is no additional formation of crosslinking.

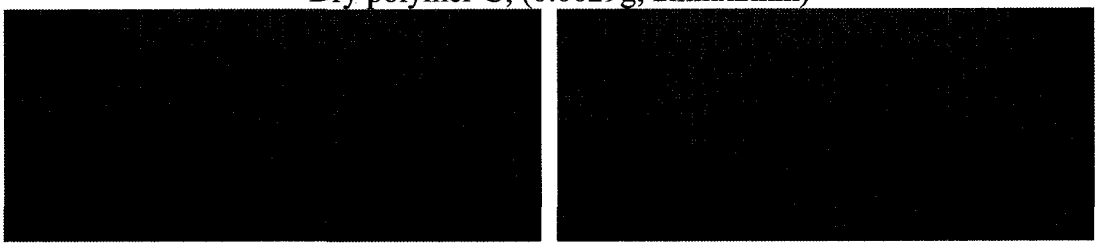
Galactose 10mmol								
Time (min)	**0**	**5**	**10**	**15**	**20**	**25**	**30**	**35**
L 1	5.266	5.315	5.216	5.184	5.084	5.092	5.029	5.054
CF 1	1	1.009	0.991	0.984	0.966	0.967	0.955	0.960
L 2	5.635	5.612	5.582	5.483	5.444	5.452	5.437	5.350
CF 2	1	0.996	0.991	0.973	0.966	0.967	0.965	0.949

L = Average length (length and width of 2-dimesional gel)

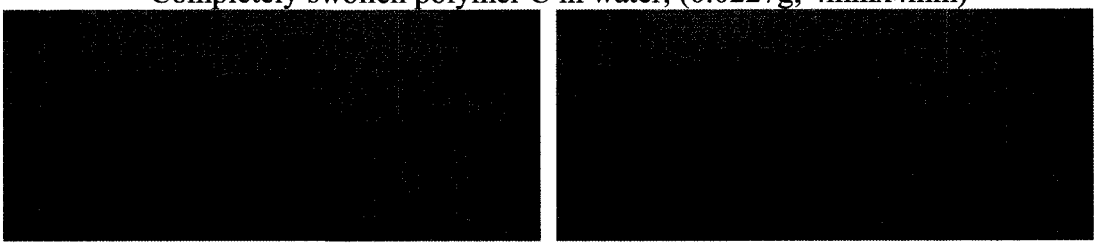
Polymer **C** in water. (mass in g, and length in mm; of dry, completely swollen and contracted in water and after the introduction glucose

SET 1:

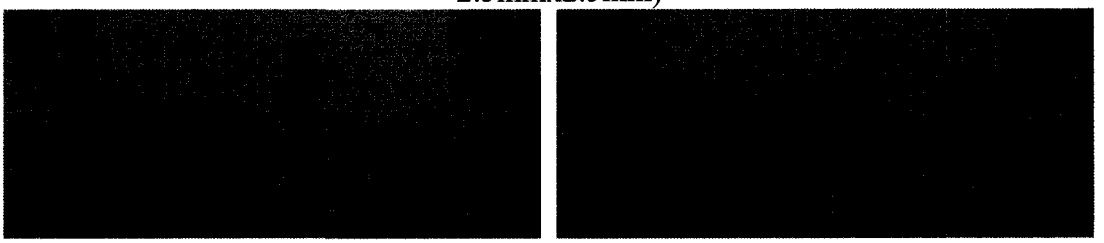
Dry polymer **C**; (0.0029g; 2mmx2mm)



Completely swollen polymer C in water; (0.0227g; 4mmx4mm)



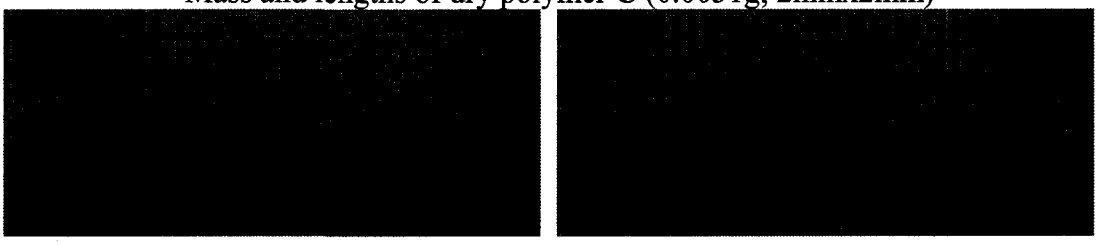
Contracted polymer **C** in the presence of 0.01193 mmol/L glucose in water(0.0092g; 2.5mmx2.5mm)



SET 2:

Length **Width**

Mass and lengths of dry polymer **C** (0.0031g; 2mmx2mm)

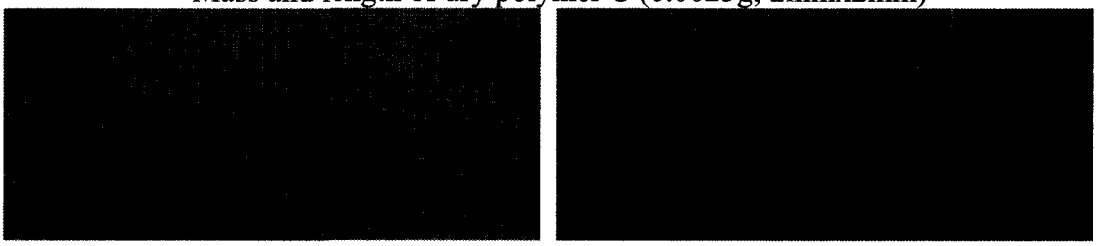


Mass and length of polymer **C** in water after (0.0307g; 4.5mmx4mm)

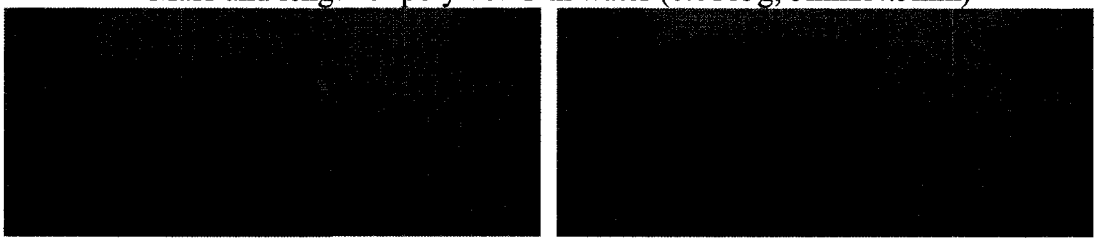
SET 3

Length **Width**

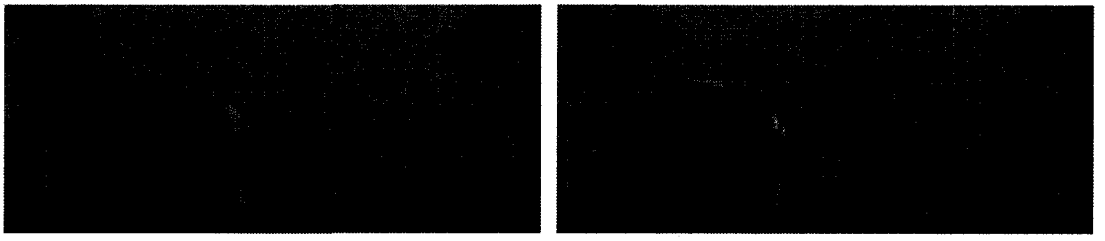
Mass and length of dry polymer C (0.0023g; 2mmx2mm)



Mass and length of polymer **C** in water (0.0183g; 5mmx4.5mm)



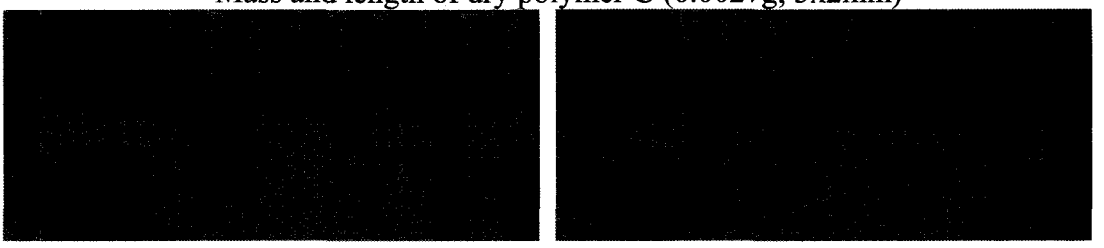
Introduction of 0.0119M glucose to swollen polymer **C** (for 20 minutes); 3mmx2.5mm



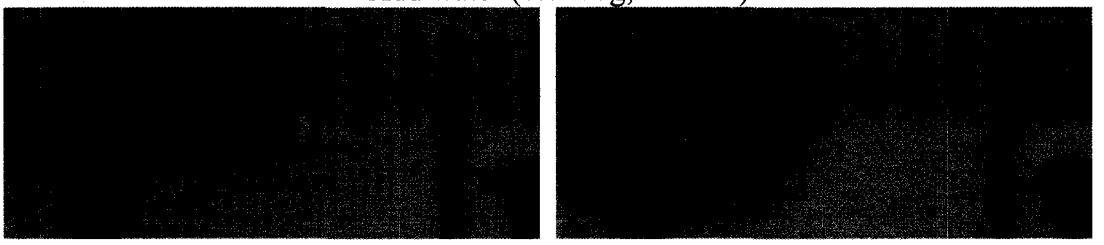
SET 4

Length **Width**

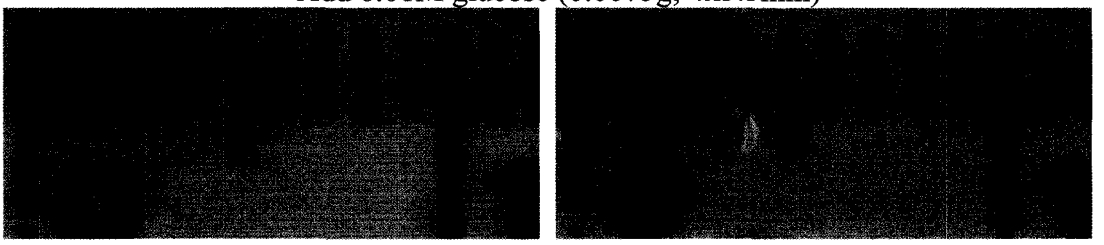
Mass and length of dry polymer **C** (0.0027g; 3x2mm)



Add water (0.0278g; 6x5mm)



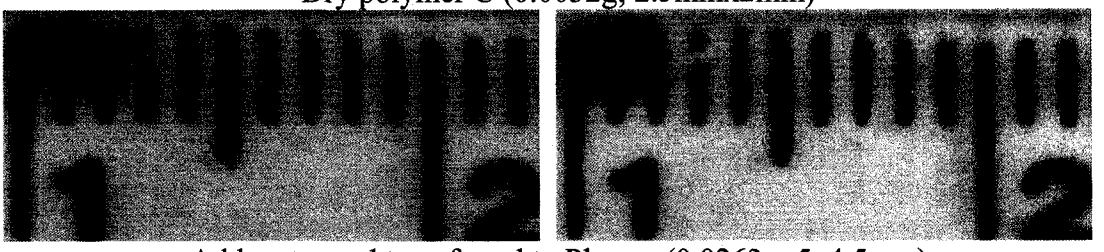
Add 0.01M glucose (0.0075g; 4x3.5mm)



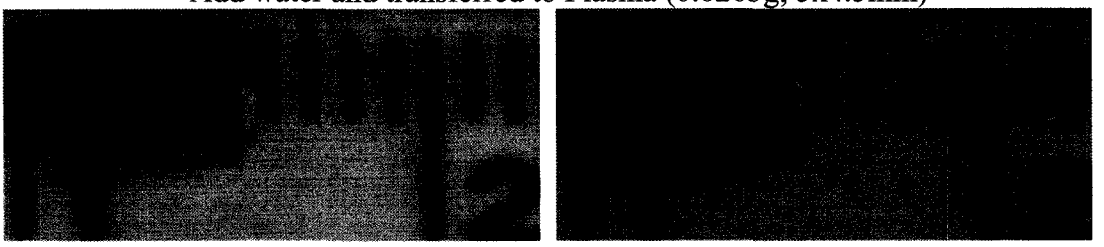
SET 5

Length **Width**

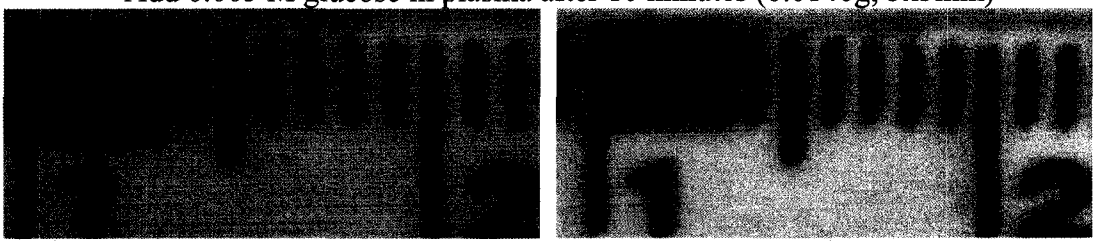
Dry polymer C (0.0032g; 2.5mmx2mm)



Add water and transferred to Plasma (0.0263g; 5x4.5mm)



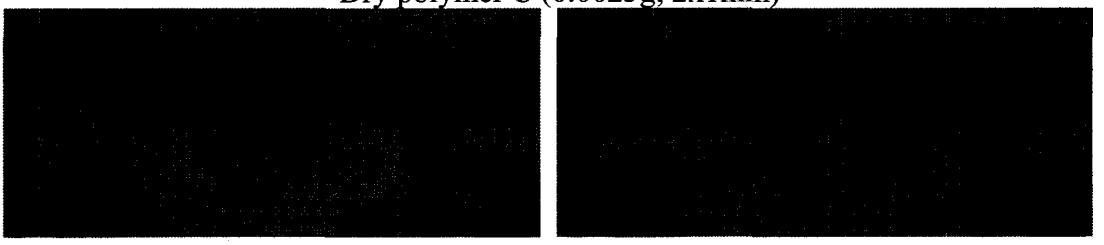
Add 0.005 M glucose in plasma after 10 minutes (0.0146g; 3x3mm)



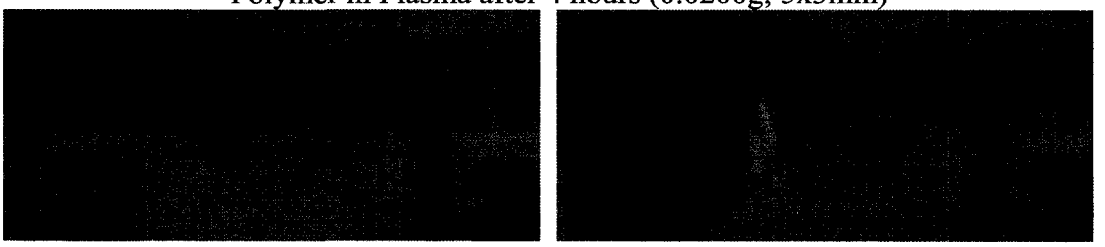
SET 6

Length **Width**

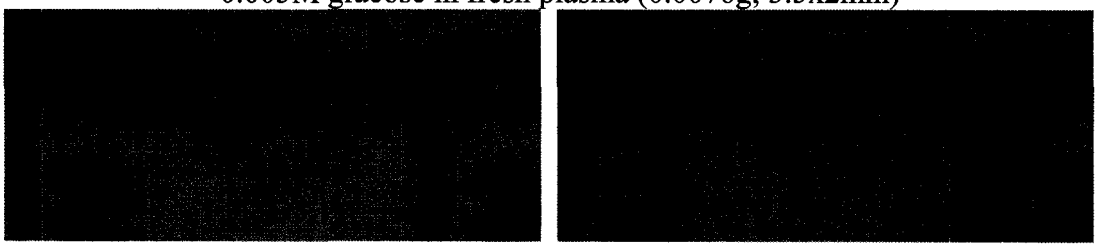
Dry polymer C (0.0023g; 2x1mm)



Polymer in Plasma after 4 hours (0.0200g; 5x3mm)



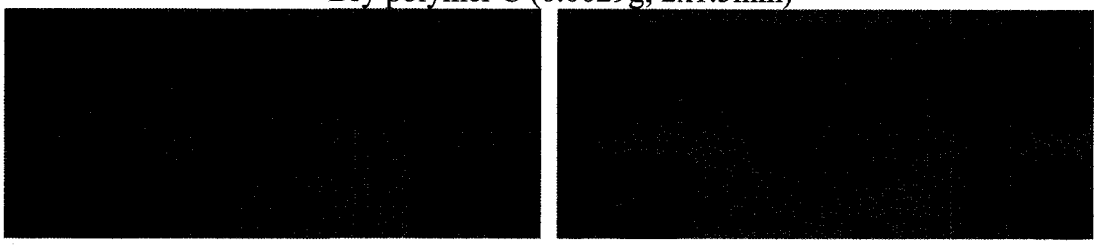
0.005M glucose in fresh plasma (0.0076g; 3.5x2mm)



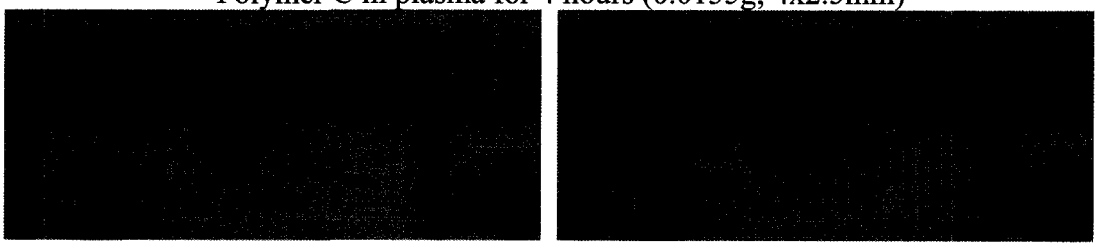
SET 7

Length **Width**

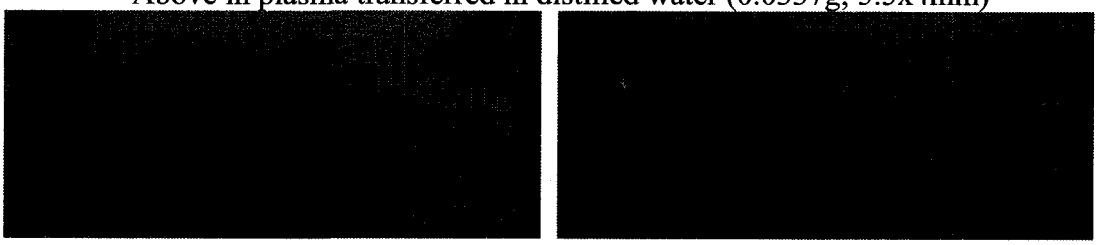
Dry polymer C (0.0029g; 2x1.5mm)

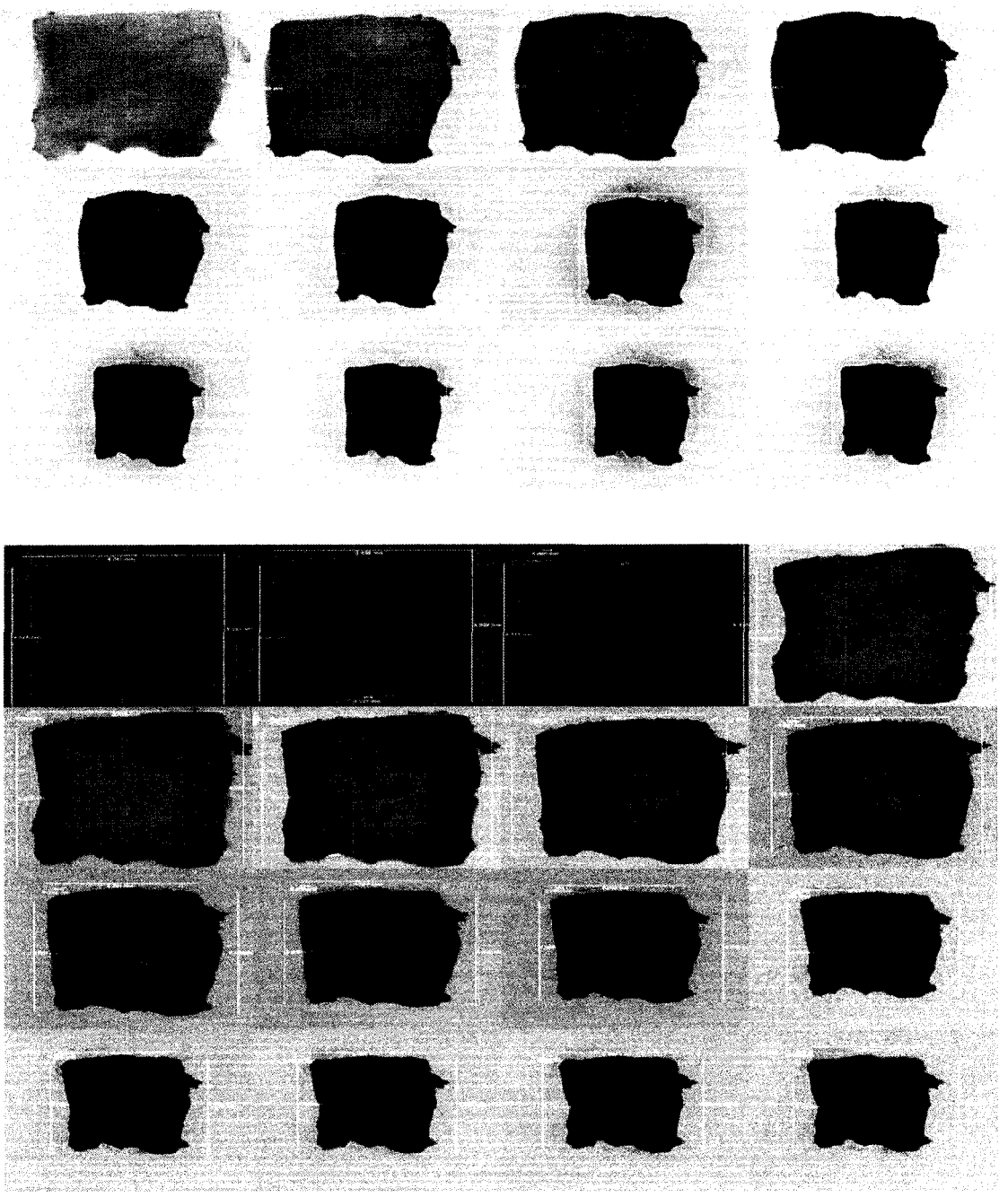


Polymer C in plasma for 4 hours (0.0135g; 4x2.5mm)



Above in plasma transferred in distilled water (0.0337g; 5.5x4mm)





Contraction-expansion of polymer C in response to glucose in water (0.005 M)

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