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Development and Optimisation of Polymeric Matrices for Use in Biomolecular Devices

By

Jeffery D. Franklin, B.Sc.

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfilment of the requirements towards the degree of
Doctor of Philosophy

The Department of Chemistry

Carleton University

Ottawa, Ontario, Canada

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for Use in Biomolecular Based Optical Devices

Submitted by
Jeffery D. Franklin, B.Sc.

in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

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ABSTRACT

Matrix development is the primary focus of this dissertation, with the intent of producing a functional polymeric matrix that displays both protein compatibility and good optical properties. The overall objectives include: (i) To prepare and optimise a PAm hydrogel formulation suitable for application in a bacteriorhodopsin based 3-D optical memory device. (ii) To design copolymers capable of post polymer grafting and photo-crosslinking that display improved long term stability and optical properties for application as a possible matrix material and (iii) To prepare novel hydroglass matrix materials displaying enhanced long term stability, optical clarity, and protein compatibility.

The aqueous polymerisation of acrylamide and crosslinking with N, N-methylenebisacrylamide afforded hydrogels displaying high levels of light scattering. Optimisation of conditions such as the reduction of particulate matter within the cuvettes, utilisation of purified water, a 12 % w/v Am concentration, a low concentration of MBA (<0.18 % w/v), a low initiator concentration, a pH of approximately 6.0 and an incubation period of 3 weeks afforded PAm hydrogels with improved optical clarity (reduced light scattering). Further enhancement to the inherent light scattering properties within a PAm hydrogel was accomplished through the implementation of “refractive index matching”. Water-soluble additives were utilised to better match the refractive index inhomogeneities throughout a given hydrogel and therefore lower the overall light scattering observed within the system. Amino acids, sugars, polymers, and other water-soluble additives such as glycerol were investigated by this methodology. Several additives showed promise at reducing the light scattering within a PAm hydrogel as correlated to increasing additive concentration. However, when considering factors such as cost, availability, water-

solubility and protein compatibility, the best candidate was found to be the additive sucrose. An optimised PAm hydrogel formulation used a 50% w/v sucrose solution that afforded a hydrogel displaying a 71% reduction in light scattering properties over that of a hydrogel formed using only water solution. Sucrose was found to remain compatible with bR at elevated concentrations and did not disrupt the polymerisation of Am. Higher concentrations of the sugar actually aided in the formation of robust hydrogels as a result of the trommsdorff effect. These optically enhanced hydrogels when applied to the bR-based device were found to function effectively, however, their long-term stability was limited to about 5 months before shrinkage or distortion of shape occurred.

The free-radical polymerisation of acrylic monomers was successful in affording both N, N-dimethylacrylamide-*co*- 2-hydroxy ethylacrylate and 2-(perfluoroalkyl)ethyl methacrylate-*co*- 2-hydroxy ethylacrylate copolymers. Increasing the mole % incorporation of HEA within these two copolymers led to measurable changes in thermal properties, solubility and refractive index. Post polymerisation esterification with cinnamoyl chloride, allowed for the successful preparation of photo-crosslinkable systems that could be photo-patterned. Incorporation of the cinnamate functionality resulted in increased T_g 's and refractive indices while causing changes in overall solubility compared to that of the parent polymers.

The synthesis of novel hydroglass structures displaying enhanced long-term stability and optical clarity was accomplished through the free-radical polymerisation of the monomers: Methacrylamide, Acrylamide, [2-(methacryloyloxy)ethyl]dimethyl(3-sulfopropyl)ammonium hydroxide inner salt hydrate, [3-(methacryloylamino)propyl] dimethyl(3-sulfopropyl)ammonium

hydroxide inner salt hydrate at high monomer concentration (> 60%). Piperazine diacrylamide was used as the crosslinking agent at a concentration range of about 7 – 10% w/v. This matrix formulation displayed a significant increase in hardness when compared to the hydrogel formulation and yielded superior optical clarity and long-term stability. When sealed appropriately, these hydroglasses displayed a shelf-life of greater than 14 months. The hydroglass formulation was prepared to contain monomers that would impart compatibility with the protein bacteriorhodopsin. This was confirmed through the successful and functional incorporation of the protein within the final matrix material.

Overall this dissertation reported: (i) The optical clarity of a PAm hydrogel can be improved through the optimisation of polymerisation conditions and can be further enhanced at significant levels via refractive index matching. The use of water-soluble additives for matching refractive index inhomogeneities within the hydrogel is a very simple and effective way to yield a matrix with a high degree of optical clarity. (ii) Photo-patternable acrylate copolymers, derived from HEA, may be prepared through the derivatization of pendant hydroxy-containing parent systems into cinnamate pendent polymers displaying changes in thermal, optical and physical properties and (iii) novel “hydroglass” matrices displaying enhanced long-term stability and optical clarity may be prepared from the aqueous free-radical polymerisation of several acrylate monomers and crosslinked with a rigid difunctional monomer such as piperazine diacrylamide.

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As I near the end of this long road towards my dissertation and the completion of my academic career, I reflect on my time spent at Carleton University and realise the importance of friends and family has never been so clear. For their vast support and time rendered on helping me keep my sanity, especially during the writing process, I would like to thank my friends Rick, Charles, Andrew, and Sean. Thank you for listening, and thank you for always being there when I needed it most.

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For the support and help received from the staff around the chemistry department and graduate studies, namely Ilona, Diane, Sheila, Mandy, Tony, and Keith – I thank you.

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"The significant problems we face cannot be solved at the same level of thinking we were at when we created them."

- Albert Einstein (1879-1955)

A handwritten signature in black ink, appearing to read "Jeff Franklin". The signature is stylized with a large, looped "J" and a cursive "Franklin".

Jeff Franklin

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List of Symbols and Abbreviations

AA	Amino Acid
Am	Acrylamide
APS	Ammonium Persulfate
bR	Bacteriorhodopsin
DMA	N,N-Dimethyl Acrylamide
DMF	N,N-Dimethyl Formamide
DSC	Differential Scanning Calorimetry
FT-IR	Fourier Transform Infrared
GPC	Gel Permeation Chromatography
HEA	2-Hydroxyethyl Acrylate
HEMA	2-Hydroxyethyl Methacrylate
MAm	Methacrylamide
MBA	N, N'-Methylenebisacrylamide
M _n	Number Average Molecular Weight
M _w	Weight Average Molecular Weight
NMR	Nuclear Magnetic Resonance
PAm	Polyacrylamide
PDA	Piperazine Diacrylate
PPM	Chemical Shift (δ)
Suc	Sucrose
TCTFE	1,1,1-Trichloro-Trifluoro-Ethane
T _d	Onset Temperature for 5% Weight Loss
TEA	Triethylamine

Tg	Glass Transition Temperature
TGA	Thermogravimetric Analysis
THF	Tetrahydrofuran
TMED	N,N,N',N'-Tetramethylene-ethylene-diamine
UV	Ultra Violet
Vis	Visible
Zonyl	2-(perfluoroalkyl)ethyl methacrylate

CHAPTER I

General Introduction

I.1 Water-Soluble Polymers

The scope of water-soluble polymers can generally range from truly water-soluble to that of hydrogels, which are crosslinked networks that swell. Certain polymers of this class can display limited water resistance owing to high molecular weight and/or their hydrogen bonding character, such as poly(vinyl alcohol) or poly(ethylene oxide). Water-soluble polymers contain functional groups that may be non-ionic or ionic in nature. Combinations of these functional groups within a given polymer are also common. These functional groups, together with molecular weight, polymer structure, and hydrophobic/hydrophilic balance help to define the material properties as well as its applications.

Water-soluble polymers can be generally regarded as being of natural origin (plant and animal based) or of synthetic origin. Polymers of natural origin can also be modified at functional sites specific to that material. Table I.1 displays some natural water-soluble polymers and polymers with modifiable functional groups found within their structures¹. Once modified, non-soluble polymers such as cellulose or chitin can become water-soluble.

Table I.1: Natural based polymers and their common functional groups

Polymer	Functional group
Starch	Hydroxyl
Starch-oxidized	Aldehyde and carboxyl
Cellulose	Hydroxyl
Cellulose-alkoxylated	Hydroxyalkyl
Cellulose-carboxyalkylated	Carboxyalkyl
Chitin	Amide
Chitosan	Amine
Pectins	Carboxyl
Hyaluronic acid	Carboxyl
Lignin	Sulfonate

The majority of synthetic polymer materials are insoluble in water and this is usually seen as a virtue. In some circumstances, however, water solubility can be advantageous. Some degree of chemical bond polarity must be present for a synthetic polymer to be soluble in water, but the controlling factors are subtle. Generally speaking, carbon chain linear polymers are only water soluble if there are polar moieties such as oxygen or nitrogen-containing side-groups attached to about one-half of the carbon atoms within the main chain ². For example, non-ionic polymers such as poly(vinyl alcohol) and poly(acrylamide), display polar, non-ionic functional groups, containing oxygen and nitrogen respectively, that impart water solubility to the polymer.

As water-soluble polymers become more widely used and investigated, their synthesis methods have reached a high degree of sophistication. There are several different routes available to produce synthetic water-soluble polymers including free-radical polymerization, condensation polymerization, and ring-opening polymerization. However, free-radical polymerization tends to offer a more broad range of polymers and copolymers. Table I.2 summarizes some common synthetic water-soluble polymers according to their preparation methods.

Table I.2: Synthetic water-soluble polymers and their preparation routes

Polymerisation Reaction	Polymer
Addition Polymerization	Poly(acrylic acid) Poly(methacrylic acid) Poly(vinyl alcohol) Poly(acrylamide) Poly(vinyl pyrrolidone) Poly(hydroxyalkyl acrylates)
Condensation Polymerization	Polyesters Polyamides Polyethers Polyacetals Polyethyleneimines
Ring-opening Polymerization	Poly(alkylene oxides) Poly(alkylene oxides – lactones) Polycarbonates Polyesters

Water-soluble polymers are used primarily to disperse, suspend (thicken and gel), or stabilize particulate matter. However, they may perform many other functions such as binding, coagulating, film forming, flocculating, lubrication and friction reducing, and rheology modification and control ^{1,3,4}.

These functions make water-soluble polymers suitable for a wide variety of applications including water treatment, paper processing, mineral processing, formulation of detergents, textile processing, the manufacture of personal care products, pharmaceuticals, petroleum production, and enhanced oil recovery and formulation of surface coatings ⁵⁻⁷. These polymers often perform more than one function in any given application. For example, a thickener for an aqueous paint may also function as a suspension agent for inert ingredients within the formulation. As of late, research has yielded many novel areas of application for water-soluble polymers. In addition to water-absorbent and cosmetic applications of water-soluble polymers, other areas of research interest include various biomedical applications ^{8,9}, biodegradable polymers ^{10,11}, and drug delivery systems ¹²⁻¹⁴. Yamada et al. have outlined the use of water-soluble polymers in water-processable positive-tone photoresists ¹⁵. While current research has also shown promise in the area of polymeric inhibition of ice growth with application to biological cryopreservation ^{16,17}.

Natural, semisynthetic (modified natural polymers) and synthetic water-soluble polymers can perform similar functions. However, synthetic polymers are often preferred in many applications because of their greater versatility.

I.2 Poly(acrylamide) and Derivatives

Water-soluble polymers have become of vital importance to a very broad range of industrial applications. An important class of these materials is the acrylamide based polymers, which are widely used as flocculants, rheology controlagents, and adhesives^{18,19}. Acrylamide polymers were first manufactured in the 1950s, and for the past 40 years or so their production has been intensively developing. As of year 2000, total worldwide production exceeded well over 400 000 tons/year.

Polyacrylamide can be prepared by the free radical polymerization of acrylamide (Scheme I.1) using bulk, solution, suspension, emulsion, inverse emulsion or precipitation techniques²⁰. Among these, homogeneous polymerization of acrylamide is usually carried out in aqueous solutions. The principle factors that make this technique popular are a high rate of polymer formation and the possibility of obtaining a polymer of high molecular weight.

Scheme I.1: Polymerization of Acrylamide

Solutions of acrylamide in water polymerize at moderate temperatures under the influence of nearly all free-radical sources, including peroxides, persulfates, redox couples, azo compounds, ultrasonic energy, uv light, ionized gas plasmas, and ionizing radiation ²¹. These are all classical chain reaction polymerizations involving the normal steps of initiation, propagation, and mutual termination of pairs of growing chains. Venkatarao and Santappa proposed that chain termination occurs mainly by disproportionation in homopolymers of acrylamide ²².

Profound changes in polymerization characteristics occur as the solvent is changed from essentially aqueous to predominantly organic ²⁰. The greater reactivity of acrylamide in aqueous solutions may be associated with the suppression of autoassociation of acrylamide monomers as they form hydrogen bonds with water molecules. However, in nonpolar solvents, incapable of forming such bonds with acrylamide, the monomer exists mainly in the associated state as a cyclic dimer and trimer, as well as in the form of linear multimolecular associates ²⁰.

A large number of N-substituted acrylamides are known. Generally, three main routes have been used to prepare such derivatives: 1) acrylonitrile-Ritter reactions ²³, 2) acryloyl halide-amine condensations ²⁴, and 3) acrylamide-formaldehyde reactions (Mannich reaction) ²⁵. Table I.3 lists some commercially important acrylamide derivatives. Thus, various acrylamide derivatives can occur via mono-, or disubstitution on the amide nitrogen, or through substitution on the α -vinyl carbon (i.e. methacrylamide). These monomer derivatives can be homopolymerized or copolymerised in the same manner as acrylamide.

Table I.3: Commercially Available Acrylamide Derivatives

Name	Structure
N-methylolacrylamide	$\text{H}_2\text{C}=\text{CHCONHCH}_2\text{OH}$
Methacrylamide	$\text{H}_2\text{C}=\text{C}(\text{CH}_3)\text{CONH}_2$
N,N'-methylenebisacrylamide	$(\text{H}_2\text{C}=\text{CHCONH})_2\text{CH}_2$
N,N'-dimethylacrylamide	$\text{H}_2\text{C}=\text{CHCON}(\text{CH}_3)_2$
N-isopropylacrylamide	$\text{H}_2\text{C}=\text{CHCONHCH}(\text{CH}_3)_2$
2-acrylamido-2-methylpropanesulfonic acid	$\text{H}_2\text{C}=\text{CHCONHC}(\text{CH}_3)_2\text{CH}_2\text{SO}_3\text{H}$

I.2.1 Properties and reactions of polyacrylamide

Polyacrylamide is a linear polymer that is white in colour, odourless, and exhibits very low toxicity. PAm is highly soluble in water, whereas it is insoluble in all common organic solvents such as, methanol, chloroform, and tetrahydrofuran (THF). With aqueous solutions of high molecular weight PAm, there seems to be “aging” effects as shown by steady reductions in the viscosity of solution with time²⁶. These effects seem to be largely physical, relating to either the disentangling or disaggregation of the polymer molecules. However, there may be some chemical contributions as well from the breaking of weak links in the polymer chain.

PAm solutions undergo the general reactions of the aliphatic amide group, with the most important reactions of the polymer being hydrolysis, Hofmann degradation, and the Mannich reaction.

The hydrolysis of PAm can be carried out in an acidic or basic medium. The reaction under slightly acidic conditions is very slow, while strong acidic conditions (i.e. less than pH 2.5) lead to a progressive insolubilization of the reaction product because of the formation of cyclic imide structures (Figure I.1)²⁷. Imidization was also reported at pH values greater than 9. Imidization is generally intermolecular, however, intramolecular imidization (cyclization) can take place if head-to-head units occur in the polymer²⁸. On the other hand, hydrolysis of PAm under basic conditions is rapid and can be used to introduce acrylate groups into macromolecules (Figure I.2).

Figure I.1: Imide structures that may form during acid hydrolysis of PAm

Figure I.2: Hydrolysis of PAm under basic conditions

A Hofmann degradation of PAm through the use of excess sodium hypochlorite and a large excess of sodium hydroxide leads to polyvinylamine²⁹ (Figure I.3). The Mannich reaction of acrylamide polymers with formaldehyde and dimethylamine may be used for obtaining polymers that contain N-methylol groups or ethers, and ethers thereof³⁰ (Figure I.4).

Figure I.3: Hofmann degradation of PAm

Figure I.4: Mannich reactions of PAm

One of the most important fundamental characteristics that relates to polymer properties and the processing of amorphous polymers is the glass transition temperature (T_g). In an amorphous polymer, motion is generally restricted to short range vibrations and rotations allowing the polymer to remain in a glass-like state. Upon increasing the temperature, the polymer undergoes a change from the glass-like

state to one that resembles a rubber, characteristically known as the glass transition temperature. The inherent T_g of a polymer plays a crucial role when considering a particular application, as it is important to consider whether the polymer will flow at the required temperature of processing and remain structurally intact through the temperature range of application.

On a molecular level, the T_g is encompassed by an increase in long-range molecular motion resulting from greater rotational freedom and consequently increases in segmental motion of polymer chains. In order for the chain segments to move, the space between the atoms, defined as free volume, must increase resulting in a greater volume or a lower density of the bulk polymer. On a macroscopic level, the glass transition results in an enthalpy change which may be measured through calorimetry. When heated, the amorphous region of the polymer softens over a broad range, losing its glass-like properties as it becomes more rubber-like. Scientists have investigated the relationship between polymer structure and T_g in order to predict polymer properties. However, the predictions are at best approximate due to the estimations in the measured values that are dependant on molecular weight, method of measurement and on the morphology of the polymer. Given that T_g is a function of rotational freedom, any structural change that restricts rotation should theoretically increase T_g . Likewise, any variation that promotes rotation or flexibility should lower T_g .

The amorphous homopolymer (PAm) shows a T_g close to 188 °C and a decomposition temperature (T_d) of approximately 290°C. A comparison of some

common acrylamide based homopolymers and their corresponding thermal properties can be seen in Table I.3³¹.

Table I.4: Acrylamide-based homopolymers and their thermal properties

Homopolymer	T _g °C
Acrylamide	188
Methacrylamide	207
N,N'-dimethylacrylamide	89
N-Isopropylacrylamide	85, 130

1.3 Crosslinking of Polymers

Crosslinking reactions are commonplace for both natural and synthetic polymers. Crosslinks act to lock the linear polymer chains and prevent free-chain movement often resulting in increased strength, but also decreased flexibility and increased brittleness. Occasionally the term *curing* is used to denote crosslinking. A number of other changes generally accompany crosslinking, for example if the polymer was previously soluble, it will no longer dissolve (except in some ionically crosslinked systems). In the presence of solvent, a crosslinked polymer will swell as solvent molecules penetrate the network. The degree of swelling depends on the affinity of the solvent and polymer for each other as well as the degree of crosslinking. Since crosslinking reactions are known to discourage segmental motion, increases in polymer glass transition (T_g) temperatures are noted. These increases in T_g are also dependent on the degree of crosslinking.

With network polymers it is common to speak of crosslink density (Γ), that is, the number of crosslinked monomer units per main chain. It is defined mathematically by:

$$\Gamma = (M_n)_0 / (M_n)_c ,$$

where $(M_n)_0$ is the number average molecular weight of uncrosslinked polymer and $(M_n)_c$ is the number average molecular weight between crosslinks³².

A polymer gel can be obtained by chemical or physical processes. Some gels are crosslinked chemically by covalent bonds (chemical gel), whereas other gels are crosslinked physically by weak forces such as hydrogen bonds, van der Waals forces, or hydrophobic and ionic interactions (physical gel). The physical gelation processes are usually reversible and called sol-gel transitions. The final gel structures and properties are sensitive to the preparation methods.

The chemical crosslinking of a polymer can be affected through various means such as; the application of heat, mechanically, vulcanation, free radical reactions caused by chemical agents or ionizing radiation, photolysis involving photosensitive functional groups, chemical reactions of labile functional groups, coulombic interactions of ionic species, or through any combination of these³²⁻³⁵. Basically, the means of affecting a crosslinking reaction can be grouped into one of two categories: (1) crosslinking during polymerization by use of polyfunctional instead of difunctional monomers and (2) crosslinking in a separate processing step after the linear (or branched) polymer is formed. The crosslinks may contain similar structural characteristics as the main chain, which is generally the case of the former category,

or they may have an entirely different structure, which is generally the case in the latter category.

I.3.1 Photo-crosslinking of Polymers

Minsk et al.³⁶ may be the first chemists to synthesize a photo-crosslinkable polymer, poly(vinylcinnamate). Today photosensitive polymers are utilized in many technologies from photoresists to microelectronic components, miniature and integrated circuits, photoengraving, precision chemical milling, and formation of protective and decorative coatings. There are two basic methods for bringing about photocrosslinking: (1) incorporating photosensitizers into the polymer, which absorb light energy and thereby induce formation of free radicals, and (2) incorporating groups that undergo either photocycloaddition reactions or light-initiated polymerization.

A wide variety of functional groups have been used to effect photocycloaddition or light-induced polymerization crosslinking³⁷⁻⁴¹. A selected few can be seen in Table 2. The groups may be present as part of the polymer backbone or, more commonly, as pendant groups. In most cases $2\pi + 2\pi$ cycloaddition occurs to yield cyclobutane crosslinks. The reactive groups may be incorporated onto the polymer during polymerization or alternatively, the group may be added to a preformed polymer through chemical modification.

When a quantum of light interacts with a molecule in the ground state, the energy is absorbed by that molecule and an electron is promoted to a higher energy

level, thereby attaining an excited state. As outlined by Turro⁴², this phenomenon obeys four laws of organic photochemistry:

- 1) Photochemical changes take place only because light is absorbed by the system.
- 2) One photon or one quantum of light activates only one molecule.
- 3) Each quantum or photon that is absorbed by a molecule has a given probability of populating either the singlet state or the lowest triplet state.
- 4) In solution, the lowest excited singlet and triplet states are the starting points for the photochemical process.

The relationship between the amount of light or the number of photons absorbed, and the number of molecules that as a result undergo a reaction, is defined as the *quantum yield*.

Table I.5: Some commonly used photo-crosslinking groups

Type	Structure
Alkyne	$R-C\equiv C-R$
Anthracene
Benzothiophene dioxide
Chalcone
Cinnamate
Coumarin
Maleimide
Stilbene
Styrene

I.4 Polymer Gels

When a polymerization reaction takes place in three dimensions, it eventually reaches a certain point when *gelation* occurs. This well defined change during polymerization is known as the gel point. At this point the reaction mixture changes from a viscous liquid to an elastic gel. Before gelation, the polymer is soluble and fusible, however, after gelation, it is neither soluble nor fusible. This is a result of restraining effects of three-dimensional space networks. Thus, another classification of polymers is possible, which is based on whether the material can form crosslinked or gelled networks. Polymers that eventually reach gelation are called *thermosetting* or crosslinkable polymers. Once past gelation, raising the temperature within these networks will no longer attain plasticity as the molecules can no longer move past each other. For the same reason they can no longer be dissolved in any solvents. A polymer gel consists of an elastic crosslinked network and a fluid filling the interstitial space of this network. The network of long polymer molecules holds the liquid in place and thus gives the gel what solidity it has. Gels are wet and soft and look like solid material but are capable of undergoing large deformation. Living organisms are largely composed of gels. Except for bones, teeth, nails, and the outer layers of skin, mammalian tissues are highly aqueous gel materials largely composed of protein and polysaccharide networks, in which water contents range up to 90%⁴³.

There are a variety of ways to classify a gel; for example, there are chemical gels and physical gels depending on the crosslinking within the network, there are natural and synthetic gels, and there are hydrogels, organogels, and xerogels according to the solvent medium the polymer network is swollen in (Table I.6)²¹.

Table I.6: Classification of polymer gels

Classification Basis	Type	Examples
Constituent phases and solvents	Solid-liquid	Hydrogel (water solvent) Organogel (organic solvent)
	Solid-gas	Lio-gel (oily solvent) Xerogel (air)
	Solid-solid	Aerogel (air) Polymer-gel (polymer)
Constituent polymers	Natural gel Synthetic gel Hybrid gel	Protein gel, polysaccharide gel Organic polymer gel, inorganic gel Protein and synthetic polymer, or polysaccharide and synthetic polymer
Crosslinkage	Covalent Noncovalent	Covalent bond linkage Coulombic interaction Hydrogen bonding Coordinate bonding

Polymer gels should, in general, be considered as composed of heterogeneous structures in different orders from a few angstroms to several micrometers. Due to this heterogeneity combined with the swellability that gives the gel network motion, detailed gel structures have not been visualized satisfactorily. The formation of the heterogeneities in crosslinked polymer gels has been of considerable interest for many years.

1.4.1 Hydrogels

In recent years, polymeric gels have been the objects of intensive study, and within this family of materials, hydrogels have generated considerable attention. Hydrogels are a unique class of polymeric materials which imbibe a significant

amount of water into their internal molecular structure yet still maintain their permanent shape. Basically defined, they are hydrophilic polymers that are crosslinked to form insoluble, but water swellable, networks. Hydrogels have special properties due to their intermediate state between liquid and a solid. Their ability to absorb and store much water and water solutions make hydrogels unique materials for a variety of applications. The use of hydrogels in an ever increasing array of applications is becoming possible as refinement of their structure/property characteristics continues. They are currently employed in applications such as soil flocculants, superabsorbents, cosmetics, toys, wound dressings, contact lenses, drug delivery systems, and artificial organs and limbs to name a few⁴⁴⁻⁴⁷. Table I.7 lists some commonly used hydrogel systems and their corresponding applications.

Table I.7: Commonly used hydrogels and their applications

Hydrogel	Structure	Application
Poly(vinyl alcohol)	$\left[\text{C} \begin{array}{c} \text{H} \\ \\ \text{H}_2\text{C}-\text{C} \\ \\ \text{OH} \end{array} \right]_n$	Drug release, artificial organs, wound dressings
Poly(N-vinyl pyrrolidone)	$\left[\text{C} \begin{array}{c} \text{H} \\ \\ \text{H}_2\text{C}-\text{C} \\ \\ \text{N} \\ \\ \text{C=O} \end{array} \right]_n$	Contact lenses, hemodialysis membranes, coatings, drug release systems, cosmetics
Poly(2-hydroxyethyl methacrylate)	$\left[\text{C} \begin{array}{c} \text{CH}_3 \\ \\ \text{H}_2\text{C}-\text{C} \\ \\ \text{C=O} \\ \\ \text{O} \\ \\ \text{CH}_2\text{CH}_2\text{OH} \end{array} \right]_n$	Contact lenses, drug delivery, wound dressings
Poly(acrylamide)	$\left[\text{C} \begin{array}{c} \text{H} \\ \\ \text{H}_2\text{C}-\text{C} \\ \\ \text{C=O} \\ \\ \text{NH}_2 \end{array} \right]_n$	Drug release, electrophoresis, soil flocculants
Poly(ethylene oxide)	$\left[\text{C} \begin{array}{c} \text{H} \\ \\ \text{H}_2\text{C}-\text{C}-\text{O} \end{array} \right]_n$	Drug release, wound dressings, contact lenses, creams

Hydrogels are very suited for interaction with biological systems, tissues, and compounds due to their high biological compatibility. Much of this compatibility comes from the fact that these networks are comprised in majority of water and from their soft, rubbery consistency which gives them a resemblance to living tissue. In addition, many of the polymers used in a hydrogel network have little to no toxicity once in the polymer form.

Much of the hydrogels commercially utilized worldwide are based on acrylic and methacrylic acids as well as their ester and amine derivatives. The main method of synthesis for such gels described in literature and used in industry is the radical polymerization of monomers with crosslinking agents and catalysts.

It is not surprising to consider that hydrogels contain a relative amount of spatial inhomogeneities, or heterogeneities in crosslink distribution ⁴⁸. These structural inhomogeneities within the gel affect its physical properties such as its optical and phase properties. Shibayama ⁴⁹ recently outlined that the properties of polymer gels, such as mechanical strength, viscoelasticity, kinetics of deswelling, and transparency, depend on their spatial inhomogeneities. For chemically crosslinked polymer gels, such as hydrogels, the relationship between light scattering and spatial inhomogeneities has been relatively well understood. Chemical crosslinks act to constrain the mobility of polymer chains in a solvent resulting in an emergence of a nonvanishing spatial variation of refractive index, thereby attributing to the observable inherent light scattering. Thus if a narrow beam of highly coherent light (i.e. laser beam) is irradiated into a gel, the scattered light shows up as “speckles” thereby outlining regions of inhomogeneities ⁵⁰.

I.5 Bacteriorhodopsin: Properties and Applications

It has been well over a quarter of a century since the discovery of purple membrane (PM) and the first bacterial rhodopsin (bR) was isolated in a wild cell culture of *Halobacterium halobium*, an extremophile bacteria that lives in unusually high salt concentrations. Since this time, many unique and interesting properties have been determined pertaining to bacteriorhodopsin structure and function.

The bR molecule is a retinal-protein complex, consisting of a 26.5 Dalton, 248 amino acid residue protein and a retinal molecule bound by a Schiff base ⁵¹.

Bacteriorhodopsin has seven transmembrane α -helices and exists in a globular shaped 3-D structure (Figure I.5) ⁵². Within the PM, bacteriorhodopsin is oriented in the form of a two-dimensional crystal surrounded with lipid portions, with all the bR molecules uniformly oriented with their carboxy termini located in the inner portion of the cell ⁵³.

In the bacterium, production of bR and hence purple membrane allows the organism to switch to photosynthesis as a means of energy production, when dissolved oxygen concentrations drop below levels sufficient to sustain respirative oxidative phosphorylation. Upon the absorption of light, a complex photochemical cycle is initiated within bR which has a duration of approximately 10 ms (Figure I.6) ⁵⁴. The molecular function of bR is that of a light driven proton pump.

Figure I.5: Tertiary globular structure of bacteriorhodopsin

The photocycle transports a proton from the intracellular to the extracellular side of the membrane. As bR does not have a refractory period⁵², after completing a photocycle under light saturating conditions, 100 protons per second could be transported by a single molecule. This light induced proton pumping, is used by the organism to generate an electrochemical gradient that the bacterium uses to chemiosmotically synthesize adenosine triphosphate (ATP), a readily available source of energy.

As seen in figure I.6, the photocycle of bR consists of several intermediates which can be identified by their spectroscopic properties, mainly their absorption maxima (wavelengths seen in brackets)^{54,55-58}. In the ground state, the retinal chromophore within the bR molecule exists in an all trans state, however, upon light

induced excitation of a bR molecule, retinal is converted to a 13-cis conformation (Figure I.7) thereby marking the formation of the first intermediate.

Figure I.6: Photocycle of bacteriorhodopsin outlining the various intermediates and their corresponding absorption maximum.

Figure I.7: Isomerization of retinal from all-trans to 13-cis conformation

This isomerization stores energy, which is then utilized to form the other intermediates at the expense of dark-dependant processes. At all pre-M state stages of the photocycle, retinal occurs in the 13-cis form, isomerising to the all-trans state at the stage of M to O conversion which then returns onto the ground state in the same isomeric form. Generally, photochemical stability is quantified as the number of times a photochromic material can be photoconverted between two species before 37% ($1/e$) of the irradiated ensemble denatures. This number is referred to as the cyclicity, and it exceeds 10^6 for bR at ambient temperature⁵². The high cyclicity of bR can be attributed to the protective features of the integral membrane protein that

serve to isolate the chromophore from reactive oxygen, singlet oxygen, and free radicals.

Each of the intermediates within the cycle is photoreactive, and may change its lifetime and relaxation pathway upon the absorption of a light quantum. An interesting example of this follows that the O state intermediate does not decay back to the ground state via the native photocycle pathway upon absorption of a light quantum, but rather follows a “branched photocycle” pathway leading to intermediates P and Q not found in the native photocycle (refer to Figure I.6). The Q state is an alternatively stable state induced within the protein photocycle. The complex can remain in this state for up to an estimated 5-20 years. Conversion of the Q state back to the ground state can be achieved via irradiation with light corresponding to its absorption maximum, 380 nm. It is this alternatively stable, and spectrophotometrically distinct Q state that is the basis for many information storage applications.

The extreme growth conditions of high salt and high temperature at which the bacterium survives, together with the crystalline lattice structure of the PM, yield a protein, bR, that has exceptional stability against salt, high temperature, photochemical degradation, chemicals, and extreme pH media^{52,59}. Thus, this stability, along with its many inherent photochromic, photoelectric, and charge transport properties, makes bR an ideal candidate biomaterial for a wide variety of applications. These include random access thin film memories, neural-type logic gates, photon counters and photovoltaic converters, reversible holographic media, artificial retinas, picosecond photodetectors, spatial light modulators, associative

memories, two-photon volumetric memories, holographic correlators, nonlinear optical filters, dynamic time-average interferometers, optical limiters, pattern recognition systems, real time holographic imaging systems, multilevel logic gates, optical computing, and branched-photocycle volumetric memories⁶⁰⁻⁶⁹.

For the integration of bR into technical systems, two obstacles must be overcome: firstly, the structuring of the biomaterial itself and, secondly, the interface of the biocomponent with the rest of the conventional system. The technical level for both these criteria is lowest for photochromic applications. No macroscopic orientation of PM patches is required. A homogeneous distribution of PM patches in an optically inert matrix material fulfills the immediate needs. Because the interface in these applications is an optical one, it is generally easy to protect the bR media. Hence, majority of applications discussed in the literature for bacteriorhodopsin pertain to its photochromic properties.

Throughout the literature, there are volumes of publications outlining potential uses/applications of bR and bR-based devices in optical memory, and optical recording media. In terms of the photochromic properties of bR and their application to information storage, the field can be narrowed to a few main groups, namely, holographic storage, associative memories, 2-D storage, and 3-D storage. For the purpose of this thesis, focus will be given to the use of bR and bR-based materials for 3-D information storage.

Present memory technologies such as magnetic disks, optical discs, and semiconductor memories, store information in a planar surface, in other words, in two-dimensions. This actually limits the storage capacity of the media. For example,

2-D optical memories have storage capacities that are limited to $\sim 1/\lambda^2$, where λ is the wavelength, which yields approximately 10^8 bits/cm². On the other hand, the development of new generation, two photon 3-D ultra high density random access memories⁵⁸ allows for a significant increase in storage capacity. Three-dimensional memories can approach storage densities of $1/\lambda^3$, yielding storage capacities in the range of 10^{11} to 10^{13} bits/cm³.

Whether or not the theoretical limits of an optical system/device based on bR can be reached depends mainly on the quality of the media, in particular their homogeneity, light sensitivity, storage time, and long-term stability. Bacteriorhodopsin based optical devices were long hindered by the availability and high cost of the protein, however, this obstacle has since been overcome. Still problems remain, organic solvents cannot be used in the preparation of bR-based media which reduces the chemical methods applicable to a great extent. For most applications, films made from bR are employed, but for 3-D storage, cubes filled with bR are used. The size of the PM relieves the problems concerning immediate rotational and lateral diffusion throughout the media, however, when considering long term information storage applications, both parameters must be considered carefully. In these applications, such as 3-D optical memory storage, any type of diffusion would cause fatigue of the stored information and reduce the useable storage density.

In most cases, the use of gelatin or poly(vinyl alcohol) as a matrix for embedding bR have been employed. For thin film applications these materials meet the necessary requirements, however, when considering use for 3-D applications, the thickness of the media becomes too great and the optical transparency is greatly

reduced. A few attempts have been made to embed bR into sol-gel glasses but hydration then becomes an issue in terms of proper functioning of the bR photocycle. Birge et al.^{54,58} reported using PAm hydrogels as a matrix for bR within their 3-D optical memory storage device. The optical transparency of the material was influenced by the fact that a significant amount of light scattering was inherent to a PAm hydrogel⁴⁹, which initially compromised the write-read efficiency and resolution. In addition, these PAm hydrogels displayed a limited “shelf life” so to speak in terms of their overall stability. Being crosslinked networks via a free-radical mechanism, there is a considerable amount of relaxation that occurs following network formation and *gel syneresis* evidently leads to large changes in gel volume. These changes in matrix volume in turn result in loss of storage/retrieval capabilities within the 3-D device.

Thus, it is evident from the literature, that for any material to function effectively in a bR-based 3-D optical memory device application, it must meet three general requirements: 1) Compatibility with the protein, 2) Optically inert – low levels of inherent light scattering, and 3) long term stability.

I.6 Rationale and Objectives

As evidence from the literature, the last 30 years or so has brought about vast knowledge of bacteriorhodopsin structure and function. Applying this knowledge to various bR-based applications and devices has yielded many interesting results. Certain bR-based devices, such as holographic applications, seem to lead the way in terms of their development and commercialization. On, the other hand, applications

such as 3-D optical memory devices, have been realised but continue to display problems which need to be overcome in order to improve their operating efficiency. Other than the speed of the device, i.e. time required to write or read information, the main obstacle for bR-based 3-D optical memory storage applications seems to be the matrix material. Issues concerning compatibility, optical properties and long-term stability of have yet to all be met by one given material.

I.6.1 Rationale for the optimization of optical properties within a bR-compatible material

In order to develop a material to function in a bR-based 3-D optical memory device in terms of its optical properties, an understanding of all the parameters influencing this property has to be made. Of course, the material must be compatible with the protein such that native photocycle functions are not disturbed. Therefore, investigations into the formation of poly(acrylamide) hydrogels (a proven compatible material) and all relevant factors influencing the inherent light scattering characteristics of these gels will be made. With a sound understanding of all influencing factors, the system may then be optimized, according to these factors, for this particular optical application. It is expected that monomer concentration, bR concentration, and crosslinking concentration will all be major contributors to the overall light scattering. However, monomer conversion, type of crosslinker, initiator concentration, and relaxation of the polymer network will also be investigated for their influence on the light scattering characteristics of the material

After probing and optimizing all relevant factors influencing the light scattering characteristics of PAm, it is likely that the material will still have a higher than desired level of inherent light scattering. Thus, it is proposed that through a methodology of Refractive Index Matching, a PAm hydrogel can be developed that will function well within the optical parameters defined by the device. It is expected that by incorporating bR-compatible, water soluble additives into the polymerization solution, the differences of refractive index between the formed polymer network (due to inhomogeneous crosslink densities) and the aqueous media can be minimized. The minimization of these RI differences should yield a more homogeneous material thereby minimizing the amount of light scattering observed. Various types of additives, and additive concentrations will be investigated for their ability to improve the light scattering characteristics of PAm. The use of this RI matching methodology will also be investigated on materials other than PAm hydrogels.

I.6.2 Rationale for the improvement of long-term stability

The other major factor to be considered when developing a material for this application is the stability. If the material is not restrictive in terms of protein motion, then the PM patches will diffuse throughout the matrix thereby compromising storage capacity as well as the write-read efficiency. Thus, the material has to effectively entrap bR and prevent long-term diffusion. In addition, the material itself must display a high degree of stability such that over time, changes in the shape/volume do not occur. Being a volumetric memory storage device, changes in recording media volume will have obvious deleterious effects.

It is proposed that by inducing crosslinks within a polymer material after the polymerization process, that a significant amount of network tension will be alleviated as the polymer chains should already be in relaxed, linear state. This should improve the stability of the material over that of the conventionally formed hydrogel. The easiest way to achieve post-polymer crosslinking, without the introduction of impurities is through a photo-crosslinking group. The post polymer modification of a water soluble copolymer of N,N'-dimethylacrylamide and 2-hydroxyethyl acrylate to contain the photoactive *cinnamoyl* group will be investigated (Scheme I.2).

Scheme I.2: Proposed copolymerisation and post polymer modification of an acrylamide based copolymer

The effects of concentration variations of cinnamoyl group incorporation on the polymers thermal properties and solubility will be investigated. As well, the photocrosslinking reactions will be employed and the resultant changes in polymer T_g according to cinnamoyl concentration will be investigated. The stability of the crosslinked system, as well as the compatability to bR will also be verified.

The use of fluoropolymers for a variety of optical applications is very evident throughout the literature. Thus, an attempt to prepare a photocrosslinkable fluorocopolymer will be made following a similar outline as to that seen in Scheme I.2. A fluoromethacrylate (Zonyl® TM) monomer will copolymerised with 2-hydroxyethyl acrylate and subsequently modified with photoactive cinnamoyl moieties (Scheme I.3).

Scheme I.3: Proposed copolymerisation and polymer modification leading to a photocurable fluoropolymer

The resultant polymer will be investigated for changes in solubility and thermal properties with changes in concentration of cinnamoyl moiety incorporation. The crosslinking reaction will be effected and the resultant gels will be investigated for changes in T_g as a function of cinnamoyl concentration.

As hydrogels are known for their lack of mechanical strength, an investigation is proposed to form a very hard, rigid crosslinked network from aqueous media that would not be susceptible to the forces of gel syneresis. Since hydrogels are very

pliable, soft and contain substantial amounts of water, they tend to be readily affected by such forces. By achieving such a rigid, “hydroglass” system, it is believed that the observed volume changes/shrinkage would be vastly improved, hence improving the long-term stability of the system. Various acrylates, methacrylates, acrylamides, crosslinking agents, initiators, and initiator concentrations will be investigated for their ability to form such a network system. Of course, once formed, all requirements: bR-compatibility, light scattering characteristics, and long term stability will be investigated.

In summary, the overall objectives of this thesis include:

- (1) The improvement of optical properties within PAm hydrogels through optimization of the polymerization conditions. Reduction of the overall light scattering properties within the hydrogel and utilization of this matrix material in a bR-based 3-D optical memory device.
- (2) The effective reduction of inherent light scattering within PAm hydrogels through the use of various additives and the methodology of Refractive Index Matching.
- (3) The investigation into photo-curable copolymers as possible materials displaying long-term stability and acceptable optical properties for device application.
- (4) The investigation into the formation of hard, rigid “hydroglass” matrices displaying improved long-term stability and light scattering properties for device application.

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CHAPTER II

Investigation and Optimization of the Factors Influencing Polyacrylamide Hydrogel Clarity and Stability

II.1 Introduction

As outlined in the preceding chapter, hydrogels are not a typical material utilized in optical applications. Many hydrogels are known to display a high degree of biological compatibility, however, they also tend to display poor optical properties and reduced stability due to relaxation of the network. Thus, these two factors; (1) *light scattering* characteristics of the material and (2) *stability* of the material are of paramount importance when considering a system for potential use as an optical matrix.

Several studies have shown that the physical properties of PAm gels such as the equilibrium degree of swelling, elastic modulus, and the clarity (light scattering) strongly depend on the conditions of gel preparation. For example, initial monomer concentration, initiator concentration, and crosslinking concentration have all been shown to influence such physical properties of PAm gels¹⁻⁵. In addition, several experiments have shown the formation of opaque PAm gels above a critical crosslinking concentration⁶. The opacity in these PAm gels is known to be a result of the spinodal decomposition of the polymer network concentration into smaller domains with two different densities, one dense and the other dilute. These concentration or density fluctuations can act to scatter light and reduce the transmission of visible light through the gel. Domain formation in polymer gels and the resulting inhomogeneities and heterogeneities are usual features of polymer networks produced via free radical polymerization. This is most likely due to the fact

that the formation of network chains by a free radical mechanism generally takes place within a short period of time when compared to that of the relatively long relaxation times of the network chains. Thus, these inhomogeneities become “frozen” into the final network structure.

Even though Am and MBA have approximately equal reactivity, it has been argued that the slightly higher reactivity ratio of MBA contributes to the network heterogeneity⁷⁻⁸. In response, others have argued that the reactivity difference between MBA and Am was too small to account for the extent of abnormalities observed and that the large scale heterogeneity in PAm gels was due to intramolecular cyclization⁹⁻¹⁰. Recently, Pekcan and Kara¹¹ studied the free radical crosslinking copolymerization of Am and MBA using an optical transmission technique where they observed that the transmitted light intensity, I_{tr} , decreased dramatically as Am is copolymerised with MBA, indicating the formation of *microgels*. Microgels are intramolecularly crosslinked macromolecules in solution with extended linear polymeric arms that are usually swollen and transparent. Microgels are molecular species that border between normal molecules and particles¹². The formation of PAm microgels is believed to occur prior to the onset of macrogelation¹⁰. As the reaction proceeds, microgels are connected to a macrogel through their peripheral pendant vinyls and radical ends due to the high extent of cyclization reactions. At this stage, highly intramolecularly crosslinked microgel particles formed in the pre-gel period act as junction points¹¹. The microgels are presented as the junction points of the final inhomogeneous network structure.

The formation of these PAm microgels is dependent on various aspects of the polymerization conditions¹³. The smaller the size/volume of formed microgels, the

less light scattering that is observed. In addition, incomplete macrogelation of microgel particles leads to greater heterogeneity in the resultant gel. Thus, no matter which school of thought one may choose to explain the inherent light scattering characteristics of a PAm hydrogel, they both seem to be greatly influenced by the conditions of gel preparation. Therefore, for purpose of thesis an understanding of all relevant factors affecting the light scattering characteristics within a PAm network would greatly aid in optimizing the hydrogel synthesis for production of optical matrix materials.

PAm stability (shape/volume) is similar to that of other hydrogels formed via free radical copolymerisation with a crosslinking agent. The process of polymerization is very quick and crosslinking occurs at the same time of chain growth. Thus, much tension or stress is inherently built into the hydrogel network upon initial formation. *Relaxation* of this system occurs in an attempt to reach a favoured stable state. However, the process of relaxation occurs slowly over time as the crosslinks within the network act to restrict chain motion. *Syneresis* is another term that can be used to explain the occurrence of gel deformation over time. The process of syneresis in gels results from the kinetics of gel formation. The initially formed gel structure may not be the most stable. The diffusion of polymer chains, which is greatly retarded in the gel, may slowly lead to the formation of a more stable compact structure. An increased pressure may then be exerted on the water within the gel, leading to its slow expulsion, a process termed *syneresis*.

Factors of polymerization, or conditions of gel formation/storage can affect the rate of relaxation in a given PAm gel. Therefore, investigations into all relevant

factors/conditions were performed in an attempt to improve the “shelf-life” of a PAm hydrogel matrix such that it may be utilized for device applications.

II.2 Determination of all relevant factors influencing the inherent light scattering within a PAm hydrogel

In order to fully understand and thus optimize the inherent light scattering characteristics within a PAm hydrogel matrix, an investigation was performed to individually identify all relevant conditions/factors that contribute to observed light scattering of this system. A logical and methodical approach to identify factors within the overall system and polymerization conditions was taken. All hydrogels compared in each experiment were prepared via the same methods, with the only deviations being the variable under investigation. It should be noted that when the term “system” is used in the context of a PAm matrix throughout this chapter and the remainder of this thesis, it is meant to encompass all of the polyacrylamide crosslinked network, the water solvent, the polymethacrylate (UV grade) cuvette, and the optical sealant.

II.2.1 Influence of Dust Particles on the Light Scattering of PAm hydrogels

The first factor investigated with the PAm system was a fairly obvious one. The cuvettes used to prepare the matrix cube were observed to contain a fair amount of dust particles. Upon investigating the difference between a PAm gel prepared in a cuvette “as is” to that of a PAm gel prepared in a clean or dust free cuvette, it was observed that the particles contained within a given cuvette contribute significantly to

the overall light scattering of the system (Figure II.1). These cuvettes contain a certain degree of dust and particulate matter upon receipt from the distributor likely as a result of the packaging conditions. Removal of the dust and particulate matter was achieved through the use of pressurized inert dusting gas.

As seen in figure II.1 removal of dust from the system reduces the light scattering characteristics of the hydrogel system by approximately 32% or by roughly one third.

Figure II.1: Influence of dust/particulate matter on the light scattering characteristics of a PAm hydrogel.

II.2.2 Influence of water purity on the light scattering characteristics of PAm hydrogels

The next relevant factor to be investigated was that of water purity. Since hydrogels are comprised of up to 90% water in some instances, the overall purity of the water should contribute somewhat to the observed light scattering characteristics

within system. In order to do this, gels were prepared using regular tap water, distilled water from the departmental resident still, and distilled/deionized water from a Barnstead Nanopure Infinity water purification system. The observed results are outlined in Figure II.2. From the figure, it is evident that water purity contributes slightly to the overall light scattering characteristics of the system. The use of tap water displays a peak with the highest amount of light scattering, likely as a result of a higher particulate content. Distilled water provides a slight reduction in the observed light scattering peak with an improvement of approximately 6% over that of tap water. The distilled/deionized water provides a further improvement over that of tap water displaying a reduction of approximately 11%.

Figure II.2 The effects of water purity on the light scattering of PAM hydrogels

II.2.3 Influence of monomer concentration on the light scattering characteristics of PAm hydrogels

Commercially, the majority of PAm hydrogels are prepared at a concentration of around 10 wt % of acrylamide monomer as outlined in the literature¹⁴. This concentration of monomer, in the presence of an appropriate crosslinking agent, provides a flexible, sturdy hydrogel suited to a variety of applications. Typically, N,N'-methylenebisacrylamide is more often than not employed as the crosslinking agent. By systematically changing the acrylamide concentration in the initial polymerization solution, an investigation into the effects of monomer concentration on the overall light scattering characteristics of the hydrogel system was carried out. The observed results can be seen in Figure II.3. The results display an obvious trend in the reduction of light scattering within a given hydrogel as the weight/volume concentration of monomer (Am) is increased.

Figure II.3: Reduction of light scattering within a PAm hydrogel as a result of increased acrylamide concentration.

The investigation covered a range of monomer concentrations from 5 wt % up to 25 wt %. The 5 wt % acrylamide concentration displayed a peak representative of the highest level of light scattering within the hydrogel system. On the other hand, 25 wt % acrylamide showed the lowest level of inherent light scattering with a reduction of approximately 93% over that of the 5 wt % concentration. Thus, it is evident that monomer concentration plays a significant role in determining the overall light scattering characteristics within a PAm hydrogel. This observed decrease in light scattering is likely due to the fact that as monomer concentration is increased, the frequency and size of crosslink aggregates is possibly reduced due to the greater abundance of reactive monomer species. Thus, the inhomogeneities produced via crosslink aggregation will likely have a reduced size/diameter, thereby resulting in a lowered level of light scattering observed within the system as the monomer concentration is increased.

II.2.4 Influence of crosslinking agent on the observed light scattering characteristics within a PAm hydrogel

Investigations into the effect of utilizing different types of crosslinking agents on the light scattering of a PAm hydrogel system were also carried out. Various spacer arm lengths were tested as well as a crosslinker displaying increased rigidity. The various crosslinkers tested can be observed in Figure II.4. The results displayed no drastic improvement of light scattering reduction going from a short chain spacer such as methylenebisacrylamide to a long chain spacer such as ethylene glycol diacrylate 400. There were slight improvements in the overall light scattering but these were observed to be minimal. Figure II.5 shows the general trend in light

scattering for PAm hydrogel systems utilising MBA , EGDA (400), and PDA as the crosslinking agents. The greatest difference in light scattering was observed when PDA was used instead of a straight chain crosslinking agent. PDA contains a piperazine ring system and thus displays a higher degree of rigidity than that of the straight chain counterparts. This rigidity is also observed in the resultant hydrogel, the gels become more durable and have improved strength when this crosslinking agent is used. However, due to the increased hydrophobic nature of PDA, the light scattering of the resultant gels is considerably higher than that of gels formed using MBA or EGDA.

Figure II.4 : Various crosslinking agents tested for their effects on the light scattering characteristics within a PAm hydrogel

Figure II.5: The effect of crosslinker type on the resultant light scattering within a PAm hydrogel system

II.2.5 Influence of the crosslinking concentration on the overall light scattering within a PAm hydrogel

It has been long known that increasing the crosslinking concentration within a given hydrogel, will result in a stronger, more durable gel as well as compromise the optical transparency of the system⁷. The more crosslinking induced within a given system, the more water that becomes expelled, thereby yielding microprecipitation of certain network regions and areas of high crosslink density. This is observed as “cloudiness” within the hydrogel. This precipitation or cloudiness results in very high levels of light scattering. However, investigations were performed to find a suitable working range of crosslinking concentration that would yield durable gels and yet contain a reduced level of inherent light scattering. MBA was chosen as the crosslinking agent since no real benefit was observed with the use of other compounds, as seen in section II.2.5. Crosslinker concentration was studied over a

range of 0.10 wt% to 0.30 wt%. Below 0.1 % the gels were too fragile and weak, and above 0.30% the gels were too cloudy/opaque. The results are displayed in Figure II.6. As expected, the amount of light scattering observed within the system increased with increasing crosslinker concentration. Figure II.7 displays these results in graphical form where it is easily observed that the range of greatest sensitivity falls between 0.2 w/v% and 0.3 w/v%

Figure II.6: The effect of MBA concentration on the overall light scattering within a PAm hydrogel system.

Figure II.7: Changes in light scattering with increasing concentration of MBA

II.2.6 Influence of initiator concentration of the overall light scattering within a PAm hydrogel

It is a well-known fact of free radical polymerization, that by varying the concentration of a free-radical initiator, the resultant molecular weight of the formed polymer will also vary¹⁵. The higher the concentration of initiator, the greater the concentration of initiating radical species and thus the lower the molecular weight will be. Conversely, the lower the concentration of initiator, the less initiating radical species there will be present to initiate chain propagation, and hence the greater the molecular weight will be. Typically, acrylamide can be polymerized with a variety of initiators, however, a commonly employed system is that of ammonium persulfate (APS) in the presence of a small amount of tertiary amine to act as an accelerator, such as N,N,N',N' tetramethyl-ethylenediamine (TMED). An experiment was set up to determine how the initiator concentration (APS), and thus molecular weight of resultant polymer chains, affects the overall light scattering within a PAm hydrogel

matrix. Figure II.8 displays the light scattering peaks observed within PAm hydrogels prepared using 0.026 wt%, 0.053 wt %, and 0.107 wt % of ammonium persulfate initiator. 0.026 wt % of APS can be considered as being a rather “low” concentration, while 0.107 wt% can be viewed as being a rather high concentration of APS. If the concentration of APS is too low, however, no polymerization reaction will take place. From Figure II.8 it can be seen that the greater the concentration of APS, the more the light scattering is reduced within the hydrogel. However, the strength of the hydrogels at greater APS concentrations seems to be compromised. This is likely a result of shorter polymer chains and hence crosslinking may not function to the effect that it should. Crosslinking of two short polymer chains makes a longer “short” chain. Therefore, the shorter chains when crosslinked may still have a fair degree of motional freedom, as compared to the crosslinking of longer chains. In addition, chain initiation of a large amount of polymer chains (i.e. high APS concentration) would lead to the formation of smaller PAm microgels throughout the resultant hydrogel. This would lead to an obvious reduction of inherent light scattering within the formed PAm hydrogels.

Figure II.8: The effects of APS concentration on the overall light scattering within a PAm hydrogel.

II.2.7 Influence of solution pH on the overall light scattering observed within a PAm hydrogel system

PAm structure is known to be influenced by changes in pH¹⁵⁻¹⁶. The kinetics of polymerization, namely propagation and termination for PAm are somewhat affected by changes in solution pH¹⁵, but to no significant degree. However, it is known that PAm can be degraded under both acidic and basic conditions. Degradation occurs more readily under basic conditions. In the gel form, PAm hydrogels are known to swell and imbibe greater amounts of water under acidic conditions. Whereas swelling is not observed under basic conditions. An experiment was performed such that the effects of pH on the inherent light scattering of PAm hydrogels could be studied. Figure II.9 displays the observed relative light scattering within PAm hydrogels formed over a range of pH. The pH variations were from pH 5

up to pH 9. It was seen that as pH of the solution is decreased, i.e. more acidic, the light scattering within the hydrogel was reduced. Table II.1 outlines the corresponding pH of the polymerization solution prior to hydrogel formation as well as the pH of the resultant hydrogels which were used to produce Figure II.9. All gels displayed a reduction in pH to some extent, when going from a solution to a gel. The observed reduction of light scattering with decrease in pH, may be explained by the fact that at lower pH values, more swelling is observed. Thus, the more swelling that occurs, the more the network may expand and dissipate some of the regions of higher density/entanglement (i.e. spread the density over a greater area).

Figure II.9: Effects of pH on the overall light scattering within a PAm hydrogel

Table II.1: pH values of PAm hydrogels before and after polymerization

pH of Polymerization Solution	pH of formed Hydrogel
5.10	4.99
5.97	5.89
7.05	6.84
8.15	7.88
9.27	9.04

II.2.8 Influence of storage time and storage temperature on the overall light scattering observed within a PAm hydrogel

Upon hydrogel formation, much tension and entanglement is introduced into the system. Over time the system “relaxes” to a more stable state. Various factors affect the rate of this occurrence by either slowing the process or accelerating it. An experiment was set up in order to examine the ageing effects that the process of gel syneresis displays on the overall light scattering within a PAm hydrogel. The PAm hydrogels were monitored over the course of 2 months (8 weeks) and the relative light scattering was recorded on a regular basis (1 week intervals). The hydrogels were also stored at different temperatures to investigate the effects temperature played on the process of gel syneresis, and hence on the overall light scattering. Figure II.10 shows the relative light scattering peaks of three hydrogels stored for 1 month at a temperature of 5°C, room temperature (23°C), or at 45°C. It can be seen from the figure that when a hydrogel is stored at higher temperatures, the more evident a reduction in overall light scattering becomes. This is likely a result of the fact that heat supplies energy which can be utilized to reach a more stable, relaxed state (i.e. aids chain mobility). In the more stable and relaxed state, there is likely less entanglements and thus less regions of high density (less inhomogeneities) than in the highly stressed, entangled gel that is originally formed. Therefore, there is an observed decrease in the light scattering characteristics of a relaxed hydrogel, compared to that of a newly formed hydrogel.

Figure II.10: Effects of storage temperature on the overall light scattering within a PAm hydrogel. (gels were incubated at temperatures for 1 month)

Figure II.11 shows the relative light scattering within the PAm hydrogels stored at 5°C, RT (23°C), and 45°C over a time frame of 8 weeks. As expected, the ageing study outlined that the gel stored at 45°C had the greatest reduction in light scattering over time, as well as the most rapid reduction (steepest slope) over the first few weeks. It seems as though this gel nearly levelled off at the 8 week mark, suggesting that the hydrogel was approaching its most stable, relaxed state. The gel stored at 5°C showed the least amount of light scattering over the 8 week period. This gel also displayed the least significant slope, indicating that the relaxation process occurs very slowly at lower temperatures.

Figure II.11: Light Scattering reduction within a PAm hydrogel stored at either 5°C, RT, or 45°C over an 8 week period.

II.3 Determination of all relevant factors influencing gel syneresis / polymer relaxation within a PAm hydrogel

The second factor of major concern when attempting to utilize a PAm hydrogel as an optical matrix is that of stability. The term “stability”, when used in the context of this thesis, refers to the ability of the originally formed material to maintain its shape, size, and volume. Therefore, any shrinkage or deformation noted within the original material will be considered as a lack of stability to some extent. The process of polymer relaxation / gel syneresis, and possibly even water loss, contribute to the observed stability of a formed hydrogel. This issue of material stability plays a crucial role in various device applications.

II.3.1 Influence of storage time on the stability of a PAm hydrogel

Since the well known processes of polymer relaxation and gel syneresis occur over time, it is not surprising that as time increases, the amount of observed deformation/shrinkage of a hydrogel should also increase as these processes work to achieve the most favoured, stable, relaxed state. Figure II.12 follows the estimated percent deformation of a PAm hydrogel with time. The value for % deformation encompasses both the vertical shrinkage within a gel cube as well as any contact departures from the surface of the cuvette (i.e. bubbling or pulling away from the sides of the cuvette). These values were totalled and compared to the original size/volume of the PAm cube yielding an estimated value for % deformation from the original structure. As prepared here, it seems that the PAm hydrogel displays a usable shelf life of approximately 2 months, after which too much distortion/deformation in its shape/volume has occurred.

Figure II.13 outlines a newly formed hydrogel with no observable deformation compared to that of a hydrogel displaying the typical deformations noted within these systems, such as vertical shrinkage and bubbling.

Figure II.12: Estimated % deformation of a PAm hydrogel over 8 weeks

Figure II.13: A) PAm hydrogel with no observable deformation and B) PAm hydrogel displaying bubbling with vertical shrinkage and C) PAm hydrogel displaying severe vertical and horizontal shrinkage

II.3.2 Influence of storage temperature on the stability of a PAm hydrogel

As previously outlined above, supplying energy in the form of heat to a newly formed hydrogel network may assist chain mobility and increase the rate of polymer relaxation (gel syneresis). Therefore, an experiment was set up to monitor the % deformation of PAm hydrogels stored at 3 different temperatures, 5°C, RT (23°C), and 45°C over the course of an 8 week period. Figure II.14 displays a bar graph depicting the estimated % deformation of each hydrogel from that of their original structure. The hydrogel stored at 45°C showed the greatest amount of deformation over the 8 week period. Thus, the rate of gel syneresis was increased with increased temperature. The hydrogel stored at 5°C showed the least amount of deformation with time, thus suggesting the process of gel syneresis was retarded.

Figure II.14: Effect of temperature on the stability of a PAm hydrogel

II.3.3 Effect of water loss on the stability of a PAm hydrogel

Since hydrogels are comprised of large amounts of water, it is reasonable to suspect that shrinkage/distortion of original gel volume may be a direct correlation to loss of water from the system through either evaporation or possibly loss to the cuvette itself. In order to test this idea, two experiments were performed. The first experiment was set up to test evaporation. Nine PAm gels were prepared, all by the same means. Three of the gels were sealed with parafilm, three gels were sealed with wax and optical adhesive sealant, and three gels were sealed with the wax and optical sealant but submerged in a water bath at room temperature. The gels were monitored for a period of 1 month and their % deformation recorded. The results showed that the gels sealed in parafilm were the quickest to deform and displayed the greatest deviation from their original structure/volume. The other two sets of gels displayed low levels of vertical shrinkage. The main point being that even the gels submerged in water, showed approximately the same amount of vertical shrinkage as those sealed in the same manner and left to stand at room temperature in air. Thus, these results help rule out the possibility that water loss via evaporation is not responsible for the observed deviations in hydrogel structure/volume with time.

The second experiment was to investigate the possibility of water loss to the walls of the polymethacrylate cuvette. In order to test this, six gels were prepared; three in cuvettes used directly from the box that were then sealed with wax and optical sealant, and three prepared in “pre-soaked” cuvettes. The cuvettes were soaked in water for 12 days, and then PAm gels were prepared inside them in the usual manner. These were also sealed with wax and optical sealant. The %

deformation was monitored over the course of 1 month. The amount of deformation seen in the two sets of gels was low, but approximately the same. Thereby indicating that water loss to the cuvette walls does not account for the observed shrinkage/deformation in the sealed PAm gel systems.

II.4 Conclusions

The outlined investigations into factors of Am polymerization and of the formed PAm hydrogel systems allowed all variables influencing the inherent light scattering and stability within these gels to be understood. For application as a possible optical matrix material, the experiments helped to set ranges or limitations in regard to certain aspects of the polymerization conditions or to the system setup. From the gathered results, an optimized formulation for preparing a PAm hydrogel system displaying the greatest possible stability and optical transparency (lowest light scattering) may be achieved.

In terms of light scattering, it seems as though an optimized formulation for the preparation of a PAm hydrogel would require a dust free cuvette, the use of purified water, a high concentration of monomer, a relatively low concentration of crosslinking agent (not too low to compromise stability), a concentration of initiator that is not too low (because greater amount of inhomogeneities produced) but not too high (gel too weak), a pH of approximately 6.0, and an incubation period of about 3-4 weeks at an elevated temperature to facilitate polymer relaxation/gel syneresis.

In terms of PAm stability it would seem that the preparation of an optimized formulation for these gels would require, a higher crosslinking/monomer concentration, and reduced temperatures of storage. In addition, for material stability

it is essential that the hydrogel system be sealed from the environment, mainly, sealed air tight with an epoxy sealant. It is evident from the results that in certain circumstances the factors influencing light scattering and stability work to oppose each other. For example, heating the gels yields better light scattering characteristics over time, yet at these elevated temperatures there is an increased rate of deformation with time.

For potential application as an optical matrix material within a biomolecular device of some sort, other factors need to be considered as well. Such as, if a biological compound is to be added to the system, it would likely act to scatter light and thus an appropriate concentration must be determined/optimized for the device application. As well, acrylamide in monomeric form is highly toxic and at elevated concentrations it would likely affect biological function. Thus utilising high concentrations of monomer to lower the light scattering characteristics of the resultant PAm hydrogel may not be possible when incorporating active biological compounds.

Unfortunately, although the light scattering and stability characteristics of PAm were investigated, the optimization of all the relevant and outlined factors yielded an improved system which still falls short of the optical clarity required by many devices. Thus further improvements/modifications to the system are required.

The questions arising from this work were therefore twofold: (1) How can the inherent light scattering within a PAm hydrogel be further improved above and beyond the polymerization/preparation conditions outlined here? And (2) How can the stability of a PAm hydrogel be improved such that the shelf life of the polymer cube system be extended to approximately that of 1 year (a common device requirement for commercialization)?

II.5 Experimental Section

Materials

All reagents such as; acrylamide, ammonium persulfate, N,N,N',N' tetramethylene-ethylenediamine, N,N' methylenebisacrylamide, Hydrochloric acid, sodium hydroxide, and ethylene glycol diacrylate were purchased from the Aldrich Chemical Company and used as received. Piperazine diacrylate was purchased from BioRad and used as received.

The polymethacrylate cuvettes, (UV- grade) were purchased from Aldrich Chemical Company, while the inert dusting gas used to clean them was purchased from Chemical Stores in the Steacie building. The dusting gas is prepared by the company TechSpray and is labelled as “Envirotech – Duster” (1,1,1,2 tetrafluoroethane).

Measurements

All light scattering measurements were performed on a Shimadzu RF-1501 spectrofluorimeter, using an empty cuvette as a blank. The samples were measured on an emission setting, meaning the system was excited at 700 nm and the emission spectrum was detected over the range of 650 – 800 nm. The 700 nm excitation wavelength was chosen as a specific device application functions at wavelengths in this region (chapter 3).

All stability measurements were done via an estimated comparison to the original gel shape/volume. The prepared gels were marked as to their height in the cuvette upon formation. Any vertical shrinkage was measured directly and assigned a percentage of the original height. All deviations, deformations along the sides of the

cuvette had to be estimated in the early stages. Each face of the polymer cube was assumed to be approximately 5% of the polymer volume such that if one complete side of the polymer cube was removed/pulled away from the side of the cuvette, then it was said to have been an estimated 5% deformation in volume. Portions smaller than this (i.e. small bubbles on the side) were estimated in relation to the total size of that side of polymer cube. Once the sides were significantly removed from the walls of the cuvette measurements could be made directly and assigned a percentage of the original width.

Hydrogel Synthesis

A typical synthesis for a PAm hydrogel used throughout this chapter is given as follows:

Stock solutions of Acrylamide (Am), Ammonium persulfate (APS), N,N,N',N' tetramethylene-ethylenediamine (TMED), and N,N' methylenebisacrylamide (MBA) were prepared as follows: a 60 wt % Am stock solution (12.0 g Am in a total 20 mL H₂O solution), a 2% APS solution (0.200 g in total 10 mL H₂O solution), a 20% TMED solution (2.6 mL in a total 10 mL H₂O solution), and a 5% solution of MBA (0.500 g in a total 10 mL H₂O solution). All solutions were prepared using distilled/deionized H₂O and all solutions were purged for 10 minutes using Nitrogen.

In each 4 mL polymethacrylate (UV grade) cuvette 0.50 mL of Am solution, 0.120 mL MBA solution, 0.050 mL TMED solution and 2.27 mL distilled/deionized H₂O were added. Finally, to the solution was added 0.060 mL of APS solution and the polymerization solution in the cuvette was then mixed quickly, sealed with

parafilm and set aside to complete polymerization. Once the reaction seemed to be finished, after about 10 minutes, wax was added to the top of the gel and a UV-cured epoxy was applied atop the wax to further seal the system. For each gel prepared, a total volume of 3 mL was used.

This procedure was applied to each section of this chapter as a general means of preparing PAm hydrogels, however, in each section certain factors were varied in order to study their effects on the overall light scattering and stability characteristics of the material. Slight variations to the PAm hydrogel protocol are as follows in each experiment:

(II.2.1) Influence of dust particles : cuvettes were used either as is or cleaned using pressurized 1,1,1,2 tetrafluoroethane.

(II.2.2) Influence of water purity: 2.27 mL of either Tap water, Distilled water, or Distilled/deionized water was added to the polymerization solution.

(II.2.3) Influence of monomer concentration: The amount of Am stock solution added was varied to yield 5, 10, 15, 20, and 25 wt % of acrylamide in the final polymerization solution. The amount of water added in each case had to be varied to compensate for a total volume of 3 mL for each sample prepared.

(II.2.4) Influence of crosslinking type: In each case, 0.120 mL of a 5 wt % solution of either MBA, EGDA, or PDA was used.

(II.2.5) Influence of crosslinking concentration: The amount of MBA stock solution added was varied in order to yield 0.1, 0.15, 0.20, 0.25, 0.30, and 0.35 wt % of MBA in the final polymerization solution. In each case the amount of water added was varied in order to compensate for a total final volume of 3 mL in each sample.

(II.2.6) *Influence of initiator concentration:* For samples prepared, either 0.030 mL of TMED and 0.040 mL of APS, or 0.060 mL TMED and 0.080 mL APS, or 0.120 mL TMED and 0.160 mL APS were used to yield PAm hydrogels prepared under various initiator concentrations. In each case the amount of water added was varied in order to compensate for a total final volume of 3 mL in each case.

(II.2.7) *Influence of pH:* In each case, dilute aqueous solutions of HCL or NaOH were used to adjust the pH of polymerization solution (prior to polymerization) to a desired value. For each sample, only 2.0 mL of distilled/deionized water was added.

II.6 References

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CHAPTER III

Refractive Index Matching to Enhance the Optical Clarity Within PAm Hydrogels

III.1 Introduction

The previous chapter outlined various factors of polymerization that affect the overall clarity and long-term stability of PAm hydrogels. After optimizing these conditions, the production of PAm hydrogels resulted in systems that displayed improved optical clarity but required further enhancement of these properties before being considered for use as an optical matrix. In order to achieve this, an understanding of the scattering process within a PAm hydrogel is necessary.

When a beam of light is passed through matter in the solid, liquid, or gaseous state, its propagation is affected in two important ways: 1) the intensity will always decrease as the light penetrates further into the medium, and 2) the velocity will be less in the medium than in free space. The loss of intensity may primarily be due to absorption, although under certain circumstances scattering may make significant contributions.

Light scattering is caused by density and concentration fluctuations. A hierarchy of the various forms of light scattering can be seen in Figure III.1. The first notable division in light scattering is elastic and inelastic scattering. For inelastic scatter, there is an energy loss associated with the scattered light (red shift) while elastic scattering displays no energy loss such that the scattered emission is identical in wavelength to the incident light¹. Thus, Rayleigh, Thompson, and Mie scattered

light has the same wavelength as the incident light, while Raman scattering displays a change in wavelength from the incident light.

Figure III.1: Breakdown of Light Scattering Phenomena

In 1871, Raleigh explained that even the purest of substances have fluctuations in their refractive index which can act to scatter light. He also showed that the intensity of scattered light is related to that of the incident light by the inverse fourth power of the wavelength ².

Polymeric materials can range from highly transparent to opaque. Loss of transparency arises from scattering processes within the material, which distort and attenuate any transmitted image. If a material is non-absorbing, and optically homogeneous, as well as isotropic, the transmitted ray of light propagates without loss of intensity. However, in real materials the transmitted ray may lose intensity as it travels forward due to scattering from refractive index inhomogeneities within the material (Figure III.2), as outlined by Rayleigh.

Figure III.2: Scattering of light on transmission through a transparent medium

As depicted in the Figure III.2, such scattering is analogous to the ray splitting which occurs at the surface p and which is repeated whenever the ray encounters a change of refractive index, δn , at points q within the material. The angular distribution of scattered light is not simple, but can be generally divided into back-scattered and forward-scattered components, B and F respectively, of the total flux ³.

The effect of scattering is to reduce the contrast between light and dark parts of an object as viewed through a transparent polymer, and thus produce a *haze* or milkiness in a transmitted image. Besides haze, scattering may produce loss of *clarity* so that distinct features of an object can no longer be distinguished in a transmitted image. Loss of clarity is caused by the forward scattering at angles very close to the undeviated beam, whereas haze is associated with scattering over a wide range of angles ³.

In polymer materials, heterogeneity of refractive index (RI) can arise from differences in density in amorphous and crystalline regions within the polymer itself,

from solid particles incorporated into the polymer materials, or from voids. As in the case of hydrogels, heterogeneity of RI may also arise from differences in density due to heterogeneities in crosslink density throughout the network. The degree of light scattering depends greatly on the variation of refractive index δn and on the size of the heterogeneities. The most efficient scattering occurs when the scattering centers are comparable in dimensions with the wavelength of light.

Generally, the refractive index of a material is estimated in terms of the molar refraction (R), which quantifies the intrinsic refractive power of the structural units constituting that material. A definition proposed for R that incorporates both of the key physical factors determining the refractive index (n), is the molar refraction according to Lorentz and Lorenz ⁴ :

$$R_{LL} = V (n^2 - 1 / n^2 + 2)$$

Where V is the molar volume. For a neat polymer one can sum the atomic and group refractions to yield the molar refraction of a repeat unit. This can then be related to the refractive index (n) by utilizing the Lorentz-Lorenz equation:

$$(n^2 - 1 / n^2 + 2)(M / \rho) = \Sigma M r_d$$

Where M is the molecular weight of the polymeric repeat and ρ is the density. The right side of the equation is represented by the sum of all the atomic and group polarizabilities ⁵.

By increasing the refractive index of the surrounding aqueous media through the incorporation of additives, it may be possible to compensate for “refractive index inhomogeneities” formed within a developing hydrogel network. The observed result should be a reduction in the amount of light the hydrogel scatters. This method has

been suitably termed “*refractive index matching*” and may utilize all forms of additives (i.e. liquids and solids) to better match the RI of two different compounds/materials whatever they may be ⁶. Applied to polymer systems interacting with solvent, this method can be adequately explained using the equation for the specific refractive index increment (dn/dc) which is a term dependent on the polymer and solvent;

$$dn/dc = (n_p - n_s) / c$$

Where n_p is the refractive index of the polymer network, n_s is the refractive index of the solvent and c denotes the concentration of polymer in solution (g/mL) ⁴. By keeping the concentration of polymer constant, one can see that minimizing the difference between n_p and n_s , yields a smaller (dn/dc) term and thus, less light will be scattered by the system. In the case of a PAm hydrogel, n_p refers to polyacrylamide network and n_s refers to water.

PAm hydrogels are clear and homogeneous on a macroscale, but on a molecular scale, the situation is more complex. Through neutron scattering and light scattering measurements it has been shown that PAm gels are not homogeneous and should be regarded as random networks ⁷. These random networks can be viewed as areas of high point density (or refractive index, RI) randomly distributed throughout the water medium. Due to these inhomogeneities of density or RI, PAm hydrogels tend to have poor optical properties and have thus been overlooked as a potential material for optical applications. However, PAm is a well-known and commonly used hydrogel that displays high compatibility with proteins. It is used in numerous biological applications such as gel electrophoresis.

Hydrogels have been extensively implicated in various biomedical applications such as soft contact lenses^{8,9}, soft tissue replacement and wound management¹⁰, some forms of prostheses^{11,12}, artificial organs¹³, as well as in controlled drug delivery and other pharmaceutical uses^{14,15}. Until recently, however, hydrogels have only been implemented in optical applications through their fabrication as soft contact lenses or intraocular lenses. Alternative optical applications for hydrogels, such as; their use in laser fabrication¹⁶, as laser surgery aids¹⁷, or as a medium for imaging biological structures via confocal scanning laser microscopy¹⁸ are being realized as refinement of their structure/property characteristics continues.

Biomolecular devices have been of growing interest as the exciting and novel characteristics of many biological compounds are now starting to be realized¹⁹. Many of these emerging bio-devices are regulated and/or function through optical processes that require a high degree of optical clarity within the matrix material. For example, bacteriorhodopsin based three-dimensional optical memory storage^{20,21} is a biomolecular device generating significant interest as of late. A device such as this requires that the current optical materials be compatible with native protein structure and function. There are several known and available optical materials to date, however, their lack of chemical compatibility with biological molecules such as proteins drastically minimizes the available selection. Thus, materials displaying high optical clarity and high compatibility to biomolecules are evidently in great shortage. In contrast to the current popular optical materials²², the development of optically transparent hydrogels may pose as an inexpensive, flexible, moldable, and

biocompatible alternative for use as an optical matrix in various biomolecular device applications. A hydrogel system displaying such characteristics would also benefit many other areas of research that would require a support matrix with high optical clarity and biological compatibility.

III.2 Refractive Index Matching: Additives Screening

As outlined in Chapter 2, the optical clarity of PAm hydrogels can be improved through optimization of the polymerization conditions. However, when considering PAm hydrogels for use as an optical matrix, further improvements to the overall optical clarity within the gel are necessary. As outlined by Lord Rayleigh, even the purest of substances have fluctuations in refractive index which can act to scatter light. Thus in a system such as a hydrogel where there exists a heterogeneous mixture of polymer network and water medium, fluctuations in RI are common. Since there is no energy loss, this type of light scattering falls under the classification of Elastic scatter but more specifically it is known as Rayleigh scattering. The main concerns when dealing with hydrogels as potential optical matrices is to minimize the fluctuations in refractive index between the polymer network and the surrounding water solvent, thereby reducing the observed scattering within the system.

This chapter outlines the method of refractive index matching to further improve the optical characteristics of PAm hydrogels. RI matching will be employed through the utilization of water-soluble additives. These additives must not disrupt or alter acrylamide free radical polymerization, and should increase the RI of the water solvent closer to that of the forming polymer network and its random inhomogeneities. As the RI of water is increased through the implication of such

water-soluble additives, the fluctuations in RI throughout the hydrogel will be reduced thereby decreasing the inherent light scattering of a given hydrogel matrix. This chapter also outlines a specific device application for an optical hydrogel matrix and introduces the concept of “biological compatibility”. The device application is that of a bacteriorhodopsin based 3D optical memory device^{20, 21} which involves the entrapment of bR protein throughout the PAm hydrogel network. Therefore, any additives to be utilized for improving the optical clarity within PAm via RI matching, must also display chemical compatibility to the protein and native protein function.

III.2.1 Additive Screening : Sugars

At first, the most logical choice to begin investigating additives for RI matching was that of sugars. Sugars tend to display high water solubility and are common compounds to biological systems. Therefore, a couple of different but readily available sugars were investigated for their ability to lower the light scattering characteristics within PAm hydrogels via refractive index matching. Figure III.3 displays the results observed when three hydrogels were prepared with a sugar concentration of 15 % (w/v) for each of glucose, sucrose, and trehalose. These were compared to a hydrogel containing no additive. As seen in the figure, all additives reduce the light scattering to some extent when compared to a PAm hydrogel prepared with no additive.

Figure III.3: Comparison of Hydrogels prepared with no additive, glucose 15%(w/v), sucrose 15% (w/v), or trehalose 15% (w/v)

All three sugars showed promise in their ability to decrease the observed light scattering within a PAm hydrogel and were therefore investigated further. Figure III.4 depicts the observed light scattering within a PAm hydrogel with increasing concentration of glucose additive. This additive initially shows great promise in reducing the observed light scattering when incorporated into a PAm network. It was observed that as the concentration of glucose increases, the inherent light scattering is reduced but eventually plateaus by a concentration of 40% w/v. At this concentration, the glucose additive does not induce any significant amount of light scattering reduction over that of the previous concentration studied. This is likely because glucose is a reducing sugar and acts to disrupt polymerisation of acrylamide at increased concentrations, possibly by quenching propagating radical species.

Figure III.4: Relative light scattering observed within PAm hydrogels with increasing concentration of glucose additive.

For the sugar trehalose, it was observed that light scattering is reduced within PAm hydrogels with increasing concentration of the additive as expected. Figure III.5 displays the observed results. The graph shows a general trend in light scattering reduction but depicts a region of greatest slope between a concentration of 15% and 20% w/v trehalose. This sugar additive does not display a plateau region within the concentration range investigated such as that which was observed with the glucose additive, likely due to the fact trehalose is a non-reducing sugar.

Figure III.6 depicts the relative light scattering observed within PAm hydrogels with increasing concentration of sucrose additive. Sucrose is a non-reducing sugar, and therefore does not seem to affect acrylamide polymerisation. The reduction of light scattering is uniform and fits nicely to an exponential curve with an R^2 value of 0.9886. At increased concentrations, the level of light scattering

reduction within the hydrogel seems to decrease and approach plateau, likely as a result of saturation limits within the polymerization solution.

Figure III.5: Relative light scattering intensity observed within PAm hydrogels with increasing concentration of trehalose additive.

Figure III.6: Relative light scattering within a PAm hydrogel with increasing sucrose concentration

Of the initial three sugar additives investigated, only sucrose was tested further for application towards preparing PAm hydrogels as an optical matrix suited to a bR based memory device. This was a result of the fact that glucose was a reducing sugar and displayed a plateau in its ability to reduce the light scattering within a PAm hydrogel. The level of light scattering reduction reached using the glucose additive prior to the plateau phase was not sufficient enough to merit further investigation. On the other hand, trehalose, although a seemingly promising additive, did not display light scattering reductions within PAm hydrogels to the extent that was observed for sucrose. In addition, trehalose was a very costly additive (more than 3x the cost of sucrose), and when being considered for application to a device, material cost plays an important role.

In Chapter 2, it was outlined that crosslinking concentration greatly affected the overall light scattering within PAm hydrogels. However, the methodology of refractive index matching can be applied to yield more optically transparent gels at higher crosslinking concentrations. Figure III.7 displays a reduction in observed light scattering with increased sucrose concentration over a range of PAm hydrogels formed at varying crosslinking concentrations. As the concentration of MBA is increased from one gel to the next, the values observed for light scattering are shifted higher, however the trend in light scattering reduction via incorporation of sucrose is common for all cases.

Figure III.7: Light scattering reduction with increased sucrose concentration over a range of PAm hydrogels formed at varying crosslinking concentration

III.2.2 Additive Screening: Amino Acids & Derivatives

In addition to sugars, amino acids are water soluble to varying extents and have known compatibility with proteins. Thus, these molecules were investigated for their ability to improve the optical clarity of a PAm hydrogel via refractive index matching. Several amino acids and amino acid derivatives were investigated, all of which displayed initial reductions in light scattering of a PAm system to some extent. Certain amino acids were limited to their use of concentration due to their solubility in water, while others displayed a tendency to inhibit polymerization of acrylamide at approximately 6% w/v, possibly as a result of pH change to the solution. Once buffered, the concentrations could be increased for some amino acids. Overall, several amino acids were effective at reducing the light scattering characteristics in PAm hydrogels at concentrations lower than what was observed with sugars. Figure

III.8 shows a representation of various amino acids and derivatives that were successfully incorporated into PAm hydrogels. These gels were then compared to that of a sucrose containing PAm hydrogel and a hydrogel containing no additive. All hydrogels prepared with amino acids or amino acid derivatives were made at a 2% w/v additive concentration while the sucrose hydrogel contained a 30% w/v additive concentration. The PAm hydrogel containing no additive was used as reference so that its inherent amount of light scattering was outlined as being 100%.

All hydrogels containing the additives displayed improved optical clarity and were thus compared in a relative manner as a percentage of the total light scattering observed within a PAm hydrogel containing no additive. As can be clearly seen from the figure, many of the amino acid or amino acid derivative additives display similar or lowered light scattering within a PAm hydrogel than that of a sucrose hydrogel at 15x the additive concentration.

Some of the more promising amino acid additives were investigated further for their ability to improve the optical characteristics of PAm hydrogels at increased concentrations. Proline, histidine, cystine, phenylalanine, threonine and glycine were the amino acid additives that initially displayed the most promising results and were thus tested further. However, phenylalanine and histidine were found to be fairly insoluble at concentrations above 4% w/v and thus were prepared as hydrochloride salts of the amine group above this concentration.

Figure III.8: Comparison of various amino acids, and amino acid derivatives in reducing the light scattering within PAm hydrogels to that of sucrose additive.

Figure III.9 outlines a typical study for a given amino acid at increased concentrations. In the figure, proline is incorporated into PAm hydrogels over a concentration range of 2-10% w/v and compared to that of a PAm hydrogel containing no additive. The light scattering study was investigated at 700 nm and as the concentration of proline was increased, the amount of light scattering observed in each gel decreased. A phosphate buffer (pH 7.2) was utilized as solvent such that higher concentrations of amino acid additives could be reached without affecting the polymerisation reaction of acrylamide.

Figure III.9: Light scattering comparison of PAm hydrogels containing various concentrations of the additive proline.

A similar investigation was performed for each of cysteine, histidine, threonine, phenylalanine and glycine. The levels of light scattering intensity within the PAm hydrogels were recorded for each concentration and plotted in graphical form. Figure III.10 represents the relative light scattering intensity for each of the outlined additives over a specified range of concentration (% w/v). As visible in the figure, glycine and threonine act to reduce light scattering within a PAm hydrogel gradually as their concentration is increased, depicted by the slight slope to their curves. On the other hand, proline, histidine, phenylalanine and cysteine seem to reduce the light scattering within a PAm hydrogel very rapidly with increased additive concentration as they display greater slopes to their curves. The greatest slope of reduction for these additives was observed in the initial stages of the graph, i.e. at lower concentrations. It should be noted that after an additive concentration of 4%

w/v for cysteine, no polymerisation reaction of acrylamide was observed, even in the presence of buffer. This is likely a result of the free sulfur group on the side chain of cysteine which may act to retard acrylamide free radical polymerisation at concentrations greater than 4% w/v. In addition, the curves depicted in figure III.10 for the additives proline, phenylalanine, and histidine were all observed to have similar trends. They all displayed a rapid decrease in light scattering up to 2% w/v but then gradually reduce the inherent light scattering within PAm hydrogels up to 10% w/v. This change in slope after 2% w/v could possibly be a result of additive solubility in the polymerization media at higher concentrations seeing as how the salts of histidine and phenylalanine had to be prepared at concentrations above 4% w/v. It should be noted that the compound histidine showed promise in reducing the light scattering within PAm hydrogels, however, over a short period of time proceeding formation of the gel, the system was observed to “yellow” at extents dependant on the concentration of histidine additive utilized.

Figure III.10: Light scattering intensity for PAm hydrogels prepared with various amino acid additives incorporated over a concentration range of 0-10% w/v.

III.2.3 Additive Screening: Alternative Additives

In addition to sugars and amino acids, other additives were tested for their ability to lower the inherent light scattering within PAm hydrogels via refractive index matching. Some polymers, of low molecular weight, were investigated for use as an additive within the PAm system, meanwhile, other additives tested were just water soluble compounds with suspected biological compatibility. These studies were performed on compounds such as glycerol, low molecular weight Polyvinylpyrrolidone (PVP) (MW ~ 10 000), Gelatin, and Ficoll 70 (a polymer of sucrose). Each of the compounds was initially tested for their ability to lower the light scattering observed within a PAm hydrogel and then further investigated over a range of additive concentration within the hydrogel.

Figure III.11 displays a representative relative light scattering for PAm hydrogels prepared with 5% w/v of the additives glycerol, Ficoll, Gelatin and PVP as compared to a hydrogel with no additive. Light scattering measurements were recorded at 700 nm as specified by the bacteriorhodopsin based optical memory device. It can be seen in the figure that all additives, with the exception of glycerol, display increased light scattering at a concentration of 5% w/v within a PAm hydrogel when compared to that of a hydrogel containing no additive. Even though many of these additives tested displayed increased scattering at a concentration of 5% w/v they were investigated further to test whether or not elevated concentrations would effectively reduce the inherent light scattering. Further investigation was performed on all additives with the exception of PVP. As can be seen in Figure III.11, the relative light scattering peak for PVP goes off scale of the spectrofluorimeter indicating a very high level of inherent light scattering for the hydrogel. This gel displayed signs of microphase separation between the low molecular weight PVP and the PAm network. A similar effect was observed when Polyvinylalcohol, PVA, ($M_w \sim 5000$) was incorporated at 5% w/v.

Figure III.11: Relative light scattering comparison of the alternative additives glycerol, gelatin, ficoll and PVP (at 5% w/v) as compared to a PAm hydrogel with no additive.

Figure III.12 outlines the range of additive concentration over which glycerol, gelatin, and ficoll was investigated within PAm hydrogels. The graph displays an initial increase in relative light scattering intensity for the additives gelatin and ficoll, however as the concentration is increased passed approximately 5% w/v the light scattering observed within the PAm hydrogels starts to decrease. At a 20% w/v concentration, ficoll starts to plateau in its light scattering reduction of a PAm hydrogel and begins to approach a saturation limit of the additive. Initially the additive gelatin, when incorporated into the hydrogel system, seems to result in a higher inherent light scattering value than any of the other additives investigated in this figure. However, it was also observed that gelatin begins to decrease light

scattering intensity within PAm hydrogels very rapidly after a 5% w/v concentration. This decrease may be representative of an increased absorbance in the range of 400-450 nm which tails out to the 700 nm region as the concentration of gelatin is increased. In comparison, the additive glycerol shows a gradual decrease in light scattering with increased concentration. Glycerol displays a very slight slope but as observed to effectively reduce the inherent light scattering of PAm hydrogel with increased concentration. Many of the alternative additives presented here do not function as well at reducing the inherent light scattering of PAm hydrogels than what was observed with the amino acid and sugar additives previously outlined.

Figure III.12: Relative light scattering intensity of PAm hydrogels formed over a concentration range of the additives glycerol, gelatin and ficoll

III.3 Concepts of RI Matching: Additive Induced Changes of RI Within PAm Hydrogels and Relation to their Inherent Light Scattering

As outlined in the introduction to this chapter, the principle behind refractive index matching is to minimize the difference in refractive index between the materials in question. In the case of PAm hydrogels, it is between the forming PAm network and that of the water solvent. By dissolving additives into the polymerization medium, the refractive index of water can be increased closer to that of the polymer system and thereby reduce the amount of refractive index inhomogeneities typically formed during the aqueous free radical polymerization of acrylamide ⁷. Therefore it becomes quite obvious that the more a given additive increases the refractive index of water, the better it would likely function as a refractive index matching agent within the PAm hydrogel system. Thus, several promising additives were dissolved in water at various concentrations and their solution refractive indices were recorded and graphed at each concentration. Figure III.13 displays the trends for each additive as well as the refractive index for water and the calculated refractive index of PAm as outlined by Swallen ⁵. All solution measurements of RI were performed using a refractometer. As can be seen in the figure, phenylalanine, histidine, and glycine seem to be the best additives at inducing the greatest changes in the refractive index over a range of concentration. This is exemplified by the fact that these additives have the greatest slopes. For example, from the results in the figure, theoretically a lower concentration (% w/v) of phenylalanine than that of sucrose, would be required to increase the refractive index of water (~1.333) equal to PAm (~1.468). By extrapolating the graph and utilizing the equation of the line for phenylalanine and

that for sucrose, it can be calculated that approximately 63.4% w/v of phenylalanine would be required to raise the RI of water to that of 1.468, while it would require approximately 95.6% w/v of sucrose to do the same. Therefore, at a given concentration, say 10% w/v, phenylalanine should reduce the amount of observable light scattering within a PAm hydrogel more so than that of sucrose or any of the other additives depicted in figure III.13, as it displays the greatest tendency to increase the RI of water at that concentration.

Figure III.13: Refractive index for aqueous solutions of various additives over a range of concentration as compared to that of water and PAm

Figure III.14 displays a bar graph for each of the additives outlined in Figure III.13 and depicts the overall light scattering within a PAm hydrogel containing 10% w/v of each of these additives. The scattering measurements are relative and compared to that of a PAm hydrogel containing no additive. As expected, for a given concentration, the additives which have a greater tendency at increasing the refractive index of water yield PAm hydrogels that display reduced levels of observable light scattering when incorporated. Thus, the PAm hydrogel containing phenylalanine displays the least amount of light scattering while the PAm hydrogel containing the additive ficoll displays the greatest amount of light scattering corresponding to the observed slopes in Figure III.13.

Figure III.14: Relative light scattering of PAm hydrogels containing various additives at a concentration of 10% w/v

Further verification of successful refractive index matching within PAm hydrogels comes from obtaining overall RI measurements of PAm hydrogel systems containing various additives. As outlined above, the greater a compound is at increasing the RI of water, then the greater its ability at yielding improved light scattering characteristics within PAm hydrogels. The RI value obtained for a hydrogel can be viewed as an average refractive index for the entire system (i.e. polymer + water). Thus, as the refractive index of the water is increased (via additive incorporation) the RI difference is minimized and an increase in the overall refractive index of the hydrogel should be observed. This increase in RI of the hydrogel would be a function of the concentration of additive used and on the ability that additive has at increasing the RI of water. The greater an additive increases the RI of water, the closer the overall hydrogel RI should be to that of PAm itself. Table III.1 displays the measured RI of PAm hydrogels containing different additives at various concentrations as compared to the overall RI of a PAm hydrogel containing no additive. All RI measurements for the hydrogels were done using an ellipsometer and displayed mean standard error (MSE) values below 8.0 indicating the model developed was in good agreement with the experimental data in each case. It can be seen from the table that when comparing PAm hydrogels containing the additives glycine, sucrose and phenylalanine at 10% w/v concentration, as expected, the hydrogel incorporating phenylalanine displays the greatest increase in overall RI when compared to the RI of a PAm hydrogel containing no additive. Figure III.15 also outlines the observed increase in overall refractive index of a PAm hydrogel with increased sucrose concentration. The data points display excellent agreement, having

an R^2 value of 0.9917. The figure compares overall RI values for the PAm hydrogels to that of RI values of aqueous sucrose solutions at the same %w/v concentration.

The RI value for PAm was calculated as outlined by Swallen⁵.

Table III.1: The overall RI measured for PAm hydrogels containing various Additives

Additive Incorporated into PAm Hydrogel	Measured Refractive Index of Hydrogel (700 nm)
No additive	1.3791
10% w/v Sucrose	1.4010
10% w/v Glycine	1.4119
10% w/v Phenylalanine	1.4224

Figure III.15: Refractive index of PAm hydrogels over a range of increasing sucrose concentration as compared to the RI of water, PAm and aqueous sucrose solutions of similar concentration.

III.4 RI Matching: PAm Hydrogels as an Optical Matrix and Application to a Bacteriorhodopsin Based 3-D Optical Memory Device

The purpose behind utilizing the method of refractive index matching was to produce a PAm hydrogel that would display improved optical clarity such that it may be incorporated in a bio-optical device application. The results explained throughout this chapter show that there are many possible additives that can be utilized for improving the optical characteristics within a PAm hydrogel. However, before any of the outlined additives can be incorporated into a hydrogel for use as a biomolecular optical matrix, they must display compatibility to the biological compound defined by the device. For example, compatibility to the protein bacteriorhodopsin (bR) is essential when the PAm hydrogel matrix is considered for application to a bR based 3-D optical memory device.

III.4.1 Additive Compatibility With The Protein Bacteriorhodopsin

All additives investigated for the purpose of refractive index matching, and that showed promise in reducing the inherent light scattering within PAm hydrogels were tested for their compatibility with bR. In the native state, bR has an absorption maximum at approximately 570 nm due to the chromophore, retinal, at the center of its tertiary structure. Therefore, all additives showing promise were each incorporated into a PAm hydrogel network at a chosen concentration along with the bR protein. These hydrogels were then compared to that of a solution of bR in water using a UV-VIS spectrophotometer. As long as the native absorption maximum was still observed for the ground state bR protein within the matrix, then both the selected

additive as well as the PAm matrix could be classified as compatible to native bR structure and function. Throughout this chapter and thesis, any reference to protein (bR) compatibility will mean this test was performed and there was no observable chemical influence or disruption of natural protein structure and/or function. Figure III.16 displays a typical UV-Vis absorbance spectrum for the testing of protein compatibility. The figure displays bR in a water solution as well as bR entrapped within a PAm hydrogel containing 30% w/v of sucrose as a refractive index matching additive. Not only was this compatibility test performed for each additive, but it was performed for each additive over a working range of concentrations (% w/v) to ensure elevated concentrations would not increase their toxicity/incompatibility. It should be noted that of all additives outlined in this chapter, only glycerol seemed to display some incompatibility to bR at elevated concentrations (i.e. greater than 25% w/v) as the absorption maximum was shifted and the native protein colour was noticeably changed. This shift in absorption maximum is an effective indicator of protein denaturation. It should also be noted that many of the amino acids could not be investigated at concentrations much above 10% w/v due to solubility issues.

Figure III.16: UV-Vis absorption maximum for a solution of bR protein in water compared to that of bR entrapped in a PAm hydrogel with 30% sucrose additive

III.4.2 Enhanced PAm Based Optical Cubes Containing bR

The protein bR when incorporated into the PAm hydrogel system, becomes entrapped throughout the hydrogel matrix. The matrix must have a pore size small enough to effectively entrap the protein and prevent its diffusion, this can be regulated by the crosslinking concentration within the network. By increasing the crosslinking concentration the pore size is reduced, however, this also causes an increase in the amount of inherent light scattering to the system. In addition, the protein itself acts as a particulate and hence works to scatter light. Therefore, outlining a greater need for improved optical clarity within PAm hydrogels and for the method of refractive index matching.

The compatibility and improved optical clarity of these PAm hydrogels is visually demonstrated in Figure III.17. The photographs, taken at 90° to the incident

angle of the laser beam (633 nm), depict PAm hydrogels containing the protein bR (purple colour). All three gels in Figure III.17 contain the same amount of monomer, crosslinker, initiator, catalyst, and bR, however, gel (A) contains 0% w/v sucrose, gel (B) contains 25% w/v sucrose and gel (C) contains 50% w/v sucrose. In each photograph, the more “hazing” of the laser beam observed as it passes through the gel, the more the material tends to scatter light. Thus, when the concentration of sucrose is increased from gel (A) through to gel (C), the beam of light passing through the matrix becomes harder to visualize as less hazing occurs, thereby indicating an improved optical clarity for the hydrogel system.

Figure III.17: Light scattering within a PAm hydrogel containing bR protein and varying concentrations of sucrose. (A) contains no sucrose, (B) contains 25% w/v sucrose, and (C) contains 50% w/v sucrose. A laser light at 633nm was used to demonstrate visually the light scattering reduction observed.

III.4.3 PAm Optical Cube Stability

The conclusion of chapter 2 outlined the fact that, as is, PAm hydrogels generally display short term stability. This stability is a factor when considering such hydrogels for use as an optical matrix within a bR based memory storage device. Outlined in the above text are several different types of additives that may be utilized for improving the overall optical clarity within a given PAm hydrogel matrix via RI matching. One of the more prominent choices for this method would be the additive sucrose. It was found that upon incorporation into the polymerization media, a sucrose concentration of approximately 50% w/v yielded optimal conditions for light scattering and bR entrapment within the product hydrogel. Stability testing of these optimized cube formulations displayed increased levels of overall stability but yet still fell short of the device requirements. Alternative means of sealing the hydrogel containing cuvettes were investigated and employed in an attempt to increase the overall stability of the system. An improved method of sealing which utilized a layer of water, a layer of wax, and a layer of epoxy sealant atop the formed hydrogel was found to improve the stability of the optimized hydrogel formulation to a shelf life of approximately 6 months. Figure III.18 outlines the stability over time for an optimized hydrogel sealed under such conditions. The stability of the system is measured through monitoring any distortions/deformations in original gel volume as outlined in chapter 2. In the case of the optimized hydrogel cubes, sealed effectively, only vertical shrinkage was observed. There were no signs of bubbling or pulling away from the sides of the cuvette.

Figure III.18: Optical cube stability over time. PAm hydrogels were sealed airtight and optimized for light scattering. Stability measured in vertical shrinkage.

III.5 Conclusions

It is evident from the outlined results that by utilizing a suitable additive for incorporation into the PAm system, the overall optical clarity of the hydrogel can be enhanced for any given wavelength. The hydrogel system and index matching method provide a foundation for further investigations into producing other “optical biomaterials”. Generally speaking, any system or device requiring optical clarity and some degree of biological compatibility would be candidate applications for these enhanced hydrogels.

One specific application presented throughout this thesis is that of a protein based 3D optical memory device. Through optimization of polymerization conditions (chapter 2) and further improvements through refractive index matching presented throughout this chapter, PAm hydrogels can be prepared such that their optical clarity is enhanced for use as an optical matrix. The PAm hydrogel matrix displays optical characteristics well within the requirements specified by the bR based memory device. Several additives and additive types were presented within this chapter as possible refractive index matching agents. Many compounds displayed promise in reducing the inherent light scattering of PAm hydrogels, however, there was one clear choice as suitable agent. When considering factors such as cost, solubility, protein compatibility over a wide range of concentration, and availability, sucrose is the optimal candidate. By investigating a wide range of concentrations as applied to the polymerization conditions outlined in Chapter 2, an optimized formulation of PAm hydrogel preparation was determined. This formulation utilized a concentration of sucrose additive at 50% w/v, which yielded hydrogels displaying low light scattering

(a reduction of 71% in scattering properties) and allowed for the incorporation of suitable amounts of bR protein.

Hydrogel stability was investigated for the “optimized” PAm system (including sucrose) and was found vulnerable to gel syneresis/ polymer relaxation as was previously seen with PAm gels (chapter 2). Alternative methods for sealing the optical cubes were investigated resulting in a method of utilizing the high absorption/swelling behaviour of PAm hydrogels. By adding a thin layer of water atop the prepared and incubated hydrogel and by sealing the cube air tight with wax and epoxy, the absorption of water and subsequent outward swelling forces of the PAm hydrogel resulted in a slowed relaxation of the system. Through this process, the shelf life of these PAm optical matrix cubes was increased from that of 2 months to approximately 6 months.

In terms of application of this optimized PAm system to the bR based 3D optical memory device, more work is needed to meet all of the device requirements. Optical clarity of these hydrogels was achieved via refractive index matching and is suitable for application as an optical matrix to this device. However, the stability of the system requires further enhancement as the long-term stability for these hydrogels reaches only half of that which is required by the device.

From the work presented, two questions arise: (1) Has the stability of the PAm hydrogel system approached a maximum through the methods invoked, or can it be further improved? And (2) What other possible polymer matrix systems can be utilized to yield a longer stability / shelf life for application to this bR based device?

III.6 Experimental Section

Materials

The reagents such as acrylamide, ammonium persulfate, N,N,N',N' tetramethylene-ethylenediamine, N,N' methylenebisacrylamide, phosphate buffer (pH 7.2), polymethacrylate cuvettes, sucrose, glucose, trehalose, all amino acids and amino acid derivatives outlined in this chapter, glycerol, and PVP (MW ~ 10 000) were purchased from the Aldrich Chemical Company and used as received.

The polymer of sucrose, Ficoll 70, was purchased from Pharmacia Fine Chemicals and used as received. The UV cured optical adhesive sealant (NOA 68) was bought from Norland Adhesives and used as received.

Bacteriorhodopsin (WT) was grown, cultivated, isolated, and supplied by the research group of Dr. R. Birge at the W.M Keck Centre for Molecular Electronics within Syracuse University. Samples were shipped as an aqueous solution at a concentration of ~9.2 mg/mL. Upon reception of the samples, the solution was filtered through a 0.8 μ m syringe filter and placed in a water bath sonicator for 30 minutes.

Measurements

All light scattering measurements were performed on a Shimadzu RF-1501 spectrofluorimeter, using an empty cuvette as a blank. The samples were measured on an emission setting, meaning the system was excited at 700 nm and the emission spectrum was detected over the range of 650-800 nm. The 700 nm excitation

wavelength was chosen as all writing and reading of information within the bR based optical memory system takes place in this region of the light spectrum.

All bR compatibility measurements of PAm and the additives used were performed on a Perkin Elmer Lambda 900 UV-Vis Spectrophotometer. Samples were measured within the UV grade polymethacrylate cuvettes in which they were prepared. All samples were compared to a blank of water and investigated over a range of 400 to 800 nm.

All refractive index measurements for aqueous solutions of additives were performed using a Fisher LR45302 refractometer. A blank of water was used in each case. All refractive index measurements of PAm hydrogels were performed on a J.A. Woollam Co. INC. M-44 ellipsometer and modelled against data for a provided cauchy material. The gels were prepared as a thin film (~0.3 cm) between two glass plates and analyzed on the ellipsometer in free standing form. All gels were investigated over a wavelength range of 400 – 1000 nm, but for this chapter, only values of RI at 700 nm are presented.

The stability measurements for this chapter were done by a comparison of an aged gel to the original gel shape/volume. The prepared gels were marked as to their height in the cuvette upon formation. Any vertical shrinkage was measured directly and recorded.

Hydrogel Synthesis

A typical synthesis for a PAm hydrogel used throughout this chapter is given as follows:

Stock solutions of Acrylamide (Am), Ammonium persulfate (APS), N,N,N',N' tetramethylene ethylene diamine (TMED), and N,N' methylenebisacrylamide (MBA) were prepared as follows: a 60 wt % Am stock solution (12.0 g Am in a total 20 mL H₂O solution), a 2% APS solution (0.200 g in total 10 mL H₂O solution), a 20% TMED solution (2.6 mL in a total 10 mL H₂O solution), and a 5% solution of MBA (0.500 g in a total 10 mL H₂O solution). All solutions were prepared using distilled/deionized H₂O and all solutions were purged for 10 minutes using Nitrogen.

In each 4 mL polymethacrylate (UV grade) cuvette 0.50 mL of Am solution, 0.120 mL MBA solution, 0.050 mL TMED solution and 2.27 mL distilled/deionized H₂O were added. Finally, to the solution was added 0.060 mL of APS solution and the polymerization solution in the cuvette was mixed quickly, sealed with parafilm and set aside to allow for complete polymerization. All gels were sealed with a layer of Norland 67 UV cured optical adhesive. For each hydrogel prepared, a total volume of 3 mL was used.

This procedure was applied to each section of this chapter as a general means of preparing and sealing PAm hydrogels, however, certain changes had to be made to the formulation of the polymerization solution depending on whether or not additives were used and in what concentration. Slight variations to the PAm hydrogel protocol were made as follows:

Sections III.2 – III.3: All additives utilized and tested throughout this chapter were prepared as a stock solution in water. Then depending on what concentration of additive desired in the resultant hydrogel, the amount of stock solution incorporated into the polymerization medium was altered. In the case of a PAm hydrogel containing no additive, 2.27 mL of water was used to make up the sample volume to a total of 3 mL. When incorporating additives, desired volumes of the stock solutions were added to the polymerization medium and the remainder of the outlined 2.27 mL volume was made up with water keeping the total sample volume at 3 mL

Section III.4 : All gels prepared throughout this section contained the protein bR. As expained above, 0.3 mL of bR was added from the filtered solution to the polymerization medium along with any RI matching additive desired. Therefore, after addition of 0.3 mL of bR, 1.97 mL of aqueous solution was available for incorporating the desired additive at variable concentrations. All differences were made up with water to 1.97 mL keeping all hydrogel samples at a total volume of 3 mL.

Section III.5: Various methods of improving the stability/shelf life of PAm hydrogels were investigated, however, the method of choice involved an incubation period and sealing with water, wax, and optical adhesive. After the polymerization reaction was completed, and the hydrogel was successfully formed, the gels were placed in an incubating oven (40°C) for approximately 5 days. Once removed from incubation, the gels were exposed to water (using a syringe) and allowed to expand back to original size/volume within the cuvette over the duration of a few hours. Then, atop the hydrogels was placed approximately 0.3 mL of water and atop the water was

poured a layer of melted wax. The wax was allowed to harden before a layer of Norland 68 optical adhesive was applied. Once this adhesive was poured atop the wax, the entire hydrogel cube was placed in a UV chamber for 2 minutes allowing the epoxy sealant to harden. All gel samples remained at a total 3 mL volume. This sealing protocol was utilized for producing optimized PAm hydrogel optical cubes for bR based memory applications.

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CHAPTER IV

Photo-crosslinkable Copolymers Derived from 2-Hydroxyethyl Acrylate and the Preparation of Optical Hydroglass Matrices

IV.I Introduction

Acrylate and methacrylate monomers are unsymmetrically substituted ethylenes containing substituents of great variety. Polymers of this class are noted for their outstanding water-clear colour and the stability of their properties upon aging under severe conditions. Acrylate and methacrylate monomers can be viewed as extremely versatile building blocks that yield polymers that may vary from those of extremely tacky adhesives, rubbers, tough plastics, and even hard powders ¹. The vast majority of commercially prepared acrylate polymers and a substantial amount of the methacrylate polymers are copolymers. This is due to the fact that acrylate homopolymers tend to be soft and tacky, while the methacrylate homopolymers are generally harder and more brittle.

The T_g of acrylates and methacrylates is mainly influenced by the nature of the alcohol group. However, methacrylates are harder polymers of higher tensile strength and lower elongation than their acrylate counterparts due to substitution of a methyl group for the α -hydrogen on the main chain ². This substitution restricts the freedom of rotation and motion of the polymer backbone. Thus, T_g values for a given methacrylate are higher than those of the corresponding acrylate. As well, for both acrylate and methacrylate homopolymers, increasing the bulkiness of the alkyl group also restricts the motion of polymer backbone chains past one and other, leading to increases in T_g .

The family of polyacrylates and polymethacrylates are commercially produced in enormous amounts worldwide ³⁻⁵, with the production of polymethyl methacrylate exceeding all others ⁶. Typically, acrylate and methacrylate polymers or copolymers are produced for use in wide variety of applications such as; organic glass, as high quality finishes in the leather and textile industries, lubricating oil additives, surface coatings, personal care products, adhesives, binders, sealers, medical aids, floor polishes and various optical applications ⁷⁻⁹. Application of these polymers as photonic materials has recently emerged as polymer waveguides and patterning become very important. Through copolymerization of an acrylate monomer containing a reactive functional group suitable for grafting and crosslinking, a photopatternable acrylic copolymer may be attained.

Photocurable acrylics-based materials have long been recognized throughout literature in areas such as dentistry, where aromatic dimethacrylates have been utilized extensively as photocurable fillings ¹⁰. Side chain modifications of many acrylate/methacrylate based polymers are easily achieved providing the proper reactive functional groups are present. For example, esterification of polymers such as poly(methacryloyl chloride) with various hydroxyalkylcarbazoles have yielded carbazole based methacrylate and acrylate polymers with application to a vast array of areas such as traditional optics, holography, electro-optics, non-linear optics, liquid crystals, and electronic data storage ¹¹⁻¹³.

One of the best studied compounds used to induce a photochemical crosslinking reaction is that of the cinnamate moiety (Scheme IV.1). Poly(vinyl cinnamate) was the earliest synthetic photopolymer ¹⁴. Its design, a polyvinyl

backbone with cinnamoyl side chains, was conceived in the hope that photo-cycloaddition between polymer bound cinnamoyl groups would crosslink adjacent molecules^{15,16}. Since this time, the practical aims of the inventors have been realized and a whole range of successful photopolymers have developed from the original idea^{17,18}.

Scheme IV.1 : Cinnamoyl photo-cycloaddition

Through utilization of various acrylic monomers, combined with basic chemical modification reactions, and the photo-cycloaddition of cinnamoyl moieties, it is possible to address one of the overall objectives of this thesis; namely to investigate photocurable polymers and polymeric glasses as possible optical matrix materials displaying long term stability. Through incorporation of methacrylate or acrylate based monomers, polymer properties such as thermal stability, T_g , refractive index, optical loss, solubility and hardness can be fine tuned for desired applications.

In focussing on improving the stability of PAm hydrogels, and their tendency to succumb to the forces of polymer relaxation / gel syneresis (Chapter 3), attention was directed towards preparing a polymer system that could be crosslinked after the polymerization reaction was complete. In this manner, the polymer chains should already exist in a relaxed state and can therefore be crosslinked as such. By carrying out this post polymerization crosslinking reaction, the system should be relieved from much of the tension that is normally formed in hydrogel synthesis, thereby reducing the possibility or onset of gel deformation due to relaxation processes. A very easy and straightforward means to induce post polymerization crosslinking is photochemically. By introducing a photoactive pendant group onto pre-synthesized polymer chain, a useful photocrosslinking reaction can be effected. Throughout this chapter, the photoactive cinnamate moiety will be utilized to induce crosslinking. Two copolymer systems will be investigated over a range of monomer feed ratio as well as cinnamate incorporation (Mole %). Both copolymers were prepared with 2-hydroxyethyl acrylate (HEA) as a comonomer, which contains a free OH group capable of post polymerization modification. The properties for the parent, modified, and crosslinked polymer systems were investigated and recorded for each copolymer series. The two copolymers to be studied are (1) 2-hydroxyethyl acrylate-*co*-N,N-dimethyl acrylamide and, (2) 2-hydroxyethyl acrylate-*co*-2-(perfluoroalkyl)ethyl methacrylate which is a fluorine containing polymer utilizing the commercially available Zonyl® TM fluoromonomer.

IV.2 Synthesis and Characterization of 2-Hydroxyethyl Acrylate Copolymers

The free radical solution polymerization of HEA with N,N dimethyl acrylamide (DMA) was performed in N,N-Dimethylformamide (DMF) in a sealed tube vessel and afforded a hydroxy containing copolymer (Scheme IV.2). The polymerization reactions were carried out over a range of mol % incorporation of HEA (0-100 mol %) in increments of 20 mol % yielding a series of polymers as outlined in Table IV.1. The free radical reaction was initiated through the use of 0.1% (mol %) 2,2'-Azobisisobutyronitrile (AIBN) and the reaction temperature was held at 75°C for a duration of 18-24 hours. The formation of polymer product was monitored by IR. After precipitation and isolation of the polymers (**IV.1 – IV.6**) from ether, the relative intensity of the carbonyl (ester) peak in HEA ($\sim 1727\text{ cm}^{-1}$) and the carbonyl (amide) peak in DMA ($\sim 1626\text{ cm}^{-1}$) were checked and compared. These relative intensities of carbonyl peaks as compared to one and other varied depending on the feed ratio of HEA that was utilized in the polymerization (Figure IV.1).

Scheme IV.2 : Free radical polymerization of HEA and DMA

Table IV.1: Measured thermal and physical properties for the DMA / HEA copolymer series.

Polymer	Feed Ratio (Mol %)	Mw	T_d¹ °C	T_g² °C	n³	Solubility
IV.1	100% DMA	52583	392	121	1.4977	DMF (heating), Chloroform
IV.2	80% DMA / 20% HEA	57251	349	84	1.4951	DMF, H ₂ O (some heating), Chloroform
IV.3	60% DMA / 40% HEA	64048	329	57	1.4915	DMF, H ₂ O, Chloroform
IV.4	40% DMA / 60% HEA	59832	318	33	1.4886	DMF, H ₂ O, partially soluble in Chloroform
IV.5	20% DMA / 80% HEA	53372	309	Not detected	1.4853	DMF, H ₂ O
IV.6	100% HEA	36645	304	Not detected	1.4679	DMF, H ₂ O

¹ measured by TGA (onset temperature 5% weight loss) under N₂

² measured by DSC under N₂

³ measured on metricron at 1555 nm

Figure IV.1: Typical IR of DMA-co-HEA polymers

Thermal properties of the HEA-co-DMA polymer series were evaluated via DSC and TGA and recorded in addition to molecular weight, and selected physical properties such as refractive index and solubility. Table IV.1, outlines the recorded values for decomposition temperatures (T_d), glass transition temperatures (T_g), refractive index (n), molecular weight (M_w) and solubility for all polymers prepared over the range of 0-100 mol % incorporation of HEA. The T_d was taken for each polymer at 5% weight loss under inert atmosphere. The T_g 's were observed to have greater deflections at higher mol % incorporation of DMA and became more difficult to visualize as the mol % of HEA was increased. A typical DSC trace for this copolymer series can be seen in Figure IV.2. The figure displays a glass transition temperature at 121.4°C for the polymer **IV.1**

Figure IV.2 : Typical DSC for the polymer **IV.1**

As can be seen in Table IV.1, the homopolymer of DMA, **IV.1**, has a greater T_d and T_g than the homopolymer of HEA, **IV.6**. Thus, the copolymer thermal properties (T_d and T_g) are observed to decrease with increasing incorporation of HEA. The bulkiness attributed to the methyl groups in DMA act to restrict the rotational freedom within the polymer chain and therefore induce observable increases in T_g as the mol % of DMA is increased. In addition to the thermal properties, the refractive index was also observed to decrease with increasing HEA incorporation as the homopolymer **IV.1** has a higher RI than that of **IV.6**.

The apparent molecular weights of the two homopolymers and the four copolymers in table IV.1 were obtained via GPC. All the polymers investigated fall within a reasonable range of molecular weight relative to one and other, with the exception of the homopolymer of HEA. This homopolymer displays an apparent molecular weight that is significantly lower than the rest of the polymers investigated for this series. It may be possible that this polymerization reaction became exposed to air. Acrylates, and methacrylates are typically very sensitive to the presence of oxygen. Oxygen inhibition of the HEA polymerization would lead to reduced molecular weights. This lowered apparent molecular weight may also be attributed to monomer polarity and monomer reactivity within the polymerization solvent.

In terms of solubility, Table IV.1 shows that this copolymer series becomes more soluble in polar solvents (i.e. water, DMF) with increasing mol % incorporation of HEA. This is an expected trend as the monomer of HEA is more polar than that of DMA. For example, it was observed that polymer **IV.1** was very soluble in

chloroform and soluble in DMF upon heating, whereas the polymer **IV.6** was very soluble in both DMF and water at room temperature but insoluble in chloroform.

Fluoro-containing polymers are generally regarded for their improved optical clarity and hence their widespread application to optics and optical devices ¹⁹. Preparation of a fluoro containing copolymer was attempted as a means of producing a photocrosslinkable polymer with enhanced optical clarity. The addition copolymerisation of HEA with 2-(perfluoroalkyl)ethyl methacrylate (Zonyl ®) yielded a fluoro containing polymer with pendant hydroxy groups (Scheme IV.3). The free radical reaction was carried out in tetrahydrofuran (THF) using 1 % (mol %) of AIBN as the initiator. The reaction was run at 75-80°C for 24 hours and monitored by IR for verification of monomer incorporation. Figure IV.3 displays a typical IR spectrum for this copolymer series. The carbonyl stretching corresponding to the Zonyl ®TM fluoromonomer incorporation is found at $\sim 1731\text{ cm}^{-1}$ and the presence of fluorine is detected in the range of $1100\text{-}1350\text{ cm}^{-1}$. While HEA is identified, as outlined above, with a carbonyl stretch at approximately 1728 cm^{-1} and an OH vibration around 3427 cm^{-1} . Similar to the other copolymer series investigated, the Zonyl ® TM fluoromonomer / HEA copolymer series was studied over a range of mol % incorporation of HEA (0-100 mol %) in 20 mol % increments thereby yielding four copolymers and two homopolymers. The six polymers were isolated from hexane, re-dissolved and re-precipitated before drying overnight under vacuum.

Scheme IV.3: Free radical polymerization of Zonyl[®] TM and HEA

Figure IV.3: Typical IR spectrum of Zonyl[®] TM-co-HEA polymer

Table IV.2 outlines physical and thermal properties studied for the Zonyl® TM / HEA copolymer series. The decomposition temperatures (T_d), glass transition temperatures (T_g), refractive index (n), molecular weight (M_w) and solubility for all polymers prepared over the range of 0-100 mol % incorporation of HEA are recorded in Table IV.2. Increases in HEA (mol %) content occur over the copolymers **IV.7** to **IV.11** inclusively.

Table IV.2: Measured thermal and physical properties for the Zonyl fluoromonomer / HEA copolymer series.

Polymer	Feed Ratio (Mol %)	M_w	T_d¹ °C	T_g² °C	n³	Solubility
IV.7	100% Zonyl	Not soluble	275	71	1.3983	1,1,1-trichlorotrifluoroethane
IV.8	80% Zonyl/ 20% HEA	7121	278	68	1.4024	1,1,1-trichlorotrifluoroethane
IV.9	60% Zonyl/ 40% HEA	9397	282	61	1.4096	1,1,1-trichlorotrifluoroethane, slightly in DMF
IV.10	40% Zonyl/ 60% HEA	11928	290	55	1.4158	DMF, slightly in Water, slightly in 1,1,1-trichlorotrifluoroethane
IV.11	20% Zonyl/ 80% HEA	13124	295	46	1.4266	DMF, Water
IV.6	100% HEA	36645	304	Not detected	1.4679	DMF, Water

¹ measured by TGA (onset temperature 5% weight loss) under N₂

² measured by DSC under N₂

³ measured on metricron at 1555 nm

The thermal properties of polymers **IV.7 – IV.11** were investigated using DSC and TGA. Decomposition temperatures were observed to increase with increasing incorporation of HEA. In terms of thermal stability, unless subjected to extreme heat conditions, acrylate polymers are durable, do not depolymerize, and degrade slowly. In contrast, methacrylates readily depolymerize at high conversion at elevated temperatures (usually near or above 300°C). This trend can be seen through comparison of the acrylate homopolymer **IV.6** with that of the methacrylate homopolymer **IV.7**. The comparison outlines a higher T_d for polymer **IV.6**, which consequently resulted in an observed gradual increase in T_d with increasing incorporation of HEA into the copolymers **IV.8 –IV.11** respectively. Comparing the glass transition temperatures, a gradual decrease in T_g is observed with increasing HEA incorporation from polymers **IV.7 – IV.11** and **IV.6** respectively. Methacrylates are known to have generally higher T_g 's than their acrylates counterparts due to the added bulk of the methyl group and its effect to reduce the free rotation within the polymer. Thus, incorporating more acrylate functionality acts to reduce the T_g by increasing segmental motion.

The apparent molecular weights for this series of copolymers are noticeably lower than what was observed for the DMA-co-HEA polymers. This was expected as 10x the initiator concentration was used in order to effectively polymerize the fluoromonomer. The apparent molecular weights in Table IV.2 were observed to increase slightly as the amount of HEA incorporated into the polymer was increased. This can likely be attributed to the difficulties associated with achieving successful

polymerizations of the Zonyl ®TM fluoromonomer, thus yielding lower molecular weight polymers at greater mol % incorporation of this monomer.

The refractive index values for the Zonyl ®TM -co-HEA series of polymers can be seen in Table IV.2. It is a well known fact that incorporation of fluorine into a polymer results in a lowered refractive index for that polymer. It can therefore be seen that the homopolymer **IV.7** has a significantly lower *n* than that of **IV.6**. The result of copolymerisation of Zonyl ®TM with HEA leads to increasing refractive index with decreasing fluorine content (from polymers **IV.7** – **IV.11** respectively).

The fluoromonomer, Zonyl ®TM, and the monomer HEA have very different polarities. The fluoromonomer is non-polar and hydrophobic while HEA is polar and hydrophilic. Thus, copolymers of these two monomers display varied solubility depending on their initial monomer feed ratios. Table IV.2 outlines the observed solubility for this copolymer series. As expected, the Zonyl ®TM homopolymer (**IV.7**) and copolymers containing high mol % Zonyl were only soluble in 1,1,1-trichlorotrifluoroethane, a rather non-polar solvent. As the mol % incorporation of HEA increased the copolymers became increasingly more soluble in polar solvents such as DMF and water.

IV.3 Post Polymerization Transformation and Characterization

The formation of an ester generally involves the nucleophilic reaction of an alcohol and an acid chloride²⁰. The presence of cinnamate esters is of general interest because it provides a route to photocurable polymers²¹. The cinnamate moiety has proven to be reactive in solution as well as in the solid state, yet not to the same

efficiency due to the required overlap of vinyl groups to facilitate [2 + 2] cycloaddition. Thus, the facile conversion of the available free alcohols within the polymers **IV.2** – **IV.6** and **IV.8** – **IV.11** to cinnamate esters by treatment with cinnamoyl chloride was carried out in the presence of triethylamine (TEA) (Scheme IV.4). Due to the range of solubility of the parent polymers, one of three solvents were employed for the modification reaction, namely, DMF, chloroform, or 1,1,1-trichlorotrifluoroethane. The derivatized polymers **IV.2d** – **IV.6d** and **IV.8d** – **IV.11d** were isolated from ether, redissolved in chloroform and filtered to remove insoluble solids.

Scheme IV.4 : Modification reaction of DMA/HEA and Zonyl ®TM / HEA parent polymers to form photocrosslinkable cinnamate ester derivatives

Modification of the pendant hydroxy groups in the polymers **IV.2 – IV.6** and **IV.8 - IV.11** with cinnamoyl chloride, afforded the formation of photocrosslinkable polymers **IV.2d – IV.6d** and **IV.8d – IV.11d** respectively. The esterification of cinnamoyl chloride with the pendant hydroxy groups on the polymers was carried out in chloroform for the parent polymers **IV.2 – IV.4**, in DMF for the parent polymers **IV.5, IV.6** and **IV.11**, and in 1,1,1-trichlorotrifluoroethane for the parent polymers **IV.8 - IV.10**. The reaction was run for 24-48 hours at room temperature under inert atmosphere and in the presence of TEA. The reaction was monitored by IR for complete exhaustion of all free hydroxy groups, the appearance of an additional carbonyl ester stretch at approximately 1717 cm^{-1} , and the appearance of an asymmetric double bond stretch corresponding to the ethyl cinnamate at around 1635 cm^{-1} (Figure IV.4). Additional confirmation was also obtained through absorbance spectrophotometry for the appearance of the UV detectable cinnamate moiety within the polymer (Figure IV.5). As can be seen the absorption maximum was observed at 280 nm. As well, $^1\text{H-NMR}$ was utilized for determining the appearance of vinyl protons at approximately 6.45 ppm as well as aromatic protons in the region of 7.3-7.8 ppm from the cinnamate moiety (Figure IV.6).

Figure IV.4 : IR spectrum comparing the parent polymer **IV.9** with its derivatized counterpart **IV.9d**

Figure IV.5: UV-Vis absorbance spectrum of the cinnamate derivatized polymer **IV.3d** in chloroform (0.38 mg / mL).

Figure IV.6: ^1H -NMR of the modified polymer **IV.10d**

Since complete exhaustion of all reactive hydroxy pendant groups within the parent polymers was achieved using cinnamoyl chloride in a 1:1 reaction, the UV-Vis absorbance maximum for the cinnamate moiety was utilized to calculate the actual mol % incorporation of HEA into the both polymer series. A calibration curve was constructed of absorbance versus concentration using *trans*-cinnamic acid as can be seen in Figure IV.7. The graph yielded a straight line with a perfect fit to the data points ($R^2 = 1$) and produced the equation of a line; $y = 140.03x - 0.0213$. Using this calibration curve, exact weighed amounts of modified polymer were diluted and analyzed for their absorbance at 280 nm. Through back calculation the actual mol % incorporation of HEA was determined for each polymer. The calculated mol % incorporation of HEA for polymers **IV.2 – IV.6** and **IV.8-IV.11** can be seen in Table IV.3 along with the thermal and physical properties of all cinnamate derivatized polymers.

Figure IV.7: Calibration curve using *trans*-cinnamic acid for calculating Mol % incorporation of HEA into all copolymers

Table IV.3: Measured thermal and physical properties for all cinnamoyl modified polymers of DMA-*co*-HEA and Zonyl ®TM -*co*-HEA

Polymer	Feed (Mol %)	Actual Mol % (HEA)	T _d ¹ °C	T _g ² °C Before cure	T _g ³ °C After cure	n ⁴
IV.2d	80% DMA 20% HEA	15.3%	348	87	144	1.5467
IV.3d	60% DMA 40% HEA	47.5%	352	73	151	1.5326
IV.4d	40% DMA 60% HEA	66.2%	355	66	186	1.5259
IV.5d	20% DMA 80% HEA	85.5%	347	61	220	1.5213
IV.6d	100% HEA	100%	349	52	249	N/A
IV.8d	80% Zonyl 20% HEA	15.5%	285	75	147	1.4181
IV.9d	60% Zonyl 40% HEA	31.5%	293	76	166	1.4247
IV.10d	40% Zonyl 60% HEA	47.6%	304	74	217	1.4389
IV.11d	20% Zonyl 80% HEA	55.3%	310	71	not detected	1.4596

¹ measured by TGA (onset temperature 5% weight loss) under N₂² measured by DSC under N₂³ measured by DSC under N₂ after 20 minute UV exposure on Novacure system⁴ measured on metricron at 1555 nm

Comparing the properties seen in Table IV.3 for each of the derivatized copolymers with data corresponding to their parent systems in Tables IV.1 and IV.2, it can be seen that the thermal and physical properties were altered as a result of the cinnamoyl modification. The calculated mol % incorporation of HEA (Table IV.3) within the DMA-*co*-HEA copolymer series, outlines a higher mol % incorporation than the feed ratio for polymers **IV.3 –IV.5**. Polymer **IV.2** was calculated to contain

slightly less HEA than the feed ratio. On the other hand, when looking at the calculated mol % incorporation of HEA within the fluoro polymers, it is obvious that with the exception of polymer **IV.10**, all copolymers in this series contain considerably less HEA than what was attempted in the feed. All modified polymers in Table IV.3 were checked by GPC for reproducibility of their molecular weights. All polymers fell within reasonable range of what was found for their parent systems, however, the polydispersity in each case was increased from the range of 1.3 – 2.1 to approximately 2.3 – 3.1. The modified polymers were detected at 280 nm on the UV-channel of the instrument. The greater polydispersity in each case is likely a result of the greater sensitivity of the UV detector as compared to the refractive index detector used for the parent polymers.

Table IV.3 displays refractive index values for all modified polymers within both copolymer series. Increases in refractive index are observed for all modified polymers when compared to that of their parent polymer. Increases are dependant on the mol % incorporation of cinnamate functionality. Figure IV.8 displays a graph of Refractive Index as a function of the mol% incorporation of HEA for the DMA-*co*-HEA parent polymer series as well as the cinnamoyl modified polymers. As can be seen, as the amount of HEA incorporated into the polymer is increased, the refractive index is decreased. This trend is observed in both the parent polymer and the cinnamoyl modified polymers. Both lines display good agreement having R^2 values greater than 0.98. However, the data point for 100% incorporation of HEA was omitted from the parent polymer series as it was an outlying value, possibly due to variation in film preparation.

Figure IV.8: Refractive index (1555 nm) over mol % incorporation of HEA for both the parent and modified polymers of the DMA-*co*-HEA series

Figure IV.9 displays a similar graph of refractive index versus mol % incorporation of HEA but for the Zonyl ®TM -*co*-HEA polymer series. Both the parent and modified polymers are displayed. In this instance, as the mol % of HEA is increased, the refractive index of the polymer is also increased. Again, modification of the polymer forming cinnamate esters yields elevated RI values over that of the parent polymer in each case, as seen in Figure IV.9.

Figure IV.9: Refractive index (1555 nm) over mol % incorporation of HEA for both the parent and modified polymers of the Zonyl ®TM -*co*-HEA series

When comparing the thermal properties of the parent polymers in Tables IV.1 and IV.2 with those of their cinnamate modified counterparts in Table IV.3, evident changes are observed. Both series of polymers displayed increases in thermal stability after modification. The observed increases come from the introduction of more aromatic character to the polymer. The two parent copolymer series investigated are of aliphatic nature which is reflected in their T_d 's. Through modification forming a cinnamate ester, aromatic character is incorporated within the polymers to various extents depending on the amount of HEA present and available for modification. This point is further exemplified by looking at the series of polymers **IV.2d – IV.6d**, where there is a gradual increase in thermal stability with

increasing cinnamate functionality incorporated. This trend was also observed over the polymer series **IV.8d – IV.11d**.

Glass transition temperatures for all modified polymers **IV.2d – IV.6d** and **IV.8d – IV.11d** increased over that of their corresponding parent polymers. The addition of aromatic, long chain bulk restricts motion within the polymer chains and thus is observed to increase the T_g . Again, increases in the amount of cinnamoyl functionality incorporated within the polymer is paralleled with increases in T_g for both series **IV.2d – IV.6d** and **IV.8d-IV.11d** when compared to the parent systems.

All noted changes in thermal and physical properties following the esterification reaction of the parent polymers with cinnamoyl chloride provide an abundance of results confirming the successful incorporation of the photoactive cinnamate moiety. However, a final test of these modified polymers should be performed through a photocrosslinking reaction. If the cinnamate moiety was successfully incorporated then all modified polymers should now carry the potential to be photocrosslinked. Crosslinked polymers are insoluble in common solvents and will display elevated T_g 's over that of their non-crosslinked polymers. The functionalized polymers **IV.2d – IV.6d** and **IV.8d – IV.11d**, as well as polymers **IV.2 – IV.6** and **IV.8 – IV.11** were dissolved in chloroform and corresponding films were spin cast. All films were treated with the same UV irradiation times (20 minutes at an intensity of 1500 mW), whereupon the cured films were then analyzed by DSC thermal analysis. It was observed that only the cinnamate containing polymers, **IV.2d – IV.6d** and **IV.8d – IV.11d**, showed an increase in T_g after UV irradiation (Table IV.3) (Figures IV.10 and IV.11). Multiple scans were performed on each of the cured

samples which finally resulted in very weak endothermic transitions at higher temperatures. Figure IV.10 displays a graph comparing the glass transition temperatures of the parent, cinnamate modified, and UV irradiated samples within the DMA-co-HEA polymer series as a function of HEA (cinnamoyl) content. Figure IV.11 displays the same comparison of glass transition temperatures as a function of HEA (cinnamoyl) content but for the Zonyl® TM-co-HEA polymer series. Increases in T_g with cinnamoyl modification and then again with UV irradiation of the modified samples is clearly evident in both figures IV.10 and IV.11. Figure IV.12 outlines a typical DSC scan of the modified polymer **IV.3d** as well as the corresponding UV cured polymer. Notice the T_g has increased from 73°C to 151°C after exposure to UV light.

Figure IV.10: Glass transition temperatures for the parent, cinnamate modified, and UV irradiated samples within the DMA-co-HEA polymer series as a function of HEA/cinnamate content.

Figure IV.11: Glass transition temperatures for the parent, cinnamate modified, and UV irradiated samples within the Zonyl® TM-co-HEA polymer series as a function of HEA/cinnamate content.

Figure IV.12: DSC thermal analysis of polymer **IV.3d** before and after UV irradiation.

All cinnamate functionalised polymers were capable of crosslinking upon UV irradiation. All crosslinked polymers were insoluble to the usual organic solvents. Photopatterning polymer films of **IV.2d** – **IV.6d** and **IV.8d** – **IV.11d** was achieved after applying a photomask and UV irradiating the desired films. Films were then developed in chloroform to dissolve uncrosslinked polymer. Figure IV.13 displays a typical photopattern for polymer **IV. 2d** achieved by UV irradiation of the functionalised polymers.

Figure IV.13 : Photopattern of polymer **IV.2d** after UV irradiation of the film under a photo-mask.

The photo induced gelation of the cinnamate functionalised polymers is usually achieved in organic solvents such as chloroform and in water with difficulty. Initially, the need for a polymer matrix displaying improved long-term stability and reduced gel relaxation /syneresis was the driving force for investigating photo-crosslinkable systems since they could be crosslinked after formation of the polymer

chains. Owing to the fact that the developed matrix would be utilized within a bR-based device, an aqueous environment is obviously required. The photoactive cinnamate functionality is rather hydrophobic, however, it was believed that by incorporating a very small quantity of these crosslinking sites (i.e. less than 1%) photo-gelation may still occur without drastically compromising the optical clarity of the system. Upon further investigation, it was found that the preparation of a photo-hydrogel crosslinked via cinnamate moieties was difficult or impossible at the concentration of desired cinnamoyl functionality. However, after several attempts, an aqueous system was successfully developed which required high concentration of cinnamate incorporation (greater than 12% cinnamoyl) within the polymer system. For example, using the parent polymer **IV.4**, partial esterification was attempted such that only 20 mol % of the available pendant hydroxy groups of HEA would be modified (assuming full yield). This allowed for the majority of free hydroxy groups to remain and impart polar functionality to the polymer, and hence aqueous solubility. After the esterification reaction with cinnamoyl chloride, and characterization of the polymer, crosslinking in aqueous solution was attempted. Thin films were spin cast from aqueous solution, dried and successfully photopatterned. Photogelation of this partially modified polymer sample was achieved over an extended irradiation time (4 hours – 1 hour / cuvette face). Figure IV.14 displays a successfully formed aqueous photogel. The photogel displayed compatibility to the protein (by UV-Vis), however, also displayed high levels of light scattering. The high levels of light scattering were to be expected due to the hydrophobic nature of the photocrosslinking groups as well as the crosslinked product. The resultant gels displayed “cloudiness” due to phase

separation caused by the formed crosslinks. The crosslinking groups as well as the formed crosslinking product are hydrophobic and thus separate from the surrounding aqueous environment.

Figure IV.14: Aqueous photo-gel of partially cinnamoyl esterified polymer **IV.4**

The use of polymers has greatly extended into the electronic environment beyond general packing and structural applications. Investigations into high performance polymers as organic waveguide materials has currently acquired a great deal of research efforts both academically and commercially²²⁻²⁸. The enhanced fabrication that spin coating and dip coating offer compared to chemical vapour deposition (CVD) and the ability for fine tuning of the refractive index, are the general reasons for intense interest. Surfaces have been patterned using microlithographic methods such as ion-beam etching, which are destructive in nature. There have been a few examples of UV photopatternable polymer light emitting devices based on poly(phenylene vinylene)s and polythiophenes²³. Polymer based

waveguide devices for wavelength division multiplexers (WDM) have also been demonstrated through the use of acrylate monomers ²⁴.

IV.4 Hydroglasses

In addition to investigating photocrosslinkable polymer systems to improve the optical matrix stability, formation of alternative aqueous-based matrices is also outlined in this chapter. Hydrogels are soft, pliable gel structures that are susceptible to the forces of polymer relaxation. However, if a very hard, rigid system could be formed in an aqueous environment, then the process of polymer relaxation may be greatly inhibited or even prevented. An unforgiving, hard, glass-like structure would be ideal for preventing/slowing gel deformation. Thus, investigations into forming “hydroglass” structures for application to the 3D optical memory device were performed. Tests for optical clarity (light scattering), bR compatibility, and long-term stability were carried out on all possible hydroglass formulations developed.

IV.4.1 Synthesis and Analysis of Novel Hydroglass Materials as an Alternative Optical Matrix

The aim of this work was to synthesize a relatively hard hydrogel with a suitable refractive index and good clarity. The synthesis and properties of hydrogel materials, especially the heterogeneity of polyacrylamide gels has been the subject of many studies ^{29, 30}. In an attempt to synthesize gels with high optical refractive index and good clarity in an aqueous environment, high concentrations of crosslinker and comonomer were employed (~60 wt %). Monomers were chosen such that there would be low water uptake by the system. All polymerization reactions were free

radical reactions, using a redox system to initiate. All monomers investigated were acrylate or methacrylate based. A starting point for these investigations was a polymer network formed from acrylamide and methacrylamide crosslinked with MBA. Polyacrylamide is a very hydrophilic polymer and so methacrylamide was chosen to make the hydrogel less hydrophilic in order to decrease the amount of water uptake, thereby strengthening the network. Crosslinker was incorporated into the polymerization solution at 7.5 mole %, while the feed ratio was 30 mole % for acrylamide (Am) and 63 mole % for methacrylamide (MAm) in the aqueous reaction mixture. The polymerization reaction was initiated with 3.24 mg of APS. The resultant gel was formed quickly with the liberation of considerable amounts of heat. The network was found to be hard and very brittle to the touch. All gels prepared with MBA as a crosslinking agent were found to display extreme brittleness. Thus, efforts were directed towards a rigid crosslinking agent with a greater spacer length than MBA as an attempt to reduce the observed brittleness. Piperazine diacrylamide (PDA) was chosen for investigation as it was found to produce PAm gels with high strength, as outlined in Chapter 3. All conditions were kept the same, with the substitution of PDA as the crosslinking agent instead of MBA. Again, the reaction was observed to be quick and very exothermic as seen in Figure IV.15. The resultant gels were found to be very hard, clear, and somewhat “glass-like”. As a result, these polymeric systems were termed “hydroglasses”. These hydroglasses did not display the degree of brittleness observed with the MBA crosslinked networks. Figure IV.15 displays the temperature profile for the formation of a hydroglass and outlines a maximum temperature of 104.2 °C at 5.25 minutes after initiation of the reaction with

APS. This temperature profile was easily obtained by submersing a thermocouple in the polymerization solution and recording temperature increases as a function of time.

Figure IV.15: Temperature profile for the exothermic polymerization of MAm and Am with PDA to form a hydroglass matrix.

Upon successful formation of hydroglass structures, various factors needed to be considered if the material was to function as an optical matrix within the bR based memory device. Firstly, compatibility with the protein was essential. Incorporation of bR into the polymerization solution resulted in a noticeable colour change from purple to a light pink (Figure IV.16). Variations of colour (purple to pink) indicate various degrees of denaturation. This toxic effect of the solution on the protein is likely due to high concentrations of monomer. Acrylamide is a known toxic compound in monomeric form, thus high concentrations were observed to result in protein denaturation over time. Formation of the polymer network, after initiation,

resulted in complete denaturation of all bR protein due to the extreme amounts of heat liberated from the reaction. Bacteriorhodopsin is a versatile protein that can withstand temperatures of approximately 80°C for very short periods of time³¹, however, at temperatures above this, quick and evident denaturation of the protein usually occurs.

Figure IV.16: Denaturation of bR within the hydroglass formulation is observed through discolouration of the native purple protein solution.

Various combinations of monomers were investigated in order to achieve bR stability/compatibility within the polymerization solution. This meant an overall reduction in the heat liberation generated by the reaction as well as a reduction in monomer toxicity towards the protein. In order to reduce the concentrations of these toxic monomers yet maintain hardness and rigidity, other monomers were investigated for incorporation into the system. Some monomers helped to impart hardness while other monomers were incorporated as protein compatible “fillers” to the network. The resultant mixture of monomers was a combination of acrylics that

allowed for bR to be incorporated into the polymerization solution without denaturation. The polymerization solution was prepared to contain all monomers outlined in Table IV.4 at the weight / volume percentages reported.

Bacteriorhodopsin was observed to remain in its native state within this polymerization mixture for extended periods of time. Protein compatibility of the polymerization solution was verified by UV-Vis.

Table IV.4 : Monomers utilized to prepare a bR compatible hydroglass matrix

Monomer	% Weight / Volume used in solution
Methacrylamide (MAm)	10 %
Acrylamide (Am)	7 %
[2-(methacryloyloxy)ethyl]dimethyl(3-sulfopropyl)ammonium hydroxide inner salt hydrate	31 %
[3-(methacryloylamino)propyl]dimethyl(3-sulfopropyl)ammonium hydroxide inner salt hydrate	8.8 %
Piperazine Diacrylamide (PDA)	8.5 %

Working with this formulation, attempts to further reduce the amount of heat produced during the exothermic polymerization reaction were investigated.

Alterations to initiator concentration were utilized to control the heat of reaction. By slowing down the reaction (less initiator) there should be an observed decrease in heat produced. Overall the reaction may generate the same quantity of heat but if the polymerization time is increased, that amount of heat will be dissipated over a longer

period of time – generating less heat at any given moment. Figure IV.17 outlines the exothermic polymerization profiles over time for various initiator concentrations within the hydroglass formulation. As can be seen, with decreasing the initiator, the maximum temperature reached becomes less. At higher APS concentration, the reaction is rapid and reaches the greatest temperature (84.3°C), while at lower APS concentration (2.05 mg) the reaction takes a greater length of time and reaches a reaction temperature maximum of only 66.8°C. Partial denaturation of bR was observed for APS concentrations of 4.10 mg and higher, however, no denaturation was observed at 2.05 mg APS or lower. All samples were prepared to a volume of 2.3 mL with a bR concentration of 2.25 mg.

Figure IV.17: Exothermic temperature profiles for hydroglass structures formed at different concentrations of APS

After successful incorporation of bR into the outlined hydroglass formulation (Table IV.4), the next important factor to test was the material's optical clarity (light scattering). Scattering measurements were carried out, as outlined in Chapter 3, using a spectrofluorimeter. Figure IV.18 displays the resultant light scattering measurements of a typical hydroglass matrix as compared to an optimized PAm hydrogel. As can be seen, the hydroglass matrix has enhanced optical clarity over that of the optically improved PAm hydrogel (Chapter 3). An advantage of utilizing high monomer concentrations, as in the hydroglass matrix, is that it reduces the chance that a polymer radical will encounter a pendant double bond on its own chain which would lead to cyclization reactions and thus a more heterogeneous network³². As well, the incorporation of a higher concentration of methacrylate monomers imparts more hydrophobic nature to the network thereby limiting the water uptake of the system. The less water uptake functions to provide a more homogeneous system as it increases the amount of polymer and decreases the amount of water, thereby minimizing the RI difference between the two components. This trend can be quantified through measurements of light scattering for a given hydroglass matrix on a spectrofluorimeter.

Figure IV.18: Light Scattering of a hydroglass as compared to an optimized PAm hydrogel

The hydroglass material was analysed via UV-Vis to ensure there was no absorbance in the range of wavelength operation for the bR based optical device. The material was scanned from 1100nm down to 400 nm (Figure IV.19). As can be seen, there is little to no absorbance of the hydroglass matrix throughout the visible spectrum. There is a slight absorbance noted at approximately 1000 nm and the beginning of significant absorption at and below 400 nm. The absorption at 400 nm may yield some difficulties in “erasing” information within the optical memory device as the erasing laser functions at a wavelength of 400 nm.

Figure IV.19: UV-Vis absorption spectrum of hydroglass matrix

Analysis of the “free-water” content within the hydroglass matrix was carried out via TGA. Both the hydroglass matrix and the hydrogel matrix were evaluated as to their H₂O content. Table IV.5 outlines the observed results. The matrix material was heated within the TGA instrument at 5°C per minute up to 150°C where the temperature was held for 20 minutes. All weight loss within this temperature profile was assumed to be a result of water loss. The hydrogel was found to contain roughly five times the free water found in the hydroglass matrix. This difference is depicted nicely in Figure IV.20, where surface photographs of a hydrogel and a hydroglass matrix are displayed. Both matrix materials contain bR protein. It is evident that the surface of the hydrogel is very smooth as a result of the high water content, while on the other hand, the surface of the hydroglass material displays some sort of network structure and texture. This network structure could be a result of water channels formed throughout the polymer matrix. The increased hydrophobicity within the system may actually result in the formation of this channel-like texture of the

material. These channels may contain higher concentrations of protein as they seem to have a more “purple” colouration than the surrounding material. Further analysis of this hydroglass formulation by X-Ray indicates that the material is amorphous and contains no crystallinity (Figure IV.21). The refractive index for this hydroglass matrix was also observed to be 1.5271 (at 700 nm) in the hydrated state, as determined by ellipsometry.

Table IV.5: Free water content within a hydroglass matrix as compared to a PAm hydrogel

Matrix Material	Weight % H ₂ O in matrix
PAm Hydrogel	74.3 %
Hydroglass	14.6%

Figure IV.20: Surface photographs of A) PAm hydrogel and B) Hydroglass. Both matrix materials contain bR protein.

Figure IV.21: X-Ray analysis of hydroglass matrix

Finally, the last and most troubling factor to consider for utilization of these hydroglass structures is that of long-term stability, or shelf life. The hardness of the formed hydroglass structures was far greater than what was observed in the previously outlined hydrogels. The polymer glass could actually be cut and formed into various sizes and shapes. However, exposure to air over time caused cracking of the hydroglass surface, likely as a result of water evaporation. Once the material was sealed with an optical sealant (Norland Adhesive) the surface remained smooth and intact as formed. The sealed hydroglass cubes containing bR were monitored over time for any shrinkage or deformation. The sealed glass-like material was observed to remain as formed for up to 16 months with no signs of volumetric deformation.

Figure IV.22 displays a hydroglass cube containing bR protein that was sealed with an optical coating and has maintained a shelf-life of 16 months. The hard, rigid nature of the formed hydroglass structures acts to retard polymer relaxation and/or gel syneresis thereby prolonging the shelf life of this matrix far beyond that which was observed for the optimized PAm hydrogels. In addition these materials display enhanced optical clarity and have demonstrated compatibility with the bR protein.

Figure IV.22: 16 month old hydroglass matrix containing bR and sealed with optical adhesive.

IV.5 Conclusions

The free radical polymerization of acrylic monomers was successful in the synthesis of the N,N-dimethylacrylamide-*co*-2-hydroxyethyl acrylate polymer series, **IV.1 – IV.6**, as well as the preparation of 2-(perfluoroalkyl)ethyl methacrylate-*co*-2-hydroxyethyl acrylate polymers, **IV.8 – IV.11**. The increase in mole % incorporation of HEA within these two polymer series led to measureable changes in thermal properties, solubility, and refractive index.

Further derivatization of the hydroxyl containing parent polymers, **IV.1 – IV.6** and **IV.8 – IV.11** with cinnamoyl chloride allowed for the formation of pendant phenyl acrylate units (**IV.1d – IV.6d**, and **IV.8d – IV.11d**) displaying increased thermal stability and T_g values. The subsequent photo-curing of these derivatized polymers further increased the T_g as a result of crosslinking. The ability to form photo-patterns on silicon wafers demonstrated that these derivatized polymers have uses as a resist of potentially a directly patternable polymer waveguide. The ability to photo-pattern polymers has become of great importance in the process design of commercial products.

Preparation of functional hydroglass networks proved successful at high monomer and crosslinking concentrations with a combination of acrylamide and methacrylate based monomers. Decreased brittleness and bR compatibility were achieved through the use of the crosslinking agent piperazine diacrylamide, slightly increased water uptake, and prolonged polymerization times. These hydroglasses display enhanced optical clarity and long-term stability over that of the optimized

PAm hydrogels, and therefore are an optimal candidate for application towards the bR-based 3D optical memory device. This polymeric glass matrix may also find uses in other optical applications such as waveguides or amplifiers.

IV.6 Experimental Section

Materials

N,N-Dimethylacrylamide, 2-hydroxyethyl acrylate, and 2-(perfluoroalkyl)ethyl methacrylate (Zonyl ® TM) were purchased from Aldrich Chemical company. N,N-Dimethylacrylamide and 2-hydroxyethyl acrylate were passed through an exchange resin (Dehibit), which was purchased from Polymer Laboratories, before use. Zonyl® TM was used as received. Cinnamoyl chloride, triethylamine, DMF, Chloroform, THF, 1,1,1-trichlorotrifluoroethane, ether, hexane, methacrylamide, acrylamide, [2-(methacryloyloxy)ethyl]dimethyl(3-sulfopropyl)ammonium hydroxide inner salt hydrate, [3-(methacryloylamino)propyl]dimethyl(3-sulfopropyl)ammonium hydroxide inner salt hydrate, and ammonium persulfate were all purchased from Aldrich Chemical company and used as received. 2,2'-Azobisisobutyronitrile was also purchased from Aldrich but recrystallized from ethanol before use.

Piperazine diacrylamide was purchased from BioRad and used as received.

Measurements

Infrared measurements were performed on a Bomem Michelson 120 FTIR spectrometer. ¹H NMR spectra were obtained on a Bruker-400 instrument using tetramethylsilane as an internal reference. UV-Vis absorption spectra were measured on a Perkin Elmer Lambda 900 spectrophotometer. Light scattering measurements were performed on a Shimadzu RF-1501 spectrofluorimeter. Gel permeation chromatography (GPC) was performed using chloroform at ambient temperature and

a flow rate of 1.0 mL per minute or using DMF at 40 °C and a flow rate of 1.0 mL per minute. Weight average and number average molecular weights were calculated relative to polystyrene standards. A refractive index detector or a UV detector set to 280 nm were used for detection. Glass transition temperatures were determined on a Seiko 220C DSC at a heating rate of 10 °C per minute under nitrogen flow (50 mL/min). Thermal stabilities of polymer samples were determined using a Seiko 120 TG/DTA analyzer run from 25 °C to 450 °C at a heating rate of 10 °C per minute under nitrogen flow (100 mL/min). Refractive index measurements on polymer films were measured using a Metricron 2010 Prism Coupler or on a M-44 ellipsometer (J.A. Woollam Co. Inc.) where it was modelled against a cauchy material. Temperature profiles for the exothermic polymerization were recorded using a Fisher Scientific Accumet Ab15 pH meter with a standard issue thermocouple.

Typical Synthesis of N,N-Dimethylacrylamide-co-2-Hydroxyethyl Acrylate Polymer

In a 100 mL 3-necked round-bottom flask, were charged N,N-dimethylacrylamide (5.46g, 55.03 mmol) and 2-hydroxyethyl acrylate (4.26g, 36.69 mmol) in DMF (70 mL, ~14 % wt/v). AIBN was added (0.0151g, 0.092 mmol) to initiate the reaction. The mixture was heated to 75°C under nitrogen. Polymer growth was monitored by GPC and the polymerization was terminated after subsequent measurements showed no change (18 –24 hours). The polymerization was cooled and precipitated into diethylether. The polymer was collected on a sintered glass filter, washed with ether, dissolved in chloroform and reverse precipitated with

ether. The coagulated polymer was filtered, dissolved in a minimum of chloroform and precipitated into ether, filtered, washed and dried under high vacuum at 80°C for 24 hours.

Synthesis of 2-(Perfluoroalkyl)ethyl methacrylate-*co*-2-Hydroxyethyl Acrylate Polymer

In a 100 mL 3-necked round bottom flask, were dissolved 2-(perfluoroalkyl)ethyl methacrylate (9.206g, 17.22 mmol) and 2-hydroxyethyl acrylate (3.0 g, 25.83 mmol) in THF (60 mL, ~20 % wt/v). AIBN was added (0.026 g, 0.158 mmol) to the solution to initiate the reaction. The reaction was heated to 75°C and allowed to run for 24 hours. The polymerization pot was diluted with THF (to 75 mL) and precipitated into hexane. The polymer was collected in a sintered glass filter, dissolved in 1,1,1-trichlorotrifluoroethane and reverse precipitated with ether. Again, the polymer was filtered, dissolved in a minimum of 1,1,1-trichlorotrifluoroethane and precipitated in ether, filtered, washed, and dried under high vacuum at 80°C for 24 hours.

Typical Post Polymerization Modification

In a 50 mL 2-necked round bottom flask, fitted with a magnetic stir bar and a nitrogen inlet/outlet, were dissolved polymer **IV.3** (1.0 g, 3.77 mmol OH assuming 100% incorporation) and triethylamine (0.38 g, 3.77 mmol) in chloroform (7 mL). Cinnamoyl chloride (0.942 g, 5.65 mmol) was dissolved in 2 mL of chloroform and added dropwise via a syringe over 10 minutes. The reaction was stirred overnight at

room temperature, filtered and precipitated into diethylether. The solids were filtered, redissolved in chloroform and the insoluble solids were removed. The chloroform solution was concentrated and precipitated into ether, where the cinnamate derivatized polymer **IV.3d** was collected on a sintered glass filter and dried at 80°C for 24 hours.

Synthesis of Polymer Hydroglass

In a glass vial were added acrylamide (0.35g, 4.92 mmol), methacrylamide (0.45g, 5.29 mmol), [2-(methacryloyloxy)ethyl]dimethyl(3-sulfopropyl)ammonium hydroxide inner salt hydrate (1.25 g, 4.47 mmol), [3-(methacryloylamino)propyl]dimethyl(3-sulfopropyl)ammonium hydroxide inner salt hydrate (0.30g, 1.02 mmol), piperazine diacrylamide (0.340 g, 1.73 mmole), and 1.90 mL of phosphate buffer (pH 7.2). The solution was mixed on low heat while being purged with nitrogen for 15 minutes. From this solution, 2.0 mL was removed and placed in a clean (dusted) polymethacrylate cuvette. Bacteriorhodopsin solution was added (0.225 mL) along with APS (1.782 mg, 0.008 mmol) and tetramethylethylenediamine (TMED) (5.7 mg, 0.05 mmol). The solution was mixed and allowed to stand at room temperature. The reaction was observed to be complete upon the formation of a hard polymer network and the liberation of heat.

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