

ABSTRACT

KLOXIN, APRIL MORRIS. Synthesis and Characterization of Antibacterial Poly(ethylene glycol) Hydrogels. (Under the direction of Richard J. Spontak and Stuart L. Cooper.)

Poly(ethylene glycol) (PEG) hydrogels containing tertiary amines were synthesized by thermally-initiated free-radical copolymerization of poly(ethylene glycol) methacrylate (PEGMA), poly(ethylene glycol) dimethacrylate (PEGDMA), and 2-dimethylaminoethyl methacrylate (DMAEMA). The mass fraction of each monomer was varied from 0 to ~1 to determine the composition for optimum mechanical properties and maximum tertiary amine content. Dry films were characterized by attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR), elemental analysis, dynamical mechanical analysis (DMA), tensile testing, swelling, and differential scanning calorimetry (DSC). The film composition with highest strength, elasticity, swelling, and amine content was found to be 30 wt% PEGDMA due to limited polymerization of DMAEMA with PEGDMA. Films composed of 10 wt% PEGDMA were subsequently quaternized with alkyl bromides of varying chain length (C_8 , C_{12} , and C_{16}) to impart antibacterial properties to the network. Quaternized films were characterized by elemental analysis, swelling, DSC, and a surface antimicrobial assay. The minimum quaternized DMAEMA concentration for antimicrobial activity was 22 wt% quaternized with C_8Br and 30 wt% quaternized with $C_{12}Br$ and $C_{16}Br$. The most potent alkyl bromide was C_8Br followed by $C_{12}Br$ and $C_{16}Br$. These quaternized PEG-co-poly(DMAEMA) hydrogels show promise as antibacterial materials for biomedical applications.

**SYNTHESIS AND CHARACTERIZATION OF ANTIBACTERIAL
POLY(ETHYLENE GLYCOL) HYDROGELS**

By

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TABLE OF CONTENTS

LIST OF TABLES	VI
LIST OF FIGURES	VII
1 INTRODUCTION.....	1
1.1 MOTIVATION OF RESEARCH.....	1
1.2 OBJECTIVES OF RESEARCH.....	2
1.3 OUTLINE OF THESIS	3
1.4 REFERENCES	4
2 LITERATURE REVIEW	7
2.1 BACTERIAL INFECTION OF MATERIALS	7
2.2 TYPES OF BACTERIA	8
2.3 PREVENTING BACTERIAL INFECTION IN MATERIALS	10
2.3.1 Biocides.....	10
2.3.2 Methods for Preventing Infection	13
2.4 HYDROGELS.....	22
2.4.1 Definition, Chemistry, and Physical Properties.....	22
2.4.2 Synthesis of Poly(ethylene glycol) Hydrogels and Applications	25
2.4.3 Hydrogels with Tertiary Amines	30
2.5 REFERENCES	32
3 SYNTHESIS AND CHARACTERIZATION OF PEG HYDROGELS	41
3.1 INTRODUCTION	41
3.2 EXPERIMENTAL METHODS	42
3.2.1 Materials	42
3.2.2 Hydrogel Synthesis	43
3.2.3 Elemental Microanalysis.....	47
3.2.4 ATR-FTIR.....	47
3.2.5 Swelling Measurements	47
3.2.6 Dynamical Mechanical Analysis	48
3.2.7 RHEOMETRY.....	48
3.2.8 Differential Scanning Calorimetry.....	49
3.3 RESULTS AND DISCUSSION.....	50
3.3.1 Hydrogel Synthesis	50
3.3.2 Mechanical Properties.....	55
3.4 CONCLUSIONS	72
3.5 REFERENCES	73
4 QUATERNIZATION AND ANTIBACTERIAL EFFICACY OF PEG HYDROGELS	76
4.1 INTRODUCTION	76
4.2 EXPERIMENTAL METHODS	77

4.2.1	Materials	77
4.2.2	Hydrogel Property Characterization	80
4.2.3	Antibacterial Efficacy Characterization.....	81
4.3	RESULTS AND DISCUSSION.....	84
4.3.1	Quaternization.....	84
4.3.2	Elemental Analysis	88
4.3.3	Swelling Experiments	89
4.3.4	Differential Scanning Calorimetry.....	92
4.3.5	Antibacterial Efficacy	93
4.4	CONCLUSIONS.....	99
4.5	REFERENCES	100
5	CONCLUSIONS AND FUTURE WORK.....	104
5.1	SUMMARY.....	104
5.1.1	Hydrogel Synthesis	104
5.1.2	Hydrogel Quaternization.....	106
5.2	FUTURE WORK	107
5.2.1	Polymerization	107
5.2.2	Quaternization and Antibacterial Testing	108
5.3	REFERENCES	110
	BIBLIOGRAPHY	111
	APPENDIX A: QUATERNARY AMMONIUM COMPOUND SYNTHESIS AND CHARACTERIZATION	119
	APPENDIX B: POLY(ETHYLENE GLYCOL)-POLYURETHANE HYDROGELS	127

LIST OF TABLES

Table 2.1. Types of biocides and their target sites.....	11
Table 3.1 Elemental Analysis of Dried Hydrogel Films [$\sim\pm 5$ % error]	54
Table 3.2 Characterization of Linear Region of Lightly Crosslinked Samples	62
Table 4.1 Degree of Quaternization of Films	88

LIST OF FIGURES

Figure 2.1 Bacterial infection of an implanted device occurs in several steps: (1) protein adhesion to the surface, (2) bacteria diffusion to the surface, (3) bacteria attachment, (4) bacteria proliferation and biofilm formation.....	8
Figure 2.2. Gram-positive bacteria cells are protected by a single-layer cell wall, whereas gram-negative bacteria are protected by a double-layer cell wall, making gram-negative bacteria more resistant to antibiotics than gram-positive bacteria.....	9
Figure 2.3. Quaternary ammonium compounds have 4 alkyl chains attached to a positively-charged nitrogen with a halogen counterion. For QACs to exhibit biocidal properties, one alkyl chain must have at least 8 carbons and a chloride, bromide, or iodide counterion.	12
Figure 2.4 Poly(ethylene glycol) (a) and heparin (b), respectively, have both been attached to polymer surfaces to improve biocompatibility.....	14
Figure 2.5 Trimethoprim (a), sparfloxacin (b), ciprofloxacin (c), and penicillin (d) are antibiotics that have been incorporated into materials make to make them antimicrobial.	17
Figure 2.6. On the left, the polymer network swells in a solvent. Small circles represent the network crosslinks. In this swollen state, a drug molecule represented by “D” can readily diffuse out of the network. On the right, the polymer network de-swells due to a change in the environment such as solvent temperature or pH, discussed below. In this collapsed state, a drug molecule cannot readily diffuse out of the network, which therefore controls the rate of drug delivery.....	23
Figure 2.7. Linear PEG has hydroxyl end groups and a repeat unit that interacts favorably with water.	25
Figure 3.1 The structure of each reactant is shown above: (a) PEGMA, (b) DMAEMA, (c) PEGDMA, and (d) AIBN.....	43
Figure 3.2 Monomer solution compositions were varied to optimize network properties. Each diamond represents a synthesized copolymer network.....	44
Figure 3.3 Shapes for (a) characterization and (b) tensile testing were cut into rubber molds with an Xacto knife as described in the text.	46
Figure 3.4 Graph A shows the monomer/film spectrum versus reaction time at 80°C. Graph B shows the difference spectra compared to the spectrum at 5 min.....	51
Figure 3.5 Analysis of ATR-FTIR spectra with SVD shows (A) the overall change in the film spectrum and (B) the change in the film spectrum in its entirety with time.	53
Figure 3.6 Equilibrium water content of hydrogels decreases linearly with increasing crosslinker concentration. Equilibrium water content shows little change with increasing	

nitrogen monomer concentration. The only exception to these observations is the 99-wt% DMAEMA sample, which was found to not be fully crosslinked.....	56
Figure 3.7 Film internal fracturing upon swelling decreased with increasing PEGDMA and DMAEMA concentrations. Each diamond represents a film composition observed to have internal fractures upon swelling in water.	58
Figure 3.8 Elastic moduli increase with increasing PEGDMA content and decrease with increasing DMAEMA content.	59
Figure 3.9 Two monomer solution compositions produced incompletely crosslinked films, where E'' , the viscous modulus, is greater than E' , the elastic modulus, where ϵ_0 is the maximum strain amplitude.....	61
Figure 3.10 Young's modulus follows the same compositional trend as the elastic modulus determined by DMA, increasing with increasing PEGDMA concentration while decreasing slightly with increasing DMAEMA concentration.....	64
Figure 3.11 Ultimate tensile stress increases with increasing PEGDMA concentration while it decreases with decreasing DMAEMA concentration only at constant 10 and 50 wt% PEGDMA.	65
Figure 3.12 Yield stress increases with increasing PEGDMA concentration and decreases with increasing DMAEMA content, a more significant dependence than seen with other properties. Any compositions omitted did not exhibit a yield stress.	67
Figure 3.13 Percent elongation shows little dependence on either monomer concentration except for the two lightly crosslinked samples.	68
Figure 3.14 Toughness increases with increasing PEGDMA concentration up to 50 wt% while its dependence on DMAEMA concentration is too noisy to be concluded.	69
Figure 3.15 Glass temperature of gels increases with increasing DMAEMA concentration.....	70
Figure 3.16 Glass transition temperatures of films compared to actual DMAEMA concentration incorporated.....	71
Figure 4.1 Quaternized hydrogels with constant 10 wt% PEGDMA exhibit color change.	86
Figure 4.2 Films dried on an elevated rack in aluminum pans (a) did not curl while films dried on flat filter paper (b) did curl during drying. Uniform drying thus eliminates film curling.	87
Figure 4.3 Equilibrium water content of hydrogels decreases with increasing quaternized DMAEMA concentration and alkyl halide chain length. Note that DMAEMA concentration of films prior to extraction is used for comparison to unquaternized swelling.....	91
Figure 4.4 Quaternization eliminates T_g dependence on DMAEMA concentration. Note that both quaternized and unquaternized films are plotted against DMAEMA	

concentration of extracted films, assuming that the T_g measured would mostly depend on DMAEMA bound to the network rather than unbound DMAEMA.....	92
Figure 4.5 Antibacterial efficacy of hydrogels increases with increasing quaternized nitrogen content while it decreases with increasing alkyl halide chain length (C_8 - C_{16}). Note that the plots are versus DMAEMA concentration prior to extraction for comparison with unquaternized films. The dashed line extends the spray CFU/cm ² and is included to help guide the eye.	95
Figure 4.6 Quaternized hydrogels with constant 10 wt% PEGDMA exposed to <i>S. aureus</i> exhibit bactericidal behavior.....	98

1 INTRODUCTION

1.1 MOTIVATION OF RESEARCH

With the increasing resistance of bacteria to antibiotics, the need to prevent bacterial infections in hospitals and in everyday life is growing at an alarming rate.^{1,2,3,4} Specifically, preventing infection of implanted and medical devices is becoming increasingly important as it is estimated that 45% of hospital infections are associated with these devices.⁵ To prevent bacterial infection of biomedical devices, we must understand how these infections begin. Bacterial infection of a biomedical device generally occurs in several steps.⁶ The first step is protein adsorption to the surface of the device, followed by adhesion of bacteria to the protein and the surface. Once attached, the bacteria proliferate and form a colony, known as a bacterial biofilm. This biofilm produces free-floating bacteria cells within the bloodstream or in tissue that can be killed by conventional antibiotics, but the film itself can be resistant to one thousand times the normal dose of antibiotics.⁷ Therefore biofilm formation often necessitates the removal of the infected device.⁸

Bacterial infection of a biomedical device can be prevented by physical modification of the material constituting the device. Several strategies have been employed for improving the antibacterial efficacy of polymeric materials: modification of surface properties to prevent protein and bacterial adhesion^{5,9}, impregnation of materials with antibiotics or biocides intended to initially kill bacteria¹⁰⁻¹⁵, and immobilization of antibiotics or biocides to permanently kill bacteria.^{8,16-20} Each of these antifouling strategies has been applied to soluble polymers and solid surfaces, but little research has

been performed on elastomeric polymer systems such as hydrogels. Hydrogels are hydrophilic macromolecular networks that swell in aqueous media.²¹ Because of their high water content and surface properties, hydrogels can be used to simulate natural tissue and often exhibit good biocompatibility, lending them for use in numerous biomedical applications such as implanted drug delivery devices²² and biosensor coatings²³. In these applications, hydrogels are likewise susceptible to bacterial infection.

In this work, we investigate the covalent attachment of antibacterial groups to hydrogels, as was done previously with polyurethane thermoplastics⁸, to produce a hydrogel with permanent antibacterial properties. Our overarching goal is to prevent bacterial infection and potentially improve hydrogel performance in implant applications. Specifically, we employ poly(ethylene glycol) hydrogels because of their known biocompatibility and hydrophilicity with quaternary ammonium compounds, which possess known antibacterial properties and have been previously used in antifouling materials.

1.2 OBJECTIVES OF RESEARCH

The primary focus of this research was to synthesize antibacterial hydrogels and characterize their properties, keeping in mind the goal above. This objective is divided into several individual tasks. The first task was to review recent work in antibacterial materials and hydrogels to identify a polymer system suitable for experimentation. The basic criteria for an acceptable polymer system are (i) it must be chemically crosslinkable, (ii) it must be hydrophilic and thus water swellable, and (iii) it must possess functional groups for chemical incorporation of antibacterial moieties. The second task

was to synthesize polymer compositions that meet the above criteria, optimizing the crosslinking conditions and bioactive group derivatization while producing adequate mechanical properties. This task formed the core research of this thesis. The final task was characterization of the hydrogels. Characterization includes determining hydrogel mechanical properties and hydrogel antibacterial properties.

1.3 OUTLINE OF THESIS

This thesis begins with a literature review of the items briefly alluded to in section 1.1 and includes recent work on in the growing field of antibacterial materials and hydrogels. The results of this investigation are covered in two chapters: *(i)* hydrogel synthesis and mechanical properties and *(ii)* quaternization of the hydrogels and antibacterial properties. Each of these chapters includes a brief introduction, experimental methods, results, discussion, and conclusions. The fourth chapter is a summary of the research findings collected here and recommendations for future work. The appendices include calculations used in the data analysis, as well as a limited investigation of small-molecule biocides and polyurethane synthesis that was not used to make the hydrogel system. References are listed at the end of each chapter, and a bibliography for the entire thesis is provided at the end of the document.

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2 LITERATURE REVIEW

The following sections provide information needed to understand bacterial infection of materials, preventative measures against bacterial infections, property development in gels, and synthetic routes to hydrogels.

2.1 BACTERIAL INFECTION OF MATERIALS

As mentioned in the Introduction, forty-five percent of hospital infections are related to the utilization of implanted medical devices.¹ Examples of biomedical devices affected include catheters, prosthetic heart valves, pacemakers, vascular prostheses, and orthopedic implants.² Bacterial infections are often unresponsive to antibiotics and necessitate removal of the implant.^{2,3} To understand how to prevent and ultimately eradicate these infections, it is important to understand the infection process.

Bacterial infection of an implant occurs over the course of several well-defined steps.^{3,4} First step initiates when the implant comes into contact with bacteria. A device can be exposed to bacteria during implantation by simply brushing up against a patient's skin or neighboring wound. Once implanted, it can likewise become contaminated by bacteria residing in the blood and other body fluids. A schematic illustration of the infection process of an implanted device is presented in Figure 2.1.

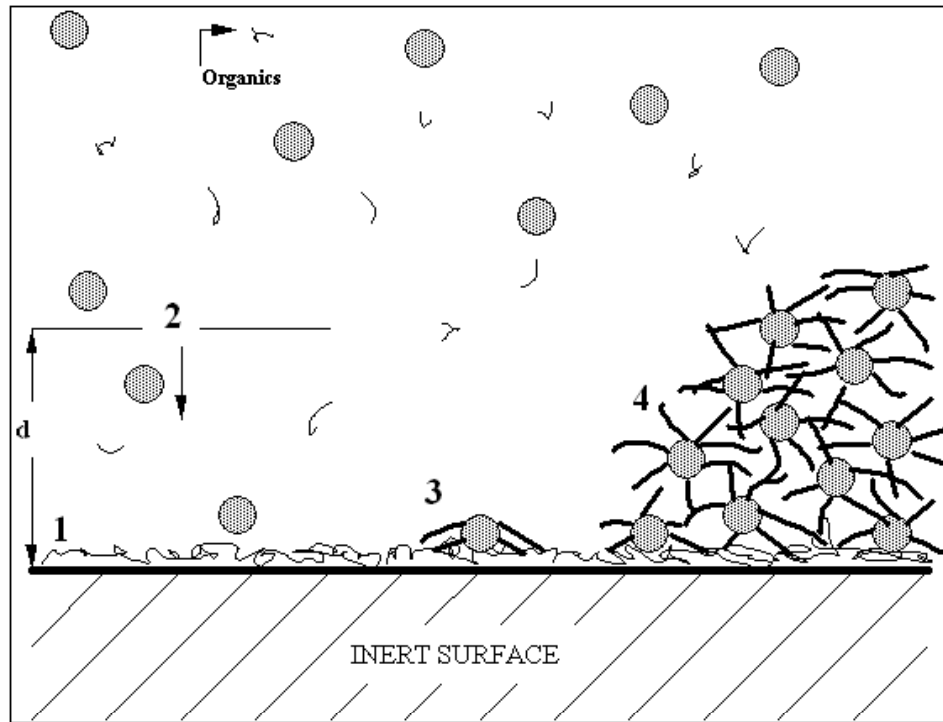


Figure 2.1 Bacterial infection of an implanted device occurs in several steps: (1) protein adhesion to the surface, (2) bacteria diffusion to the surface, (3) bacteria attachment, (4) bacteria proliferation and biofilm formation. (Figure taken from Grapski⁵.)

Proteins and other organics diffuse from the blood and adhere to the surface of the device, **1** in Figure 2.1. Bacteria subsequently diffuse to the surface for nutrients offered by the adhered proteins, **2**. Once on the surface, bacteria attach to the device as well as to the adsorbed proteins, **3**. The attached bacteria proliferate and excrete a protective layer of polysaccharides, forming a biofilm that is resistant to antibiotics in acceptable doses, **4**. The biofilm produces free-floating, or planktonic, bacteria cells that travel through the bloodstream, thereby promoting systemic toxicity.

2.2 TYPES OF BACTERIA

Bacteria can be classified into two general categories on the basis of their cell wall structure. The cell walls of gram-positive bacteria are rich in peptidoglycan, which

selectively absorbs violet dye.⁶ In this case, gram-positive bacteria appear violet when exposed to violet dye followed by red dye. Gram-negative bacteria, on the other hand, turn red when exposed to the same dye protocol because their cell walls are more structurally complex and contain less peptidoglycan, which is located in a periplasmic gel. The differences in the cell walls of the two types of bacteria are shown schematically in Figure 2.2.

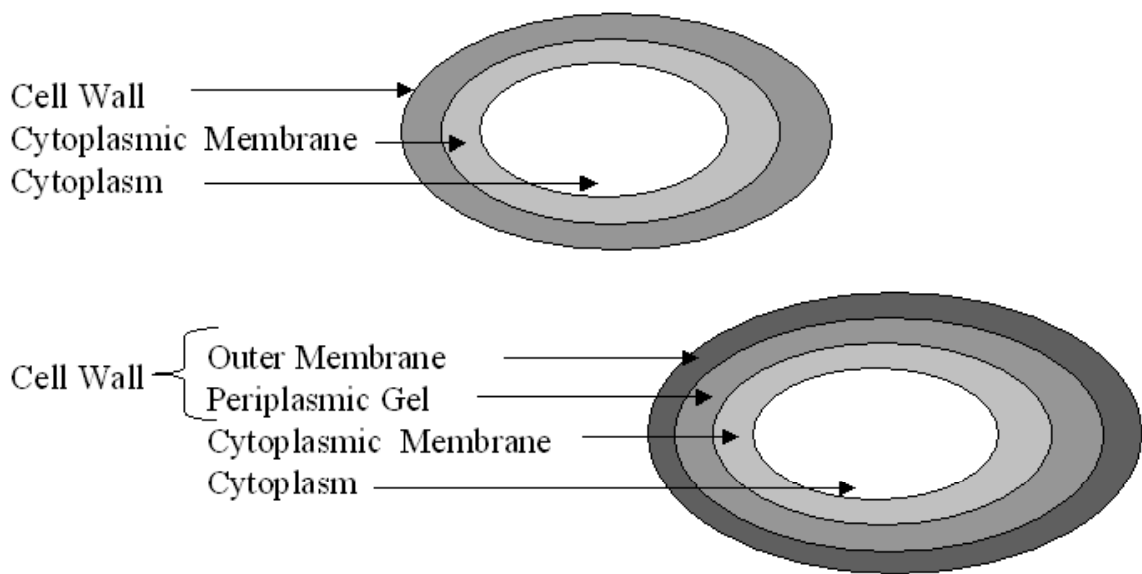


Figure 2.2. Gram-positive bacteria cells are protected by a single-layer cell wall, whereas gram-negative bacteria are protected by a double-layer cell wall, making gram-negative bacteria more resistant to antibiotics than gram-positive bacteria.⁶

Due to differences in their cell walls, gram-negative bacteria are typically more resistant than gram-positive bacteria to host defenses, antibiotics, and biocides. *Staphylococcus aureus*, a skin bacterium, and *Bacillus anthracis*, a soil bacterium commonly known for causing Anthrax, are two examples of gram-positive bacteria. *Escherichia coli*, an intestinal bacterium, and *Vibrio harveyi*, a marine bacterium, are two types of gram-

negative bacteria. Note that *S. aureus* is a common cause of infections on biomedical devices.²

2.3 PREVENTING BACTERIAL INFECTION IN MATERIALS

2.3.1 BIOCIDES

2.3.1.1 Types of Biocides and Antibacterial Activity

A biocide is defined as a substance that is destructive to many different types of organisms. These organisms include bacteria, viruses, fungi, and other microbes. In the case of the present work, the interest is in biocides used against bacteria. Biocides can be either bacteriostatic or bactericidal. A bacteriostat only inhibits the growth of bacteria, whereas a bacteriocide actually destroys bacteria.

A biocide has several routes by which it can inhibit or destroy bacteria cells.^{7,8} These routes involve three cellular regions wherein the biocide can interact with the bacteria: the cell wall, the cytoplasmic membrane, and the cytoplasm, as shown in Figure 2.2. The cell wall provides the cell with structural integrity and is often the site for antibiotic attack but not biocidal attack. The cytoplasmic membrane is the main target of biocidal attack, which, if successful, may promote several cellular responses: (i) physical disruption of the membrane causing leakage of intracellular components, (ii) dissipation of the proton motive force stopping active transport across the membrane, or (iii) inhibition of membrane-associated enzyme activity and related reactions. Although the cytoplasm contains critical cellular machinery, biocides typically do not target the cytoplasm because cell entry, implying prior disruption of the cytoplasmic membrane, is

required. Depending on the number of cellular functions disrupted and the severity of the disruption, a biocide may exhibit bacteriostatic to bactericidal behavior.⁷ Bacteriostatic effects are normally observed at lower biocide concentrations due to reversible activity with the cytoplasmic membrane and membrane-associated enzymes, while biocidal effects would be observed at higher concentrations, which produce permanent cellular damage.⁸ Depending of the chemical nature and structure of the biocide, different cellular functions will be affected. A list of biocides, including their target sites and type of interaction, is provided in Table 2.1.

Table 2.1. Types of biocides and their target sites⁷

Biocides	Target Site	Type of Interaction	Neutralizing Process
Bronopol	Enzyme-SH	Chemical	Cysteine
Quaternary Ammonium Compounds (QACs)	Membrane	Ionic	Lecithin + Tween 80
Formaldehyde	Amino Groups	Chemical	Glycine
Biguanides	Membrane	Ionic	Lecithin + Tween 80
Sorbic Acid	Enzyme-SH, Proton Gradient	Chemical, Physical	Dilution + Tween 80
Ethanol	Membrane	Physical	Dilution
Phenols	Membrane	Physical	Dilution
Aromatic Alcohols	Membrane	Physical	Dilution

In the case of an immobilized biocide, the target site is limited to the cell wall and cytoplasmic membrane since the biocide is not free to enter the cell. As will be discussed in Section 2.3.2.3, previous efforts employing immobilized biocides have focused on quaternary ammonium compounds (QACs) due primarily to their cell membrane activity and antibacterial efficacy. The biocide of choice for use in this study is also a QAC.

2.3.1.2 Quaternary Ammonium Compounds

Quaternary ammonium compounds consist of a positively-charged nitrogen with 4 covalently-bound alkyl groups and a halogen counterion, shown in Figure 2.3.⁹

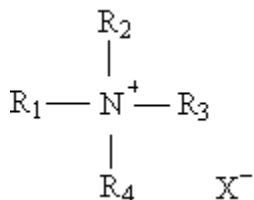


Figure 2.3. Quaternary ammonium compounds have 4 alkyl chains attached to a positively-charged nitrogen with a halogen counterion. For QACs to exhibit biocidal properties, one alkyl chain must have at least 8 carbons and a chloride, bromide, or iodide counterion.¹⁰

These compounds are formed by a nucleophilic substitution reaction, S_N2, when an alkyl halide reacts with a tertiary amine to quaternize the nitrogen with its alkyl chain. The halogen counterion is from the alkyl halide reactant and is typically a chloride, bromide, or iodide.

Quaternary ammonium compounds inhibit the growth of algae, bacteria, and fungi at low concentration while killing these same organisms at moderate concentrations.^{11,12} These biocides are effective against both gram-positive and gram-negative bacteria, although they are less effective against gram-negative bacteria due to their double-layer cell wall morphology mentioned in Section 2.2

The antibacterial efficacy of QACs is related to the length of the alkyl chains attached to the quaternary nitrogen, with potency occurring when one chain has at least 8 carbons.¹⁰ Cutler et al.¹³ have reported that, as alkyl chain length increased, the driving force for the QAC to penetrate into to the cell membrane increased while the overall mobility of the QAC and its interaction with the cell decreased. This observation suggests

the existence of an optimum chain length for membrane penetration and cell interaction, with the most effective antibacterial chain length corresponding to about 14 carbons in the specific case of alkyldimethylbenzyl ammonium chlorides.

The biocidal activity of QACs arises from their interaction with the cytoplasmic membrane of the cell, inducing leakage of intracellular components in the following order: potassium, inorganic phosphates, amino acids, nucleic acids, and proteins.⁸ It is thus hypothesized that the QAC combines with the membrane phospholipids, causing disruption of the cytoplasmic membrane and eventually cell lysis.

2.3.2 METHODS FOR PREVENTING INFECTION

With the mechanistic steps of bacterial adhesion to surfaces known, studies employing different strategies have been performed to prevent bacterial infection of biomedical materials. These strategies include *(i)* modification of surfaces properties, *(ii)* impregnation of materials with antibiotics or biocides, and *(iii)* immobilization of antibiotics or biocides. Each of these strategies will be briefly elaborated in the next sections, with a particular emphasis on the latter strategy. Extensive reviews of materials designed to possess antimicrobial properties are also available in the literature,¹⁴⁻¹⁶ and the interested reader is encouraged to examine them as prerequisites to ongoing developments in this rapidly expanding and exciting branch of materials research.

2.3.2.1 Modification of Surface Properties to Prevent Adhesion

One strategy developed to prevent biofilm formation relies on reduced adhesion of proteins and bacteria through modification of surface properties such as hydrophobicity, surface energy, and roughness. Many variations of this approach have

been studied, including surface modification with poly(ethylene oxide),^{2,17,18} heparin,^{1,19} and other biologically-active molecules.^{20,21} Two examples of this method are described below, but, since this method was not considered for this research project and the field is so broad, an extensive review is not provided. The interested reader can consult one of the many books on the topic of surface modification and biomaterials including *Polyurethanes in Biomedical Applications*,²² *Antimicrobial/Anti-infective Materials: Principles, Applications, and Devices*,²³ *Biomaterials Science*,²⁴ and *Biomaterials, Medical Devices, and Tissue Engineering: An Integrated Approach*.²⁵

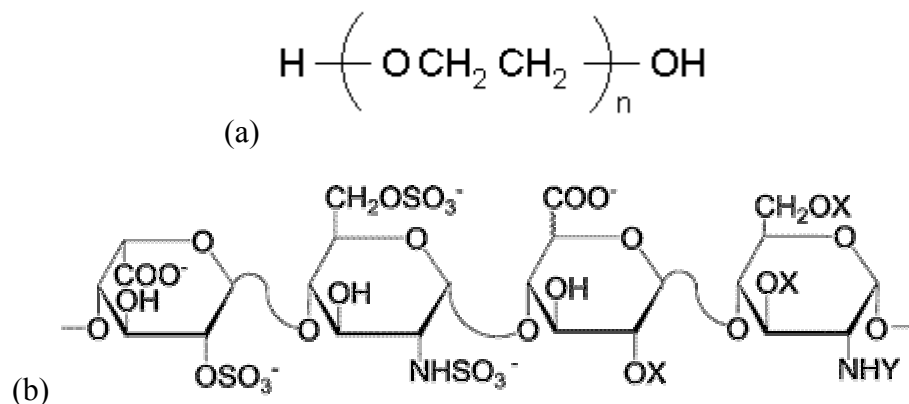


Figure 2.4 Poly(ethylene glycol) (a) and heparin²⁶ (b), respectively, have both been attached to polymer surfaces to improve biocompatibility.

Hubbell and co-workers² treated the surface of poly(ethylene terephthalate) (PET) films with 18.5 kDa poly(ethylene oxide) (PEO) to increase the hydrophilicity of the inherently hydrophobic PET film surface and thus decrease the adsorption of proteins, specifically fibrinogen, and bacteria (*S. aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginos*). The PET films were modified by a surface physical interpenetrating network (SPIN) technique wherein the PET and PEO are mutually swelled in a common solvent allowing PEO to diffuse into PET matrix. The PET is

exposed to a non-solvent, which extracts the swelling agent thereby trapping the PEO inside of the film and along the film surface. Bacteria adhesion to the PET film *in vitro* was reduced between 70 and 95% after modification with PEO². On the basis of these results, it was hypothesized that reduced fibrinogen adhesion promotes decreased bacteria adhesion. Later, Razatos et al.²⁷ used atomic force microscopy to demonstrate that *E. coli* cells were separated by the length of a single poly(ethylene glycol) (PEG) chain from a glass surface treated with a PEG-lysine dendron copolymer. In this case, PEG eliminated the attractive interaction between the bacteria and the surface while creating repulsive steric effects.

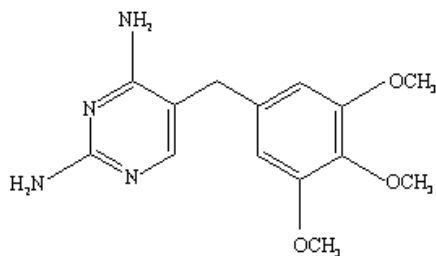
Arciola et al.¹ explored bacteria adhesion to heparin-treated poly(methyl methacrylate) (PMMA) films, which are likewise inherently hydrophobic. As-made PMMA and heparin-surface-modified PMMA contact lenses were used in the study. As with PEG-modified materials, the adhesion of *S. aureus* was significantly reduced by surface treatment with heparin, an anticoagulant.

These surface-modification techniques are initially effective at reducing protein and bacteria adhesion, but the surface agents may diffuse away over time, leading to bacteria adhesion and infection. A more permanent modification of the biomedical device may be anticipated to be more effective at preventing bacteria infection long-term.

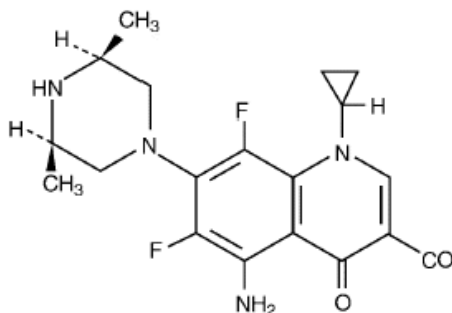
2.3.2.2 Impregnation with Antibiotics or Biocides

An alternative strategy for preventing infection in biomedical devices is impregnation of the material with antibiotics or biocides to destroy bacteria around or on the surface of the device. Examples of such antibiotics and biocides used for this purpose

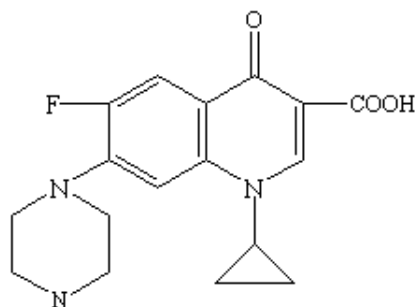
include rifampin, trimethoprim, sparfloxacin, ciprofloxacin, and penicillin, the chemical structures of which are displayed in Figure 2.5. Impregnation of ventricular grafts and catheters with individual, as well as combinations of, antibiotics is achieved by soaking of the device in an antibiotic/solvent solution, attachment of a hydrogel imbued with antibiotic to the device surface, and binding of antibiotics with surfactants to the device surface. Each of these methods will be briefly discussed below.



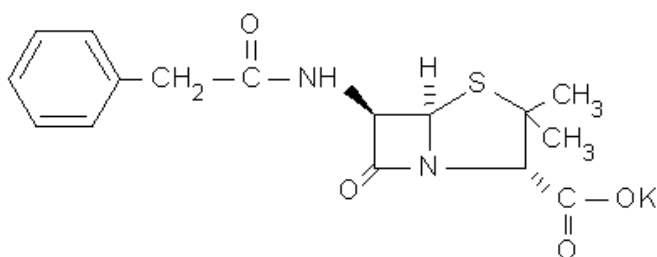
(a)



(b)



(c)



(d)

Figure 2.5 Trimethoprim (a), sparfloxacin (b), ciprofloxacin (c), and penicillin (d) are antibiotics that have been incorporated into materials make to make them antimicrobial.

Silicone ventricular catheters were imbibed with mixtures of antibiotics to improve antibacterial efficacy and likewise reduce the risk of bacteria resistance to each

individual antibiotic. To incorporate the antibiotic, 2-cm segments of silicone catheter were soaked in an antibiotic/chloroform solution between 5 and 37 g/L for 1 h at 60°C and subsequently dried under reduced pressure for 2 days at 60°C. Impregnated catheters were exposed to a bacteria/broth solution, incubated in fresh, and sonicated to remove attached bacteria. The detached bacteria were plated on blood agar and incubated overnight to determine the number of viable colony forming units (CFU). The antibiotic combination of rifampin and trimethoprim was effective at reducing colonization of *S. aureus* by 99.97% within 3 hours *in vitro* while rifampin and sparfloxacin were observed to decrease the colonization of *S. epidermidis* for 1 year *in vitro*.^{28,29} These results show promise for short-term and long-term (1 year) prevention of catheter-related infections.

An alternative method of incorporating antibiotics onto the surface of catheters is by the addition of a hydrogel layer containing antibiotics. DiTizio et al.³⁰ covalently bound a PEG-gelatin hydrogel via urethane linkages to the surface of silicone catheter. First, the catheter was immersed in a gelatin-buffer-binder (1,3-dicyclohexylcarbodiimide) solution for 1 h and dried at 40°C for 2 h. The gelatin solution was bound the surface by irradiation with short wave UV for 3 min. A mixture of liposomes containing the antibiotic ciprofloxacin and PEG-gelatin was then spin-coated on the surface of the gelatin-treated catheter, incubated at 4°C for 15 min, and polymerized by immersion in borate buffer for 1h, binding the hydrogel to the catheter surface. Liposomes held within the hydrogel-coated catheter were released *in vitro* over the course of 7 days. Bacteria adhesion was inhibited over the entire antibiotic release assay. This coating technique can be used to modify the surfaces of other implantable devices and allow for incorporation of other biologically active compounds. Note that

the previous two studies have only been tested *in vitro*. Efficacy *in vivo* is often different from *in vitro*, as will be shown in the next study.

Greco et al.³¹ have non-covalently bound antibiotics to ventricular grafts and catheters in studies over several years. Dacron ventricular grafts were treated with a surfactant, typically benzalkonium chloride, to bind penicillin or cefozolin. When implanted in rats, the grafts slowly released the antibiotics to the surrounding tissue. Poly(tetrafluoroethylene) (PTFE) ventricular grafts treated with benzalkonium chloride or tridodecylmethylammonium chloride and penicillin were implanted in dogs as replacements for *S. aureus*-infected grafts. The antibiotic-treated grafts significantly reduced the number of infections versus the control, and penicillin remained bound to the treated grafts for 3 or more weeks.³² Silicone dialysis catheters were treated with tridodecylmethylammonium chloride and penicillin.³³ In rats, these catheters were more resistant to infection than controls when bacteria were introduced, but 95 % percent of the penicillin had left the catheter within 48 h. Despite these successful results and others, these antibiotic-treated dialysis catheters showed no difference from controls in the occurrence of catheter-tract infections during a large (86-patient) randomized clinical trial.³⁴

Impregnation of biomedical materials with antibiotics suffers from the drawback of long-term efficacy as the antibiotics or biocides diffuse away from the surface over time. Other considerations are that diffusion of biocides from the device into the host could be detrimental to the surrounding tissue and that diffusion of antibiotics into the host may lead to increased bacterial resistance.

2.3.2.3 Immobilization of Antibiotics or Biocides

Considering the limitations of surface modification and antimicrobial impregnation, permanent immobilization of the antibiotic or biocide on the material surface has been explored. To create lasting antibacterial materials for biomedical devices, researchers have permanently attached antibiotics or biocides to polymer backbones or polymer surfaces via covalent bonds. Some examples of this protocol include covalent bonding of various antibacterial salts to soluble polymers,³⁵⁻⁴² covalent bonding of quaternary ammonium salts within crosslinked polymers,⁴³⁻⁴⁸ and covalent bonding of polycations to surfaces.⁴⁹⁻⁵³

Nurdin et al.³⁵ prepared polybutadiene-based polyurethane films with covalently bound quaternary ammonium salts of varying alkyl chain length: 8-, 10-, 12-, 14-, and 16-carbons. Films of each alkyl chain length showed biocidal activity against gram-positive and gram-negative bacteria, yeasts, and molds.⁴² Quaternary ammonium salts with bulkier side chains less active relative to those with short side chains, indicating steric hindrance affects antibacterial efficacy.

Grapski and Cooper³⁶ prepared quaternized polyurethane elastomers with varying concentrations of quaternized moieties as well as varying alkyl halides: C₈ iodine, fluorinated-C₁₀ iodine, and C₁₈ iodine. The antibacterial efficacy of these polymer films against *S. aureus* was quantified via fluorescence microscopy. The C₁₈ chain was most effective with 95% reduction in number of viable adherent cells followed by the C₁₀ chain with 80% reduction and the C₈ chain with 16-36% reduction. Only the C₁₈ chain demonstrated antibacterial activity against *E. coli*, possibly because the bulky fluoro-

carbons of the C₁₀ chain sterically hindered interaction of the biocide with the bacteria cell membrane.

Kanazawa et al.³⁹ investigated the use of polymeric phosphonium salts with 10- to 18-carbon chains. These polymers exhibited biocidal properties against *S. aureus* and *E. coli in vitro*. While the antibacterial efficacy of these salts decreased with increasing alkyl chain length, the antibacterial efficacy of the corresponding unbound phosphonium salts increased with increasing alkyl chain length, confirming that steric hindrance affected the activity of the immobilized salts. The antibacterial activity of mixtures polymeric phosphonium salts and polymeric ammonium salts was also studied and revealed synergistic effects, whereas the antibacterial activity of copolymers containing phosphonium and ammonium salts increased monotonously with increasing phosphonium salt incorporation.⁴⁰ Moreover, phosphonium salts covalently bound to cotton fibers and cellulose filters also showed antibacterial activity.^{38,41}

Klibanov and co-workers have derivatized the surface of glass slides⁴⁹ and various polymer films^{50,52}, including polyethylene, polypropylene, nylon, and poly(ethylene terephthalate), with a poly(vinyl-N-alkylpyridinium bromide). They have also modified glass slides, nanoparticles⁵³, and fabrics⁵¹, including cotton, wool, nylon, and polyester, with alkylated polyethylenimine. The protocol for surface modification varied depending on the presence of a reactive group on the surface. The general steps undertaken were (i) attachment of an amine, (ii) attachment of a polyamine, and (iii) quaternization of tertiary amines along the backbone of the polyamine with alkyl bromides. These derivatized materials were effective at killing gram-positive bacteria, gram-negative bacteria, and fungi. Cen et al.⁵⁴ also explored this method and arrived at

similar results. Although a distinct advantage of this method is that almost any material can be made antibacterial, it still has limitations, however, as its sequential surface reaction steps may be difficult to scale up for use in bulk manufacturing.

Imazato et al.^{43,45,46} have incorporated antibacterial moieties into crosslinked dental resins. Methacryloyloxydodecylpyridinium bromide (MDPB) was crosslinked into a dental resin and tested against 7 major oral streptococci mutans. These modified resins before and after crosslinking exhibited bacteriostatic properties. Incorporation of the biocide did not affect the mechanical properties of the resin.⁴⁵

Kawabata et al.⁴⁷ used crosslinked poly(vinylpyridium bromide) to remove bacteria from water. When water infected with bacteria passed through a column containing the polymer, 99% percent of *E. coli*, *Salmonella typhimurium*, *Streptococcus faecalis*, and *S. aureus* was removed between 2 and 6 h. Bacteria were irreversibly trapped alive in the column and continued to grow when bound to the polymer. Since bacteria could not be removed from the column, an alternative water purification method was tried. Non-woven cloth coated with a thin layer of the modified polymer was used to effectively remove bacteria, bacteriophage, and viruses from the contaminated water.⁴⁸

2.4 HYDROGELS

The second principal subject of this thesis is the polymer system and its properties. The chemistry and synthesis of gels, specifically hydrogels, is reviewed below.

2.4.1 DEFINITION, CHEMISTRY, AND PHYSICAL PROPERTIES

Polymer gels are three-dimensional networks composed of flexible polymer chains connected by chemical bonds or strong physical forces, denoted crosslinks,

crosslink sites, or junction points.⁵⁵ Chemical gels consist of networks that are covalently bonded and are therefore permanent, whereas physical gels are stabilized by intermolecular interactions such as hydrogen bonding or electrostatic interactions and are temperature- or deformation-reversible. Throughout the remainder of the thesis, only permanent chemical gels will be considered further. When a polymer network contacts a theta or good solvent, the polymer swells, absorbing the solvent without dissolving. Swelling of chemical gel is analogous to a solvent spontaneously mixing with a pure polymer to form a homogeneous polymer solution except for the fact that the gel is insoluble due to the presence of crosslinks.⁵⁶

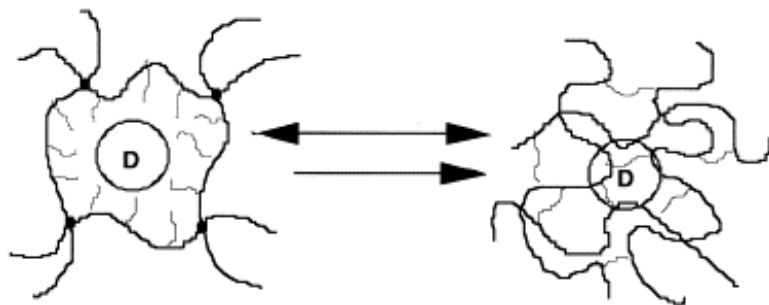


Figure 2.6. On the left, the polymer network swells in a solvent. Small circles represent the network crosslinks. In this swollen state, a drug molecule represented by “D” can readily diffuse out of the network. On the right, the polymer network de-swells due to a change in the environment such as solvent temperature or pH, discussed below. In this collapsed state, a drug molecule cannot readily diffuse out of the network, which therefore controls the rate of drug delivery.⁵⁷

Novel chemical and structural characteristics of gels make them interesting to study and useful in a broad range of applications. The polymer backbone comprising the network, as well as its functional substituent groups, control the response of the polymer to solvents and other environmental stimuli including, but not limited to, pH, temperature, pressure, ionic concentration, stress, and electric field.⁵⁸ Functional groups

that control the gel response typically exhibit hydrophobic-hydrophilic, van der Waals, hydrogen-bonding, or ionic interactions. These interactions govern the stimulus that triggers the network swelling. The degree of swelling in response to a solvent is generally continuous. Swelling induced by an environmental change can, however, trigger a dramatic, discontinuous change in the volume. Such a volume change is designated the volume-phase or volume-change transition. The volume-change transition and its corresponding environmental stimulus can be used to turn the solvent absorbing power of a gel “on” or “off,” thereby making polymer gels valuable as smart separation media, molecular sieves, drug delivery systems, actuators, and sensors.

Hydrogels, in particular, are hydrophilic polymer networks that swell specifically in aqueous environments.⁵⁹ These crosslinked polymers swell in water because of hydrophilic functionalities such as -OH, -COOH, -CONH₂, -CONH, and -SO₃H on the polymer backbone or on substituent groups. Because of their high water content in the swollen state, hydrogels behave surprisingly similar to natural tissue and are often used in tissue engineering applications, as well as in drug delivery devices, biosensors, and membranes. For biomedical applications such as these, crosslinked poly(ethylene glycol) (PEG) is a suitable material due to its biocompatibility and hydrophilicity.⁶⁰ The uncrosslinked PEG precursor, shown below, is a linear heteroatom oligomer or polymer with a simple polyether structure. It is commercially available over a wide range of number-average molecular weights (M_n) up to *ca.* 20,000 g/mol.⁶⁰

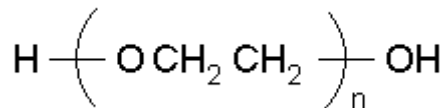


Figure 2.7. Linear PEG has hydroxyl end groups and a repeat unit that interacts favorably with water.

Despite its simple structure, PEG is uniquely effective at excluding other polymers in the presence of an aqueous environment. It rejects proteins, forms two-phase systems with other polymers, and resists recognition by the immune system.⁶⁰ This non-ionic polymer is soluble in water and most organic solvents. In addition, PEG is non-toxic and is rapidly cleared from the body.⁵⁷ Due to its functional end groups, PEG can be covalently attached to another material so as to “transfer” its properties, making a toxic system nontoxic or a hydrophobic system hydrophilic.⁶⁰

The synthesis and application of crosslinked PEG, the polymer system selected for use in this thesis, are addressed below.

2.4.2 SYNTHESIS OF POLY(ETHYLENE GLYCOL) HYDROGELS AND APPLICATIONS

While a variety of strategies have been developed to physically crosslink PEG⁶¹⁻⁶⁴, two synthesis techniques are most widely used for crosslinking PEG: radiation crosslinking and chemical crosslinking. Each of these techniques is discussed further below.

2.4.2.1 Radiation Crosslinking

Radiation crosslinking of PEG and its high molecular weight counterpart poly(ethylene oxide) (PEO) is the oldest technique for producing PEO hydrogels. Union

Carbide holds many patents on the technique dating back to 1968.^{65,66} Under these patents, either PEG or PEO is diluted between 2 and 6 (w/v) % in water.⁶⁶ Depending on the polymer concentration and molecular weight, a specified dose of γ -radiation from a ^{60}Co source is applied. The irradiation of PEG causes simultaneous chain scission and chain branching, creating a weak polymer network. The resultant PEG gel can be reinforced with a plastic porous sheet for use as wound dressing. Stringer and Peppas⁶⁷ and Kofinas et al.⁶⁸ further studied the radiation crosslinking of linear PEO in water for applications in drug delivery. More recently, Lowman and Peppas⁵⁷ have explored radiation crosslinking of PEG star polymers. These PEG star polymer gels had a large number of free -OH arms compared to linear radiation-crosslinked PEG, offering opportunities for additional functionalization. Overall, radiation crosslinking is expensive and inconvenient because of limited access to a ^{60}Co γ -radiation sources.⁶⁹ The gel structure is also difficult to control because chain scission and chain branching under radiation are stochastic processes.⁵⁷

Chemical crosslinking of PEG is less expensive than radiation crosslinking and offers more opportunities for incorporation of different functional groups. Two primary reaction schemes have been developed for chemically crosslinking PEG on the basis of (i) urethane linkages or (ii) free radical polymerization upon thermal or UV-initiation.

2.4.2.2 Chemical Crosslinking via Urethane Linkages

Graham et al.⁶⁹⁻⁷³ have studied the chemical crosslinking of PEG via polyurethane chemistry. In this case, PEG is reacted with a diisocyanate and triol at 70°C and poured into a mold to finish the reaction at 100°C for 15 h⁷⁰. During this reaction, each hydroxyl

endgroup of virgin PEG reacts with an isocyanate group to form a urethane bond. Ideally the resultant difunctional isocyanate then reacts with one of the triol hydroxyl groups, thus producing a branched PEG-polyurethane hydrogel. All reactants must be thoroughly dried under vacuum prior to crosslinking to prevent reaction of the diisocyanate with water, which yields carbon dioxide and bubbles in the gel. Because the reaction is fast and must be water-free, it is difficult to reproduce reliably. Successful reactions result in an excellent PEG hydrogel, however, with a high degree of swelling and the ability to controllable change swelling properties by varying temperature between 25 and 100°C.

Bromberg⁷⁴ has recently modified the reaction of Graham and coworkers⁷⁰ to control the reaction speed and network structure. Rather than crosslinking with a diisocyanate and a triol, independent synthesis of a cyclic isocyanate crosslinking agent eliminates the need for a triol and, hence, reduces the variability in the crosslinking step to help control the network structure formed. The reaction is also conducted in solution at 10% w/w PEG/solvent for 2-3 days rather than in a melt state for just a few minutes. Performing the reaction in solution reduces diffusion limitations, resulting in a more uniform network structure. PEG hydrogels synthesized by this method are used for protein and drug delivery, such as vaginal delivery of prostaglandin E₂ to induce labor.^{70,74}

2.4.2.3 Chemical Crosslinking via Free Radical Polymerization

An alternative method of chemically crosslinking PEG is to both hydroxyl groups with acrylate or methacrylate endgroups and initiate free radical polymerization with peroxides and heat or UV radiation. This crosslinking method is more controlled than the

urethane method described above since the reactants can be mixed at ambient temperature without reaction and followed by crosslinking *in situ*.

Patel et al.⁷⁵ polymerized poly(ethylene glycol) diacrylate with molecular weights of 575 (PEG575DA) or 700 g/mol (PEG700DA) with 0.5 wt% initiator (2,2'-azobisisobutyronitrile, AIBN) at 80°C for 18-20 h. The monomer/initiator mixture was agitated at ambient temperature until homogenous, placed in a vacuum to remove air bubbles, and poured between two glass plates before crosslinking. Defect-free films measuring between 40 and 200 μm in thickness were produced and tested as membranes for CO_2 separation from H_2/CO_2 gas mixtures. Surface-modified fumed silica (FS) with methacrylate groups was also incorporated prior to crosslinking to increase the mechanical strength of the inherently soft network. With the addition of up to 10 wt% FS, dynamic elastic tensile modulus (E') increased by 60% for the PEG700DA system.

Peppas et al.⁵⁷ copolymerized poly(ethylene glycol) dimethacrylate (PEGDMA) and poly(ethylene glycol) monomethacrylate (PEGMA) both with a molecular weight of 1000 g/mol. The two oligomer precursors were mixed in ratios ranging from 20/80 to 80/20 w/w and were subsequently diluted to 50 wt% in water. A small amount of photoinitiator, 1-hydroxycyclohexyl phenyl ketone, was added, and the solution was cast to form 0.9-mm thick films that were placed under a UV lamp operated at $1 \text{ mW}/\text{cm}^2$ for 30 min. The hydrogels were subsequently rinsed with deionized water and dried under vacuum at 37°C. This technique and others like it allow incorporation of drugs, mammalian cells, and other biologically active substances into the solution prior to crosslinking.⁷⁶⁻⁸⁰ The polymer/bioactive solution can then be injected in the delivery location and safely crosslinked under UV light. In an aqueous environment, the gel

swells, releasing the entrapped drug or allowing the tissue cells to grow. Peppas et al.⁵⁷ successfully incorporated 10 wt% diltiazem, a 4.24-Angstrom drug, into a PEG hydrogel by this method and observed Fickian diffusion of the drug *in vitro*.

Ward et al.⁸¹ investigated the kinetics of UV-initiated copolymerization of PEGMA and PEGDMA. Specifically, the effect of PEGMA chain length on the reaction rate was explored with kinetic gelation modeling and kinetic analysis. Poly(ethylene glycol) methacrylate with PEG molecular weights of 200 (PEG200MA) or 400 g/mol (PEG400MA) were crosslinked with 200-g/mol poly(ethylene glycol) dimethacrylate (PEG200DMA) and the initiator 2,2-dimethoxy-2-phenyl acetophenone (DMPA). In this study, PEG200DMA concentration was varied from 2.5 to 75 mol% while the concentration of DMPA was held constant at 0.1 wt%. Monomer solutions were bubbled with N₂ to remove dissolved O₂ and placed between glass slides separated by a 0.9-mm Teflon spacer. Polymerization was performed in an inert environment with a spot cure UV light source of 10 mW/cm² for 10 min. The propagation of polymer chains became increasingly diffusion-limited with increasing PEGMA chain length, as the longer PEG chains decrease mobility and hinder reaction of pendant double bonds with neighboring chains. Increased PEGMA chain length thus decreased the rate of crosslinking, delaying the gel point, increasing the reaction of radicals with pendant double bonds on the same chain, and thus creating a more heterogeneous network. Increasing the amount crosslinker increased the reaction rate, however, and the effect of PEGMA chain length was decreased. These kinetic effects were accompanied by a decrease in both mechanical strength and T_g with increasing PEGMA chain length for those films tested (only films

with >50 wt% PEGDMA were examined). Increasing PEGMA chain length affords benefits for biomedical applications, but creates weaker, less uniform materials.

2.4.3 HYDROGELS WITH TERTIARY AMINES

Any elastomeric system that incorporates a tertiary amine is also pertinent to this thesis. Several efforts that studied antimicrobial properties of such materials were listed in Section 2.3.2.3, but did not include those materials not intended for antimicrobial use. Incorporation of 2-dimethylaminoethyl methacrylate (DMAEMA) into a hydrogel is of particular interest.

Prausnitz and co-workers⁸² copolymerized 2-hydroxyethyl methacrylate (HEMA) and DMAEMA in butanol. The HEMA, 30 mol% DMAEMA, 0.5 mol% ethylene glycol dimethacrylate (EGDMA), 1.7 mg/mL AIBN, and varying amounts of butanol were mixed and degassed under vacuum for 15 min. The monomer solution was injected between silanized glass plates separated by a 0.48-mm Teflon gasket and heated to 60°C in a water bath for 24 h. The resultant hydrogels were soaked in fresh butanol for 2 weeks to remove soluble fractions and dried in the fumehood for 1 week. Elemental analysis indicated that, on average, 11% of the DMAEMA present prior to polymerization was not incorporated into the network. These basic hydrogels exhibited pH dependent swelling, with hydrogel swelling decreasing with increasing pH. Such pH-dependent swelling arises from protonation of the DMAEMA tertiary-amine groups at low pH levels, increasing the charge density of the network, and therefore raising the ion osmotic pressure within the hydrogel.

Vassilev et al.⁸³ prepared quaternized DMAEMA networks for catalysis experiments. A monomer solution of 0.95 wt% AIBN, 3.8 wt% divinylbenzene (DVB), and DMAEMA was prepared and added to 20 mL of benzene. The reaction mixture was stirred at 75°C for 1 h, followed by washing with benzene and drying under vacuum at 40°C. The network was subsequently quaternized in benzene with 1-bromododecane at 70°C for 40 h, washed with benzene and ether, and dried in the dark under vacuum at 40°C. A corresponding network of irradiation-crosslinked PEO and poly(vinyl pyridine) (PVP) was also prepared. The PEO-PVP network was quaternized in nitromethane-methanol solution (2:1) with 1-bromododecane at 60°C for 30 h, followed by washing with ether and drying under vacuum at 40°C. Synthetic strategies such as these could be applied to polymer systems of interest in antibacterial applications.

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3 SYNTHESIS AND CHARACTERIZATION OF PEG HYDROGELS

3.1 INTRODUCTION

Hydrogels are polymer networks that swell in aqueous environments, thereby allowing them to be used in biomedical applications such as drug delivery devices, biosensors, anti-fouling coatings, and tissue replacement. Poly(ethylene glycol) (PEG) hydrogels are of particular interest for these applications since PEG is established as non-toxic, biocompatible, and similar to natural tissue in its crosslinked form.¹ Research efforts have explored various synthetic strategies, such as chemical insertion of urethane linkages into hydroxy-terminated PEG^{2,3} and free-radical polymerization of poly(ethylene glycol) dimethacrylate (PEGDMA),⁴ to generate PEG networks. The latter approach offers additional opportunities for incorporation of acrylic functional groups into the backbone of the network and complex biological assemblies such as cells⁵ or pharmaceutical species^{4,6} into the bulk of the network. Some functional groups to be considered for PEG modification in biomedical applications are either acidic or basic, which can promote a stimulus response⁷⁻⁹ or an antimicrobial capability which prevents bacterial infection.^{10,11} One such functional group that imparts basic, pH-sensitive, as well as antimicrobial, properties is a tertiary amine. Tertiary amines have been introduced in hydroxyethyl methacrylate (HEMA) hydrogels to promote pH-dependent swelling.⁷ Tertiary amines have also been incorporated into crosslinked networks of poly(ethylene oxide) (PEO), poly(vinyl pyridine) (PVP), and dental resin and subsequently quaternized to impart novel catalytic activity¹² or bacteriostatic properties.¹³⁻¹⁸

The focus of the present work is to integrate tertiary amines into PEG networks with properties that could be tailored by controlling the crosslink density. For this purpose, we have generated PEG-amine networks via thermally-initiated free-radical polymerization of poly(ethylene glycol) methacrylate (PEGMA), dimethylaminoethyl methacrylate (DMAEMA), and PEGDMA. Synthesis parameters have been systematically varied to optimize the mechanical properties and maximize the amine content. Resultant network characteristics and properties are investigated by elemental analysis, swelling, dynamical mechanical analysis, rheometry, and differential scanning calorimetry.

3.2 EXPERIMENTAL METHODS

3.2.1 MATERIALS

The macromonomer PEGMA ($M_n \sim 526$ g/mol), amine monomer DMAEMA, and crosslinker PEGDMA ($M_n \sim 800$ g/mol) were obtained from Aldrich Chemical Co. (Milwaukee, WI) and used as-received. The initiator was 2,2'-azobisisobutyronitrile (AIBN) provided by Sigma Chemical Co. (St. Louis, MO) and used without further purification. All reactants were stored at 4°C until used. The reactants are shown in Figure 3.1. Silicone rubber, styrene-butadiene rubber (SBR), nitrile rubber, and neoprene rubber, were all obtained from the Raleigh-Durham Rubber and Gasket Co. Inc. (Raleigh, NC) in 1.5 mm and 0.75 mm thick sheets, whereas Teflon sheets measuring 0.25 mm and 0.75 mm thick were purchased from Modern Plastics (Bridgeport, CT).

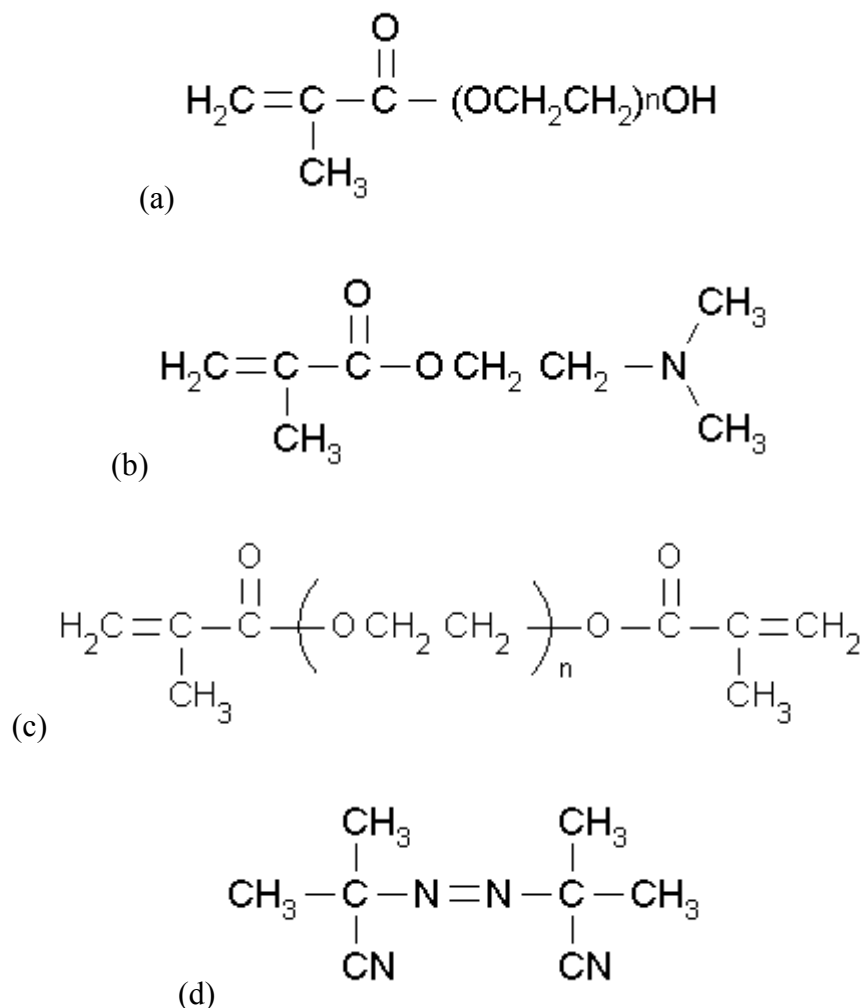


Figure 3.1 The structure of each reactant is shown above: (a) PEGMA, (b) DMAEMA, (c) PEGDMA, and (d) AIBN.

3.2.2 HYDROGEL SYNTHESIS

3.2.2.1 Crosslinking Reaction

Transparent, homogenous PEG-based hydrogels were synthesized by free-radical copolymerization of PEGMA, DMAEMA, and PEGDMA. Bulk copolymerization of the monomer solution was initiated with AIBN at a constant concentration of 0.5 wt%. Initial experimentation was based on the synthesis protocol of Patel et al.¹⁹ and consisted of a

high PEGDMA concentration. Due to the stiffness and brittleness of these materials and the resulting difficulty in handling during subsequent steps, however, a full range of monomer composition combinations was probed to determine the composition yielding both optimum mechanical properties and maximum amine content. With the AIBN concentration held constant, the mass fractions of PEGMA, PEGDMA, and DMAEMA were varied from 0 to ~1. A ternary diagram of the network compositions examined is provided in Figure 3.2. Note that the PEGMA weight fraction lines are horizontal and the DMAEMA weight fraction lines are diagonal with a negative slope.

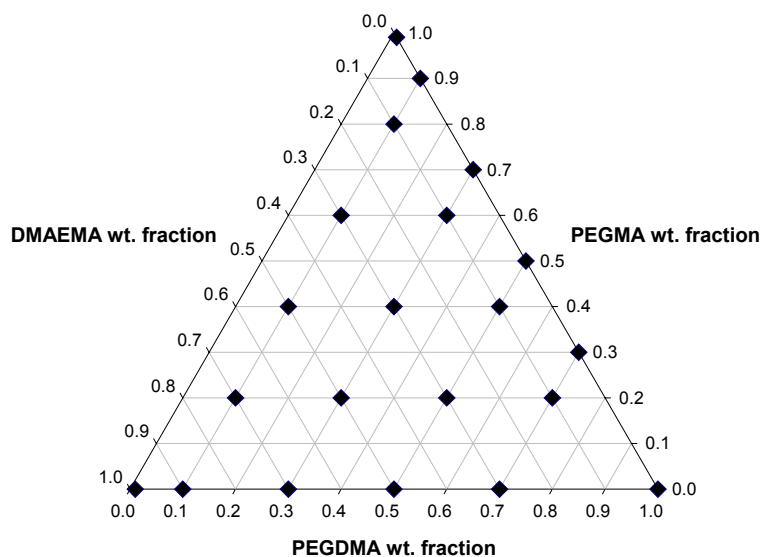


Figure 3.2 Monomer solution compositions were varied to optimize network properties. Each diamond represents a synthesized copolymer network.

According to the compositional plan displayed in Figure 3.3, predetermined quantities of PEGMA, DMAEMA, PEGDMA, and AIBN were slowly mixed in a scintillation vial or jar with a magnetic stir bar to limit bubble formation during mixing.

The solution was sonicated to dissolve the solid AIBN and remove air bubbles. To further remove oxygen, which inhibits free-radical polymerization, the solution was degassed under vacuum for 15 min. The solution was subsequently syringed into the rubber molds sealed between glass plates that were treated with an anti-adhesive agent. During the injection, a second 22-gauge needle was inserted on the adjacent side of the mold to vent air within the mold. The filled molds were heated to 80°C. After 34 h, the oven was slowly cooled to ambient temperature over ~8 h. Resultant hydrogels were rinsed with deionized water, dried in air for 24 h and under vacuum for an additional 24 h. All films were subsequently stored in a desiccator until further used. Hydrogels subjected to swelling were first placed in deionized water, which was refreshed every 24 h for 3 days to remove residual monomer and initiator. Other hydrogel samples were not extracted in this fashion prior to testing due to the formation of edge microcracks, only visible under an optical microscope, propagating through some of the samples during the course of swelling and de-swelling. Such artifacts would produce untestable film shapes and inconsistent mechanical property measurements.

3.2.2.2 Glass Plate Preparation

Standard window glass and silicone rubber sheets measuring 1.5 mm thick were cut into 13 cm x 15 cm rectangles. Within each silicone sheet, individual shapes were cut out with an Xacto knife: 3 microscope-slide size rectangles for antimicrobial analysis, 1 long rectangle for mechanical property testing, and 5 small squares for swelling, thermal calorimetry, and elemental analysis. Silicone rubber was also cut into microscope-slide size rectangles for molding individual tensile specimens. A schematic of the two types of

rubber molds is provided in Figure 3.3. Prior to use and after use, the glass plates were washed with an aqueous detergent solution, rinsed with water, soaked in a potassium hydroxide/isopropanol bath, rinsed with tap water, rinsed again with deionized water, and dried. The plates were subsequently treated with a silicone-based, anti-adhesive agent that preferentially bonds to glass to prevent film adherence.

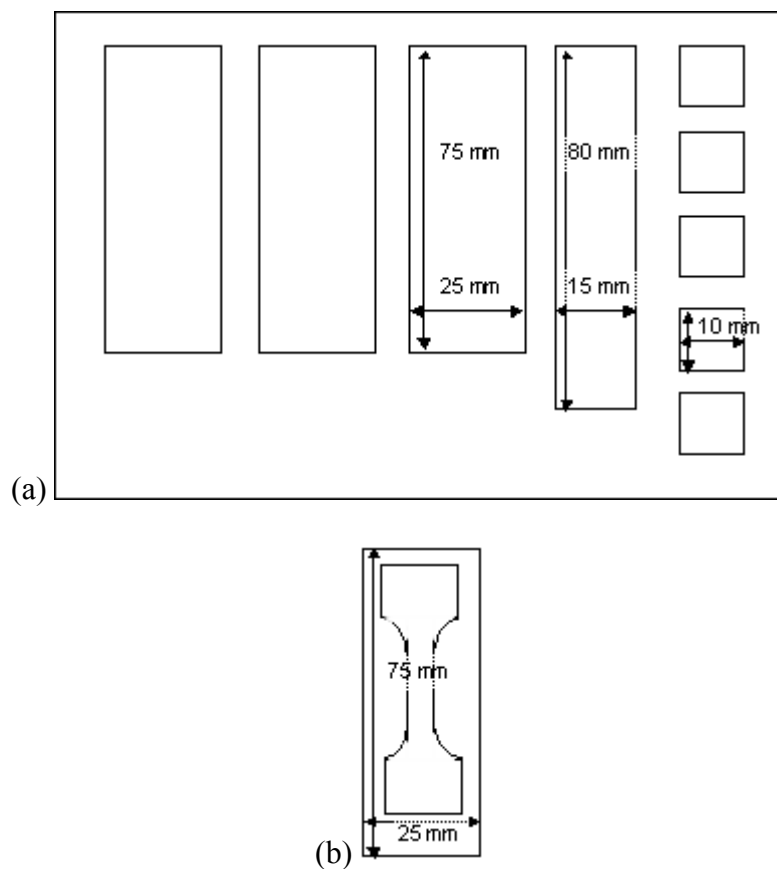


Figure 3.3 Shapes for (a) characterization and (b) tensile testing were cut into rubber molds with an Xacto knife as described in the text.

3.2.3 ELEMENTAL MICROANALYSIS

Carbon, hydrogen, nitrogen, and bromine elemental microanalysis were performed by Atlantic Microlab, Inc. (Norcross, GA) on dried hydrogel samples prior to and after excess solvent/initiator extraction.

3.2.4 ATR-FTIR

The crosslinking reaction progress of one hydrogel composition with 10 wt% PEGDMA and 30 wt% DMAEMA was monitored by attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) with a Digilab FTS3000 FTIR Spectrometer (Randolph, MA). Samples were prepared by pipetting monomer solution into a circle (8 mm diameter) cut in a sheet of SBR measuring 0.75 mm thick and sandwiched between glass slides. Multiple samples were prepared in this fashion and heated to 80°C. Individual specimens were removed according to the following schedule: every 5 min up to 30 min, every 10 min up to 1 h, every 30 min up to 3 h, every 1 h up to 5 h, every 5 h up to 15 h, and at 20 and 34 h. Upon removal, each specimen was submerged in ice, placed in a freezer maintained at –20°C for 10 min, and stored at 4°C until ATR-FTIR analysis. Sample spectra were collected with 64 scans at 4 Hz resolution and were baseline-corrected. The changes in peak heights and shapes (especially the C=C peak located at 1635 cm⁻¹) were all progressively monitored.

3.2.5 SWELLING MEASUREMENTS

Hydrogel samples were weighed, placed in 10 or 50-mL beakers, and exposed to deionized water in triplicate. The deionized water was replaced every 24 h for 72 h to

remove residual monomer and/or initiator. Each sample was subsequently removed from the bath, pressed between Kimwipes under a 100 g mass for 30 s, and weighed. Samples were then dried under vacuum for 24 h and reweighed to confirm reversibility.

3.2.6 DYNAMICAL MECHANICAL ANALYSIS

Specimens prepared for dynamic mechanical analysis (DMA) were cut into strips measuring 6 mm x 35 mm x 1.5 mm and examined in triplicate using the film setup on a Rheometrics Solids Analyzer RSA II (Piscataway, NJ). Each specimen was subjected to strain amplitudes ranging from 0.01 to 2.0% at a constant frequency of 1 Hz, and values of the dynamic elastic and viscous tensile moduli (E' and E'' , respectively) were recorded.

3.2.7 RHEOMETRY

Specimens produced for uniaxial tensile deformation were molded into the dogbone shape depicted in Figure 3.3. Previous unsuccessful attempts to cut dry samples into this shape generated edge microcracks and promoted crack propagation throughout the samples. Each molded sample was dried and measured prior to testing on an Instron 5542 (Canton, MA). To prevent specimen slippage during testing, the Instron clamps were covered with double-sided tape, and a small drop of Loctite 401 instant adhesive (Fisher Scientific, Pittsburgh, PA) was placed on each tab before the handles were clamped together tightly. All tests were conducted at an extension rate of 2 mm/min until failure occurred.

Since the analysis of tensile data can vary somewhat, a brief description of the analysis employed here is provided below. From extension versus load data collected for

each sample, the engineering stress (σ) and engineering strain (ϵ) were calculated.^{20,21} The Young's modulus (E), defined as the initial slope of the stress-strain curve, was computed by linear regression of the first 20 or so data points through zero. The yield stress, defined as the stress at which the material exhibits irreversible deformation and non-linear stress-strain behavior, was discerned by extrapolating a straight line from the initial, linear portion of the stress-strain curve and identifying the stress at which the line and data diverge. The ultimate tensile strength (stress), the maximum stress the material experiences prior to failure, was determined directly from the maximum in the stress-strain curve. The percent elongation at break was also ascertained here according to

$$\%elongation = \left(\frac{L_f - L_o}{L_o} \right) \times 100 \quad (1)$$

where L_f and L_o denote the final and initial specimen lengths, respectively. Toughness, a measure of the capacity of a material to absorb energy up to failure (given as the area under the stress-strain curve), was calculated by numerical integration of the data.

3.2.8 DIFFERENTIAL SCANNING CALORIMETRY

A ~15 mg piece of each dry hydrogel was cut, inserted into an Al differential scanning calorimetry (DSC) pan, and tested on a TA Instruments DSC Q100 calorimeter (New Castle, DE) according to the following cycle: the specimen was cooled from 40°C to -80°C at 60°C/min, equilibrated for 5 min at -80°C, and heated from -80°C to 40°C at 5°C/min. Resulting plots of heat flow versus temperature were used to identify the glass transition temperature (T_g) of each material. Dry and dry/extracted hydrogels were tested.

3.3 RESULTS AND DISCUSSION

3.3.1 HYDROGEL SYNTHESIS

Using the synthesis procedure described in the previous section, the gelation time of the reaction mixture, as qualitatively discerned from visual/tactile verification of the point of solidification, appears to vary with both mold geometry and monomer composition. Thinner molds measuring 0.75 mm thick promote faster gelation than thicker molds measuring 1.5 mm thick. This experimental observation is attributed to an increase in heat transfer in the bulk and, thus, an increase in reaction rate with decreasing mold thickness. With the thicker molds, solidification of all hydrogels differing in composition is achieved between 18 and 20 h at 80°C. To ensure a uniform polymerization history and curing protocol for all compositions, the synthesis parameters are held constant at 34 h (cure time) and 80°C (cure temperature) in molds measuring 1.5 mm thick unless otherwise indicated.

3.3.1.1 ATR-FTIR

The rate of the polymerization reaction in one hydrogel composition (10 wt% PEGDMA, 30 wt% DMAEMA, 0.5 wt% AIBN and ~59 wt% PEGMA) has been investigated by ATR-FTIR. In this case, gelation of specimens in the molds measuring 0.75 mm thick is observed after only 10 min at 80°C. The corresponding change in the FTIR spectrum from 5 to 240 min is presented in Figure 3.4. Figure 3.4A shows the as-acquired spectra as a function of increasing cure time, whereas Figure 3.4B illustrates the

difference between the spectrum at each time and the spectrum of the liquid solution evaluated at 5 min. Note that little change in the spectra is observed after 40 min at 80°C.

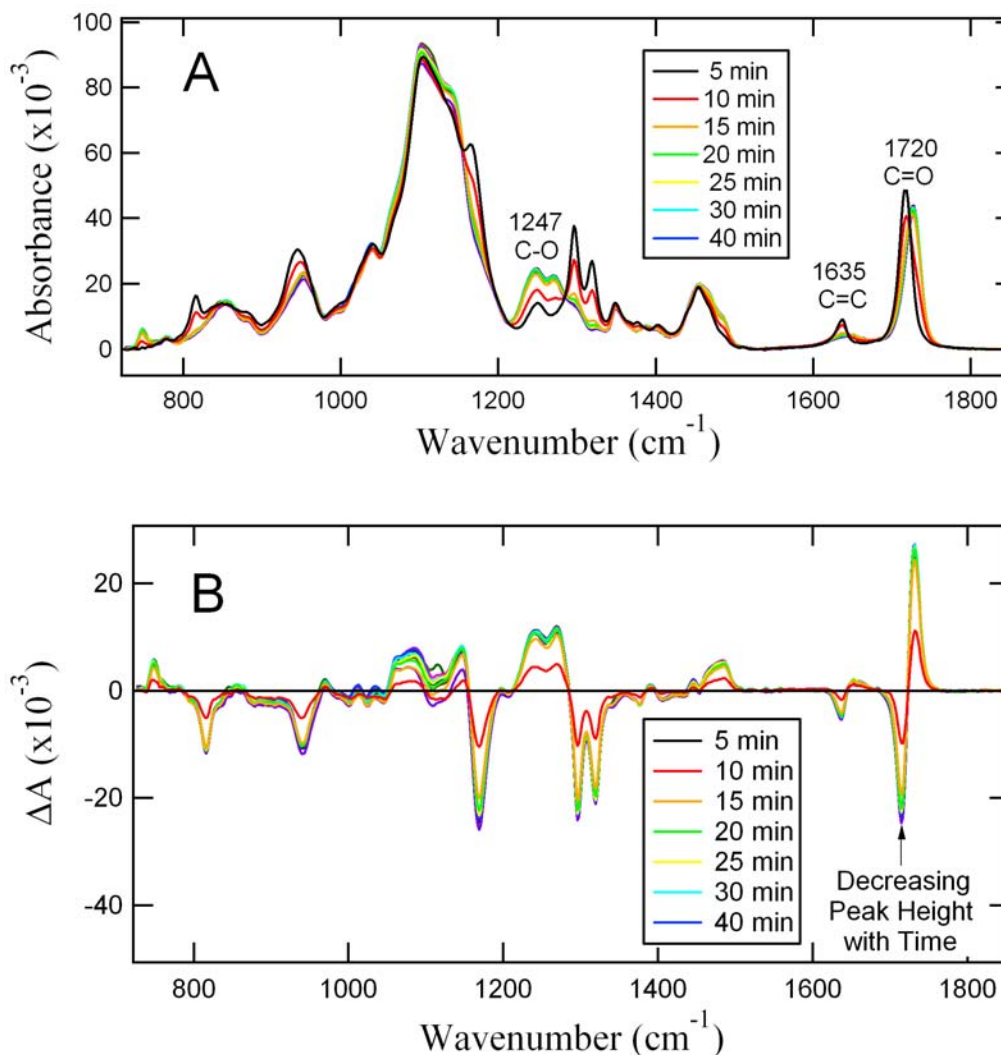


Figure 3.4 Graph A shows the monomer/film spectrum versus reaction time at 80°C. Graph B shows the difference spectra compared to the spectrum at 5 min.

According to the data displayed in Figure 3.4, the peak signifying the C=C bond (used in the free-radical polymerization) at 1635 cm^{-1} decreases to a constant value by 40 min, further indicating that (i) the polymerization is completed at the specimen surface and (ii) a quantity of unreacted residual monomer remains. Since the reaction of interest here is a

bulk copolymerization, residual monomer is expected due to diffusion limitations. Figure 3.4 furthermore reveals that the COO and CO peaks shift, indicating some rearrangement of molecules on the surface as the polymerization progresses.

Evaluating the change in the entire FTIR spectrum by singular value decomposition (SVD) reveals the overall progress of surface polymerization, as demonstrated in Figure 3.5.

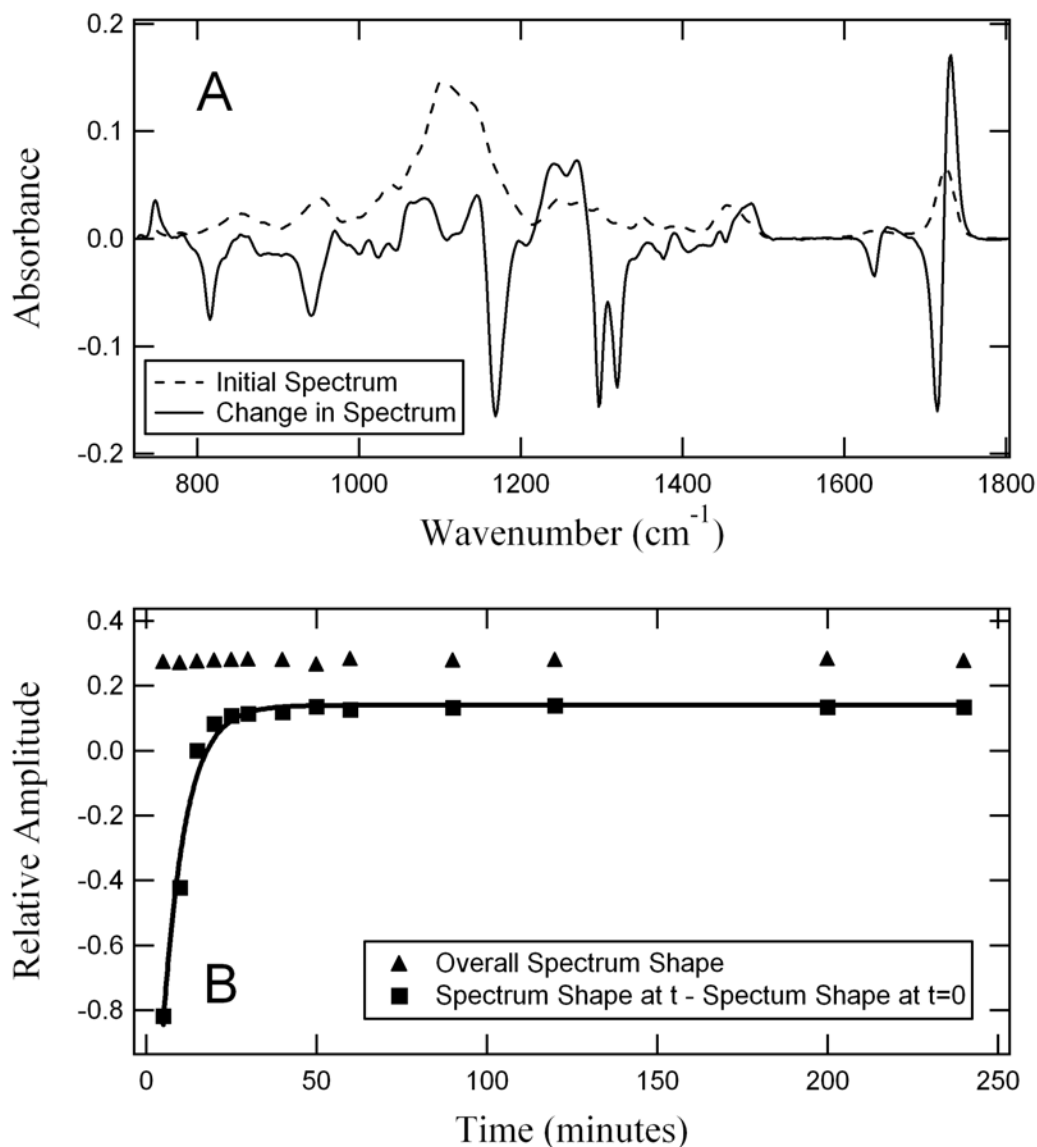


Figure 3.5 Analysis of ATR-FTIR spectra with SVD shows (A) the overall change in the film spectrum and (B) the change in the film spectrum in its entirety with time.

These data confirm that the surface polymerization is finished after 40 min at 80°C. While this result provides the cure time for the hydrogel surface, it does not, however, indicate the cure time for the bulk polymerization, since ATR is only capable of probing a few microns into the specimen from the surface. Transmission FTIR spectroscopy has been attempted without success to obtain equivalent information regarding the bulk

polymerization kinetics. Although residual monomer remains in the hydrogel after the apparently complete cure time, it presumably remains unreacted and trapped until extraction. Increasing the reaction temperature during the polymerization will certainly change the reaction rate, but additional reaction time is not expected to change the extent of network formation nor the ultimate network properties.

3.3.1.2 Elemental Analysis

Elemental analysis is used here to determine the film composition before and after monomer/initiator extraction of the samples containing 10 wt% PEGDMA. Table 3.1 shows the results of this analysis.

Table 3.1 Elemental Analysis of Dried Hydrogel Films [± 5 % error]

Sample	Treatment	Theoretical (g DMAEMA / g film) %	Actual (g DMAEMA / g film) %	Difference
C	none	0	0.00	0.00
D	none	10	9.99	0.01
D	extracted at 60°C with DMSO	10	10.10	0.10
E	none	30	28.84	1.16
E	extracted at 25°C with water	30	31.87	1.87
E	extracted at 60°C with DMSO	30	22.45	7.55
F	none	50	49.16	0.84
F	extracted at 25°C with water	50	45.90	4.10
F	extracted at 60°C with DMSO	50	22.45	27.55
G	none	70	67.34	2.66
G	extracted at 25°C with water	70	67.34	2.66
G	extracted at 60°C with DMSO	70	28.73	41.27

From the data above, all film compositions are in favorable quantitative agreement with the known monomer solution compositions. The error is less than 3% prior to extraction and only *ca.* 4% or less after extraction in water at ambient temperature. Swelling films in dimethylsulfoxide (DMSO) for 96 h at 60°C, fresh DMSO for 72 h at ambient

temperature, and *n*-hexane for 72 h also at ambient temperature has a more pronounced effect on hydrogel composition, as this extraction sequence removes a significant amount (as much as ~42 wt%) of unreacted monomer over a total extraction time of 10 days. As the DMAEMA concentration in the monomer solution is increased, the fraction of DMAEMA covalently incorporated into the crosslinked network decreases. In fact, the concentration of DMAEMA in the film appears to plateau at about 30 wt% in the presence of 10 wt% PEGDMA. This result is consistent with the reported findings of Prausnitz and co-workers,⁷ who crosslinked HEMA in the presence of 30 mol% DMAEMA in butanol and observed an 11 mol% loss (on average) of DMAEMA upon extraction in butanol over the course of 2 weeks.

3.3.2 MECHANICAL PROPERTIES

Due to fracture of some films during monomer/initiator extraction (most probably a consequence of significant monomer loss and microcrack propagation), mechanical tests have only been performed on unextracted, dry films. Unreacted monomer is anticipated to act as a plasticizing agent and influence thermal and mechanical properties in known ways.

3.3.2.1 Swelling Experiments

Hydrogel swelling exhibits a strong dependence on PEGDMA (crosslinker) concentration, but relatively little dependence on DMAEMA (nitrogen) concentration. These results are presented in Figure 3.6.

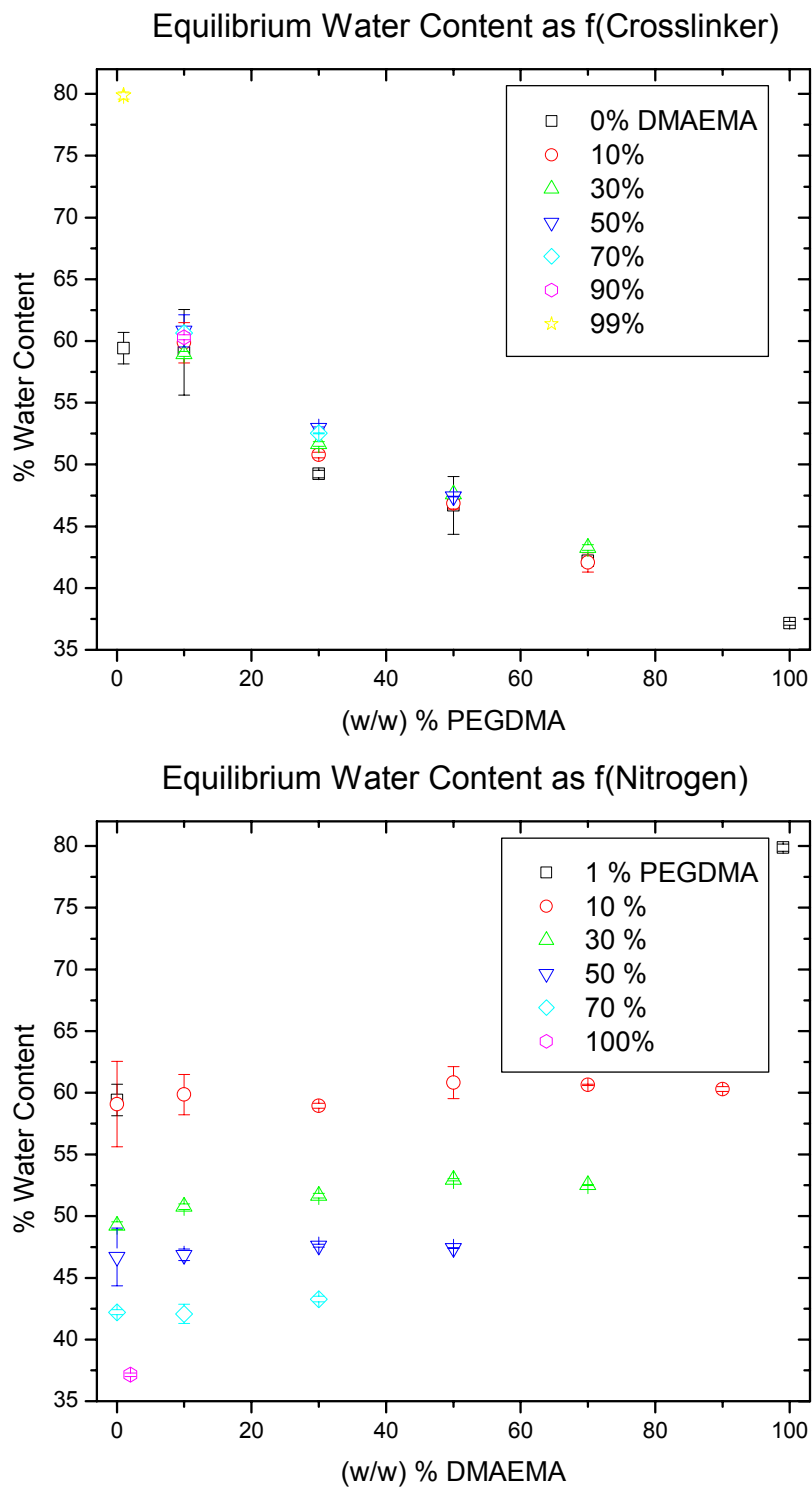


Figure 3.6 Equilibrium water content of hydrogels decreases linearly with increasing crosslinker concentration. Equilibrium water content shows little change with increasing nitrogen monomer concentration. The only exception to these observations is the 99-wt% DMAEMA sample, which was found to not be fully crosslinked.

The equilibrium water content of the hydrogels decreases almost linearly with increasing PEGDMA concentration, which reflects an increase in crosslink density and the accompanying reduction in M_c . It is interesting that the equilibrium water content increases slightly with increasing DMAEMA concentration. This slight dependence may be attributed to two effects: protonation of the nitrogen groups or decreased crosslinking with increased DMAEMA composition. In the first case, each tertiary amine possesses two unpaired electrons that it can share or use to withdraw protons from neighboring molecules. In this case, water can be envisaged to behave as a weak acid and donate a proton to the tertiary amine, thereby giving the amine a positive charge, changing the osmotic pressure of the network, and increasing the degree of network swelling. If this were the case, however, a more substantial change in swelling is expected. Tests conducted at varying pH are needed to understand the pH response of this new network. Alternatively, increased DMAEMA concentration in the monomer solution may promote a decrease in crosslink density and an increase in M_c . As detected by elemental analysis, the percent DMAEMA reacted/incorporated within the network decreases with increasing DMAEMA monomer concentration. Residual unreacted DMAEMA may result in network defects (e.g., voids) that permit a slightly elevated level of swelling.

Some specimen films develop small internal fractures upon swelling in water. A qualitative degree of internal fractures visualized from such specimens is provided as a function of composition in Figure 3.7. Internal fracture formation appears to decrease with increasing PEGDMA and DMAEMA content, which implies that a reduction in PEGMA is responsible for enhanced film stability. The macromonomer PEGMA has

been found²² to inhibit polymerization between neighboring chains, creating a less homogeneous molecular network. Internal fractures may arise from such heterogeneous polymerization, which eventually produces discontinuities in the polymer network and hydrogel.

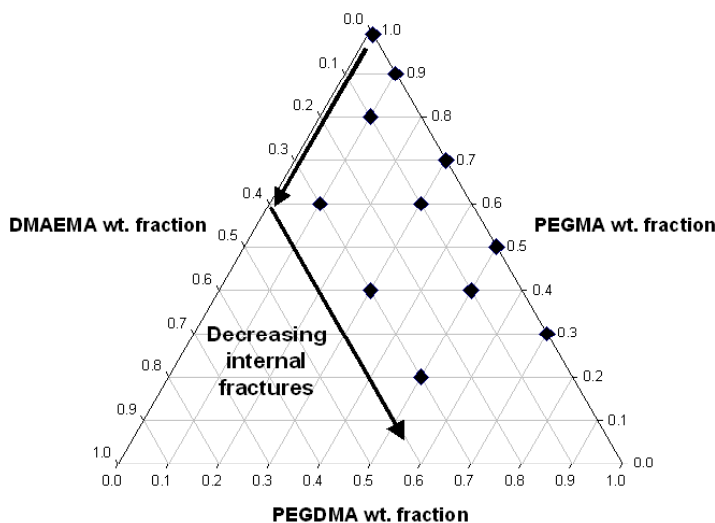


Figure 3.7 Film internal fracturing upon swelling decreased with increasing PEGDMA and DMAEMA concentrations. Each diamond represents a film composition observed to have internal fractures upon swelling in water.

3.3.2.2 Dynamic Mechanical Analysis

Dynamic mechanical analysis has been performed as a non-invasive test method on dry, unextracted films to ascertain their mechanical properties. Hydrogels at each composition except the 90 and 99 wt% DMAEMA formulations exhibit a constant dynamic elastic modulus (E') that is at least one order of magnitude greater than its corresponding dynamic viscous modulus (E''), confirming that the materials can be considered elastomers with a negligible viscous component.

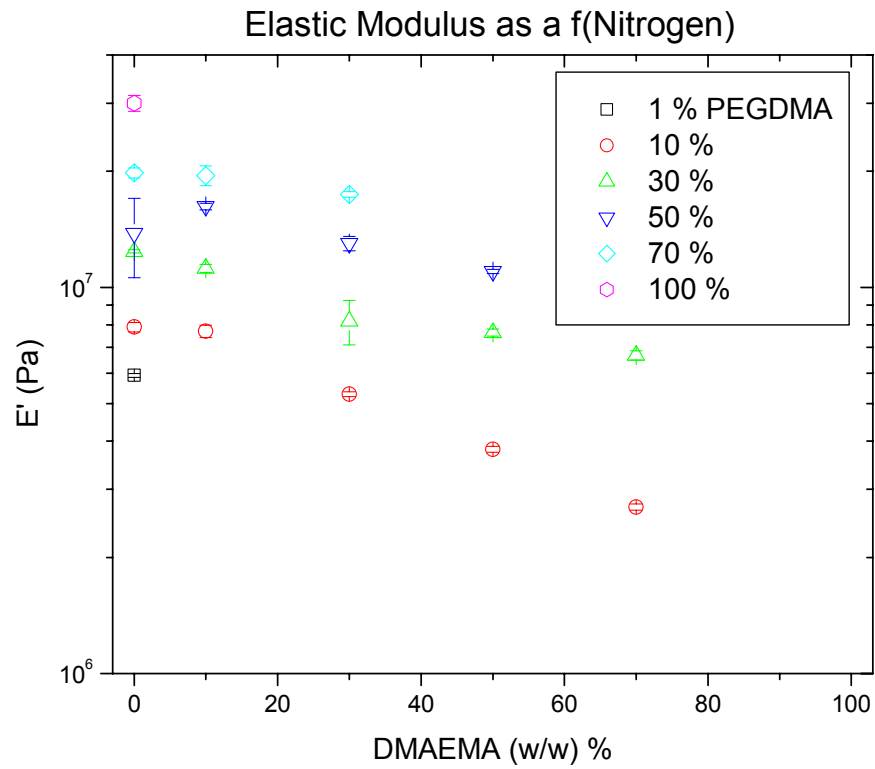
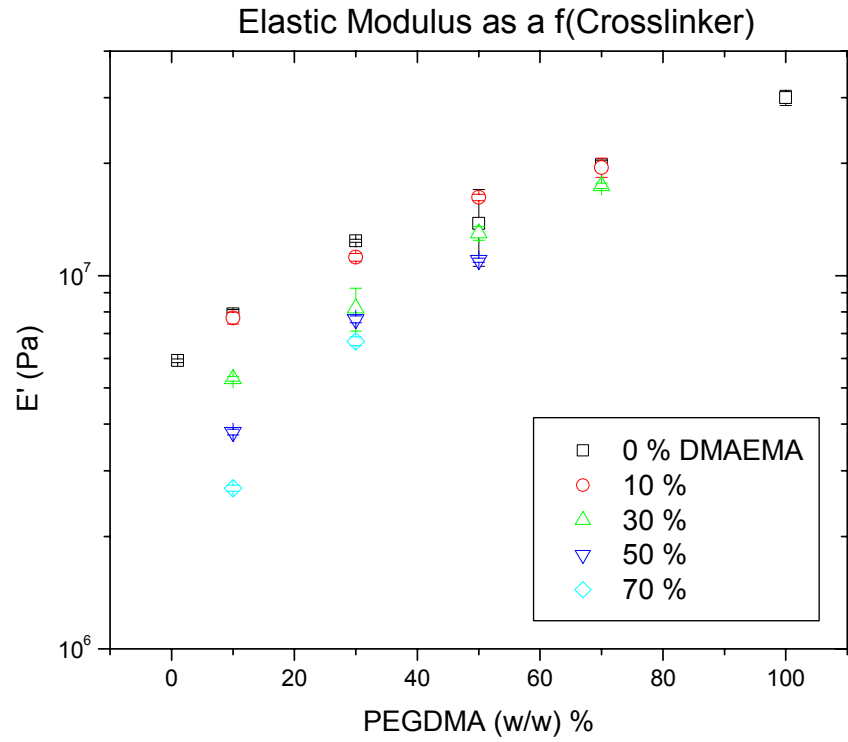


Figure 3.8 Elastic moduli increase with increasing PEGDMA content and decrease with increasing DMAEMA content.

The elastic modulus of each sample depends on both PEGDMA concentration and DMAEMA concentration, as illustrated in Figure 3.8. Due again to the corresponding increase in crosslink density, E' is observed in Figure 3.8A to increase with increasing crosslinker concentration. The apparent reduction in E' with increasing DMAEMA concentration in Figure 3.8B, on the other hand, suggests a nontrivial decrease in the crosslink density, which likewise serves to decrease the network strength and, ultimately, E' . The extreme of this scenario occurs in the two samples containing the most DMAEMA: 90 wt% DMAEMA with 10 wt% PEGDMA and 99 wt% DMAEMA with 1 wt% PEGDMA. These two formulations do not completely crosslink and thus exhibit values of E'' that are greater than those of E' . The dependence of E' and E'' on strain amplitude is included for the sake of completeness in Figure 3.9. Since these samples contain a large amount of unreacted monomer, the observed behavior may be due to the formation of liquid layer on the film surface during testing, giving anomalous results. A parallel plate geometry should be used on these samples to further understand their behavior and eliminate any liquid-layer formation.

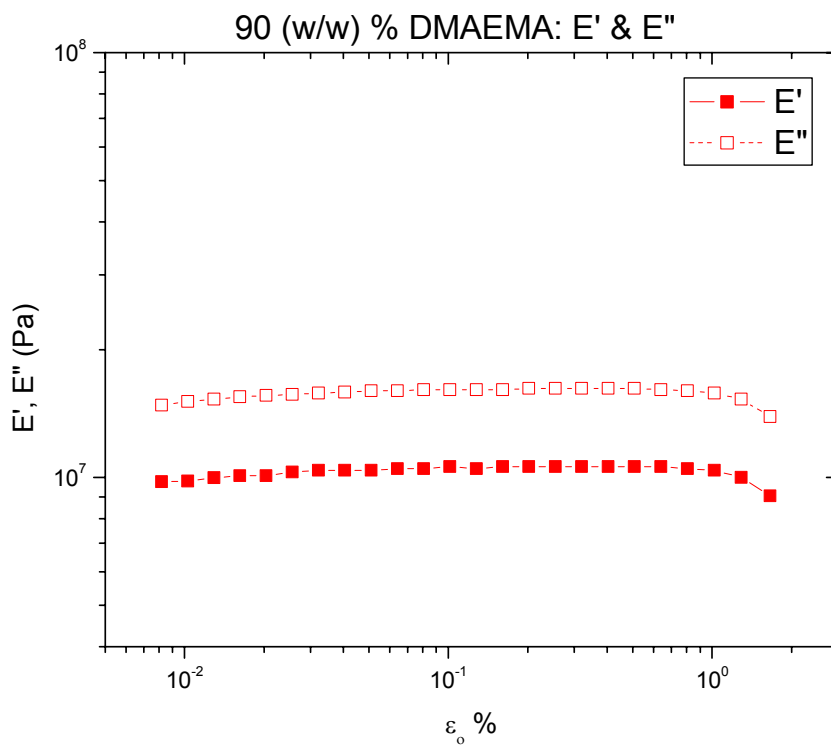
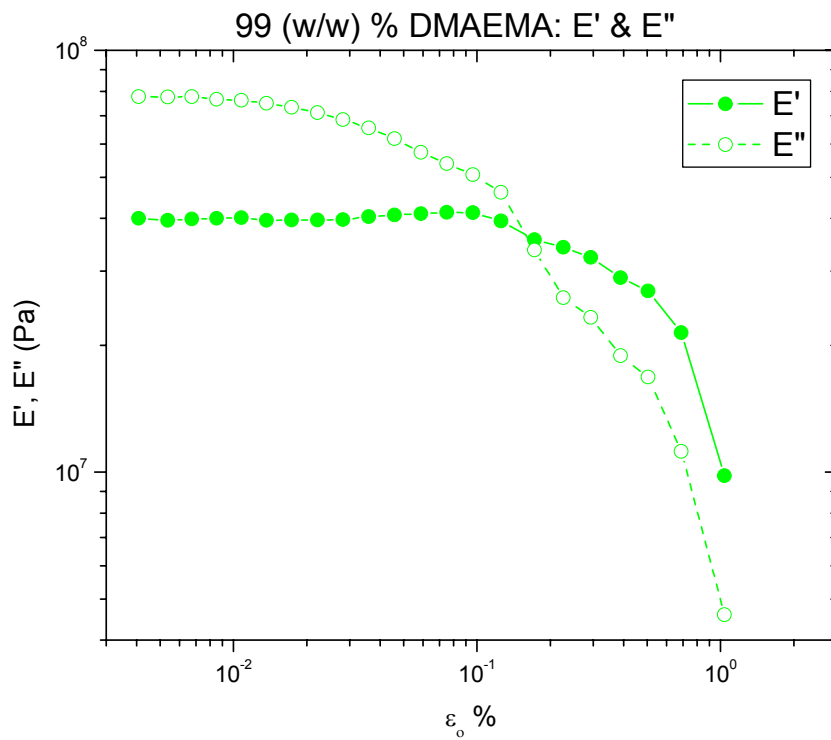


Figure 3.9 Two monomer solution compositions produced incompletely crosslinked films, where E'', the viscous modulus, is greater than E', the elastic modulus, where ϵ_0 is the maximum strain amplitude.

These samples have been examined in triplicate to yield an average value of E' and the strain amplitude identifying the extent of the linear viscoelastic regime (wherein E' is independent of ϵ_0). Corresponding values are reported in Table 3.2. These lightly crosslinked hydrogels are stiff and tacky, reminiscent of an incompletely cured adhesive. No T_g is detected for these two formulations between -80°C and 40°C .

Table 3.2 Characterization of Linear Region of Lightly Crosslinked Samples

Sample (w/w) % DMAEMA	E' (Pa)	Linear Viscoelastic Regime	
		ϵ_0 (%)	E' (Pa)
99	3.77E+07	0.63	2.72E+07
90	9.39E+06	1.0	8.39E+06

3.3.2.3 Rheometry

Uniaxial tensile tests have been performed on dry, unextracted hydrogel films to determine the Young's modulus, yield stress, ultimate tensile strength, percent elongation at break and toughness. As demonstrated in this section, the Young's modulus and ultimate tensile stress increase with increasing PEGDMA concentration, while M_c decreases with increasing PEGDMA concentration. In marked contrast, each of the former properties *decreases* substantially with *increasing* DMAEMA concentration at *constant* PEGDMA concentration (10 wt%), but *decreases* slightly (if at all) with *increasing* DMAEMA concentration at *other constant* PEGDMA concentrations. The dependence of these properties on PEGDMA concentration indicates, as it did in the case of E' , that an increase in PEGDMA content increases the crosslink density, decreases M_c , and thus increases the network density. The dependence on DMAEMA strongly suggests that, at 10 wt% crosslinker, an increase in DMAEMA concentration hinders

copolymerization, thereby resulting in fewer crosslinks, a larger M_c , and compromised network strength. It appears from these data that the deleterious influence of DMAEMA on copolymerization is diminished at crosslinker concentrations of 30 wt% or higher. Note that this effect is not as pronounced in the DMA results, but the same trend is evident. Values of E measured from rheometry are comparable to E' values obtained by DMA, but they are not exact. The mean percent difference between these values is 45%. Such error may be indicative of insufficient sampling or experimental methodology (e.g., the crosshead speed in rheometry can have a profound effect on the value of E measured and can exaggerate the relative contribution of voids in the hydrogel network).

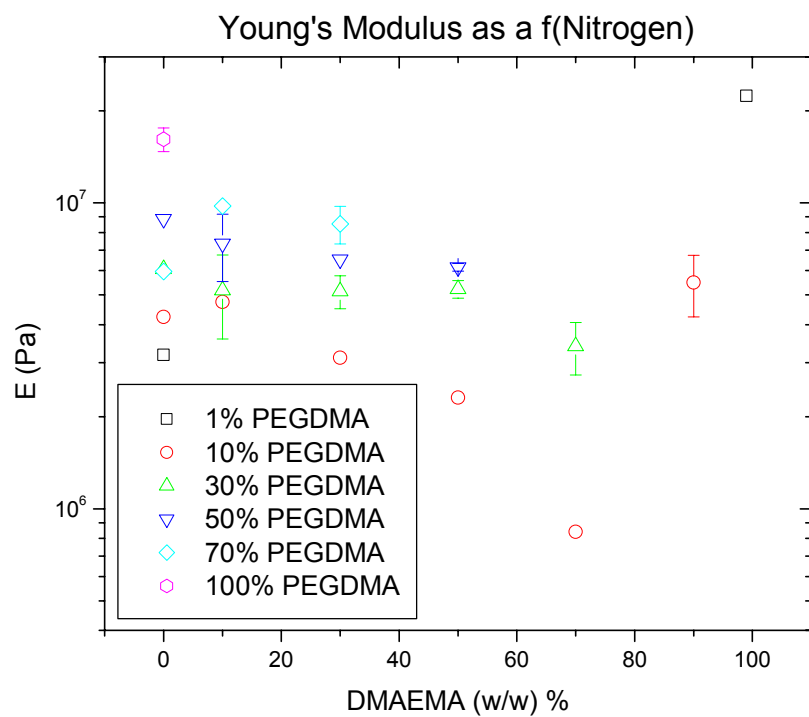
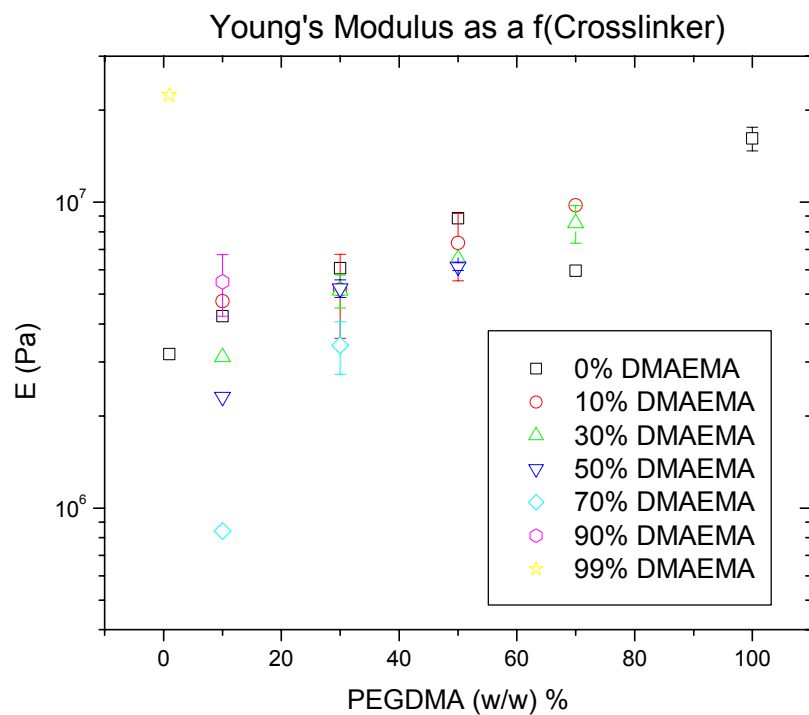


Figure 3.10 Young's modulus follows the same compositional trend as the elastic modulus determined by DMA, increasing with increasing PEGDMA concentration while decreasing slightly with increasing DMAEMA concentration.

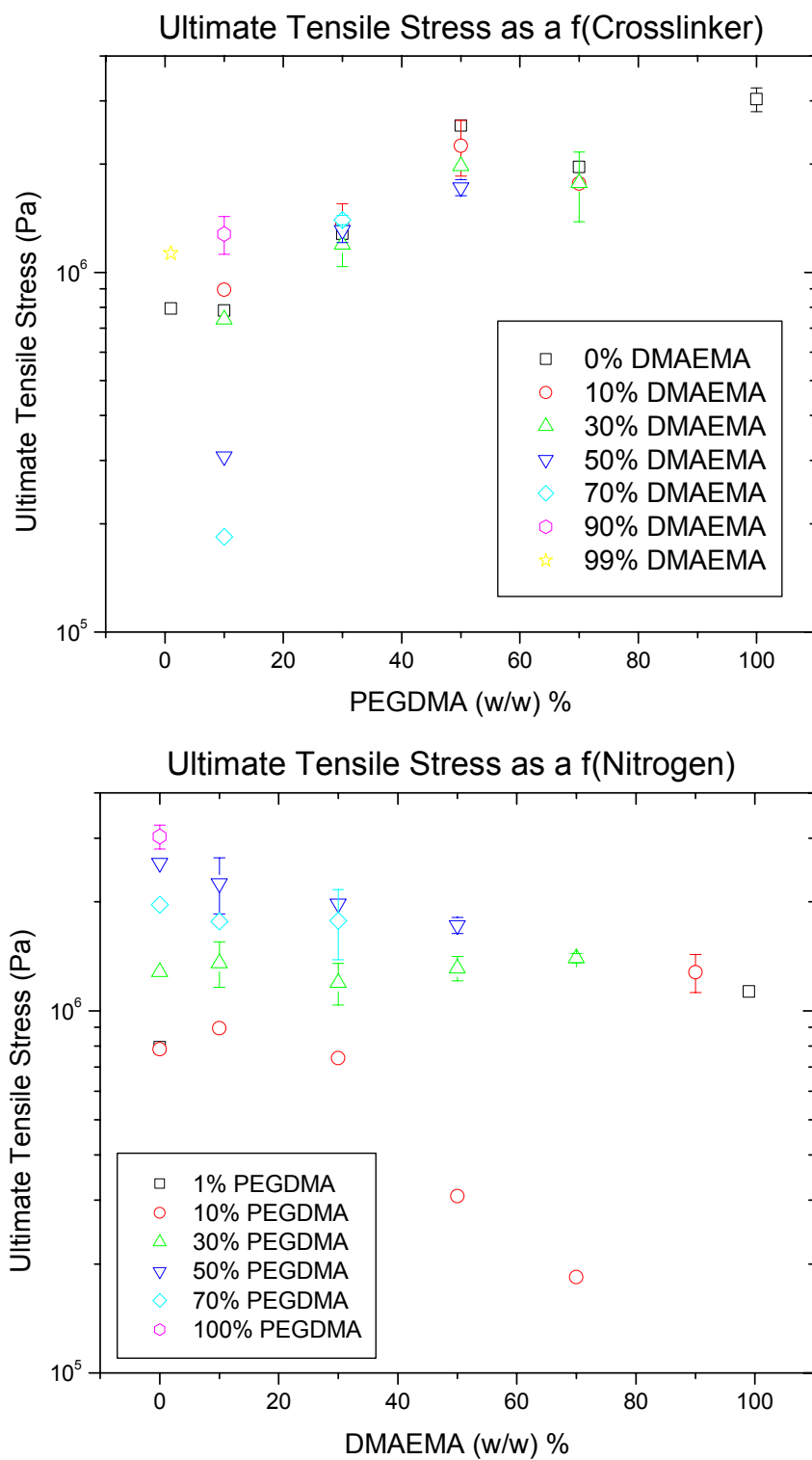


Figure 3.11 Ultimate tensile stress increases with increasing PEGDMA concentration while it decreases with decreasing DMAEMA concentration only at constant 10 and 50 wt% PEGDMA.

As reported here, the yield stress appears to increase with increasing PEGDMA concentration but decrease with increasing DMAEMA concentration. Note that samples with a PEGMA concentration greater than 60 wt% do not exhibit a yield stress. This property dependence again confirms that PEGDMA increases the crosslink density and, consequently, the rigidity of the network. The percent elongation exhibits no conclusive dependence on either PEGDMA or DMAEMA concentration except for the two incompletely crosslinked formulations, which exhibit significantly greater elongation than all the other samples examined. Toughness increases initially with increasing PEGDMA concentration but exhibits no discernible dependence beyond 50 wt% PEGDMA. In light of the experimental uncertainty in the data, we conclude that toughness is not strongly dependent on DMAEMA concentration. The apparent trends in both of these ultimate properties indicate that the PEG networks, although exhibiting variation in rigidity, possess limited deformability prior to failure.

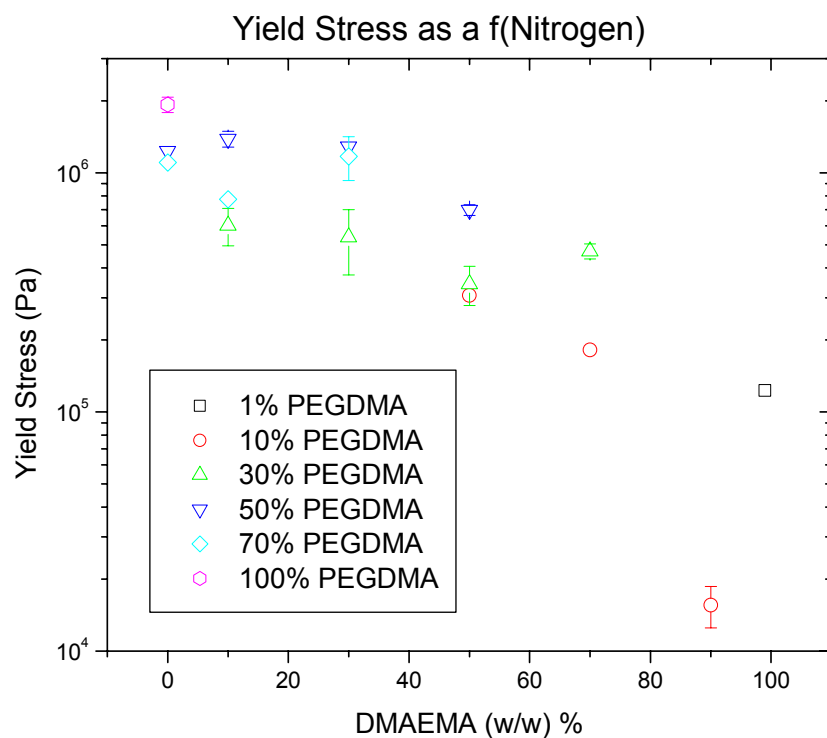
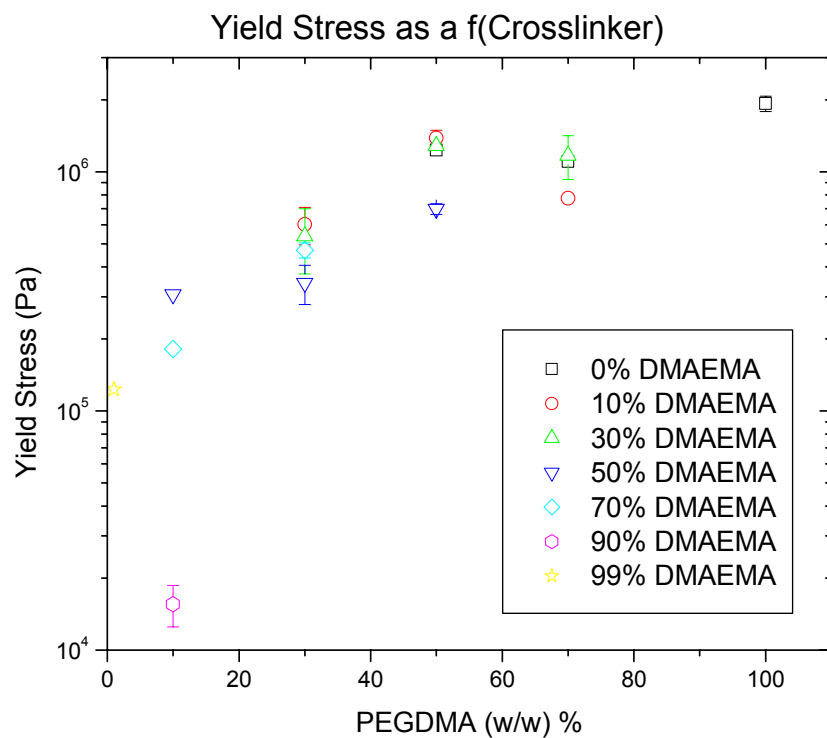


Figure 3.12 Yield stress increases with increasing PEGDMA concentration and decreases with increasing DMAEMA content, a more significant dependence than seen with other properties. Any compositions omitted did not exhibit a yield stress.

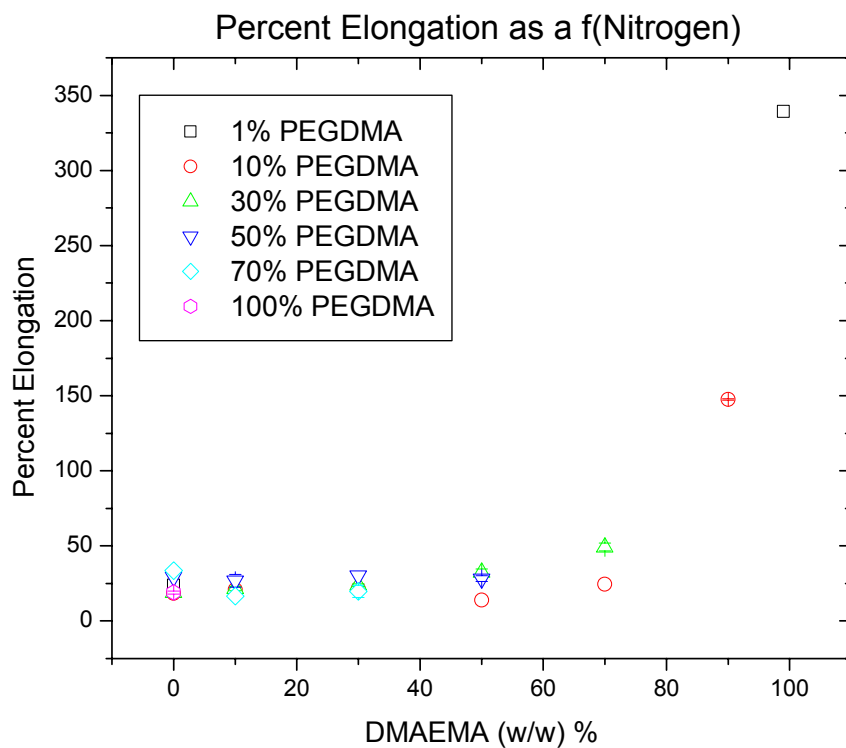
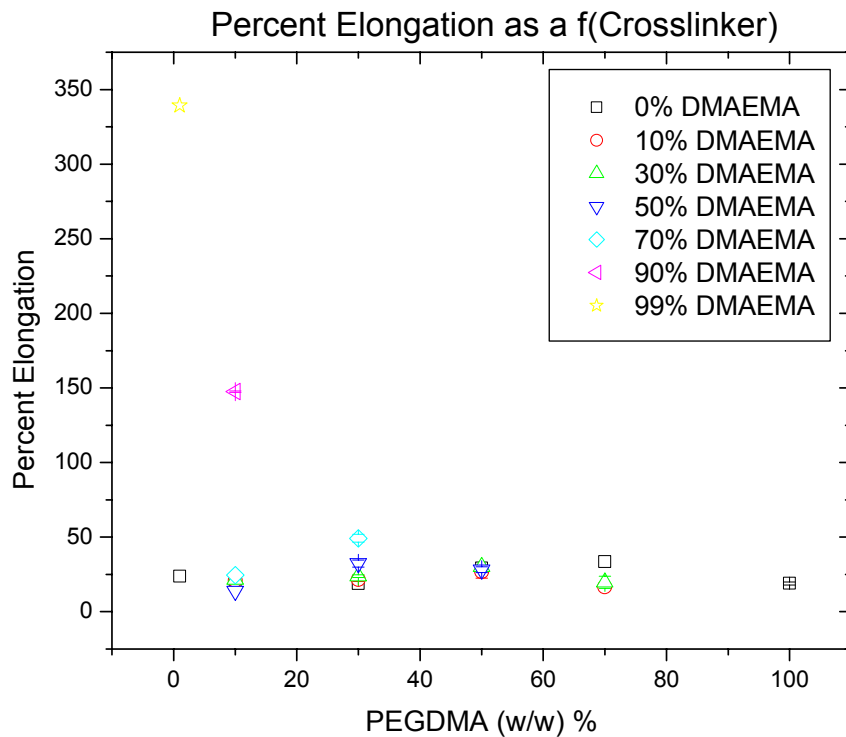


Figure 3.13 Percent elongation shows little dependence on either monomer concentration except for the two lightly crosslinked samples.

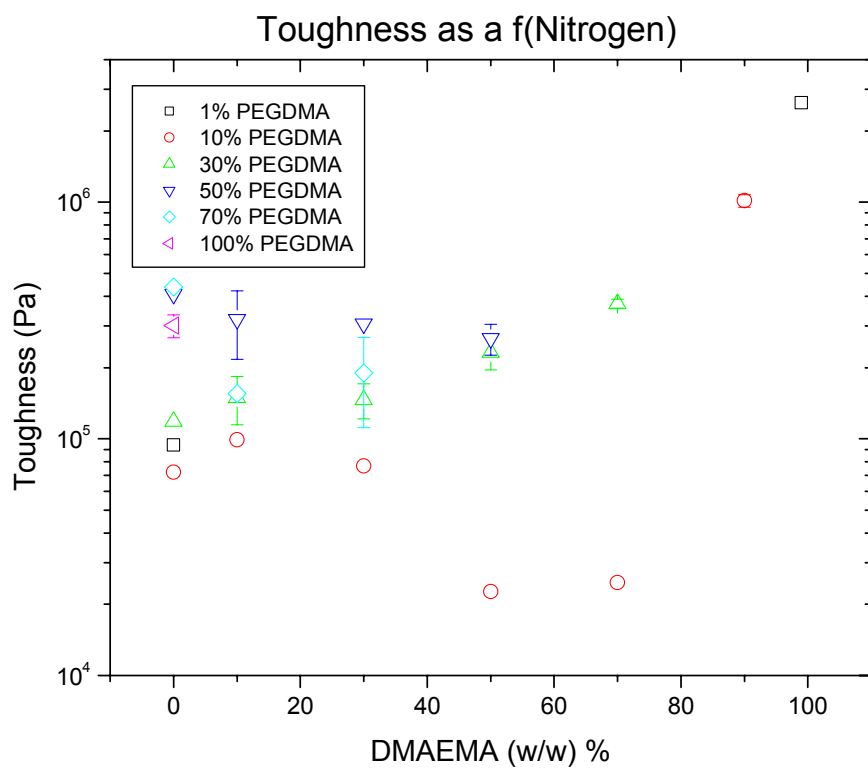
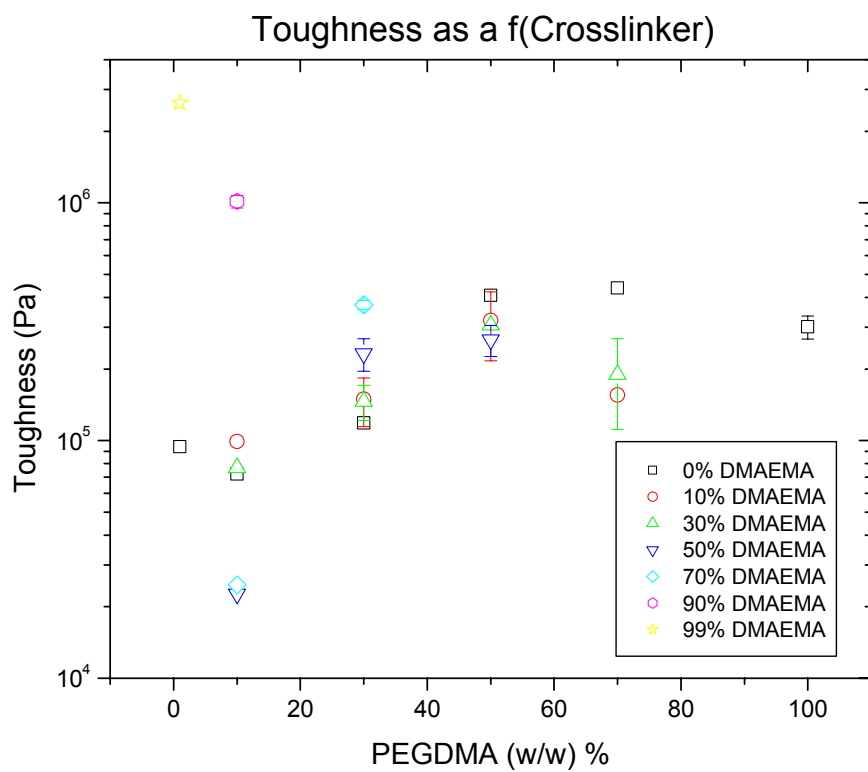


Figure 3.14 Toughness increases with increasing PEGDMA concentration up to 50 wt% while its dependence on DMAEMA concentration is too noisy to be concluded.

3.3.2.4 Differential Scanning Calorimetry

The glass transition temperatures of dry, unextracted films with constant PEGDMA concentration of 10 wt% were measured with DSC. The glass transition temperature of these films showed a linear dependence on DMAEMA concentration, increasing with increasing DMAEMA content as shown in Figure 3.15.

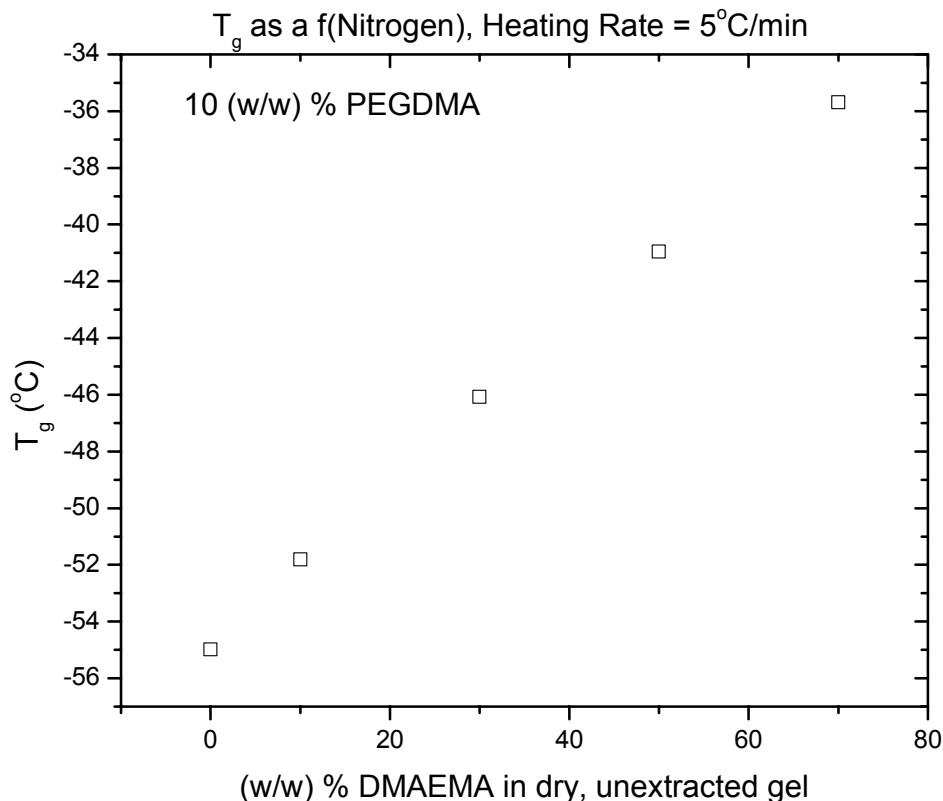


Figure 3.15 Glass temperature of gels increases with increasing DMAEMA concentration.

Without knowledge of the other analyses performed during this study, one might hypothesize that an increase in DMAEMA content increases nitrogen content, thereby increasing the propensity for hydrogen bonding, decreasing network flexibility and segmental mobility and, hence, increasing T_g. However, from elemental analysis, the amount of DMAEMA chemically incorporated into the PEG network is far less than

expected, especially at high DMAEMA concentrations. If the T_g data are plotted as a function of the DMAEMA content discerned by elemental analysis, the trend evident in Figure 3.17 remains, as illustrated in Figure 3.16.

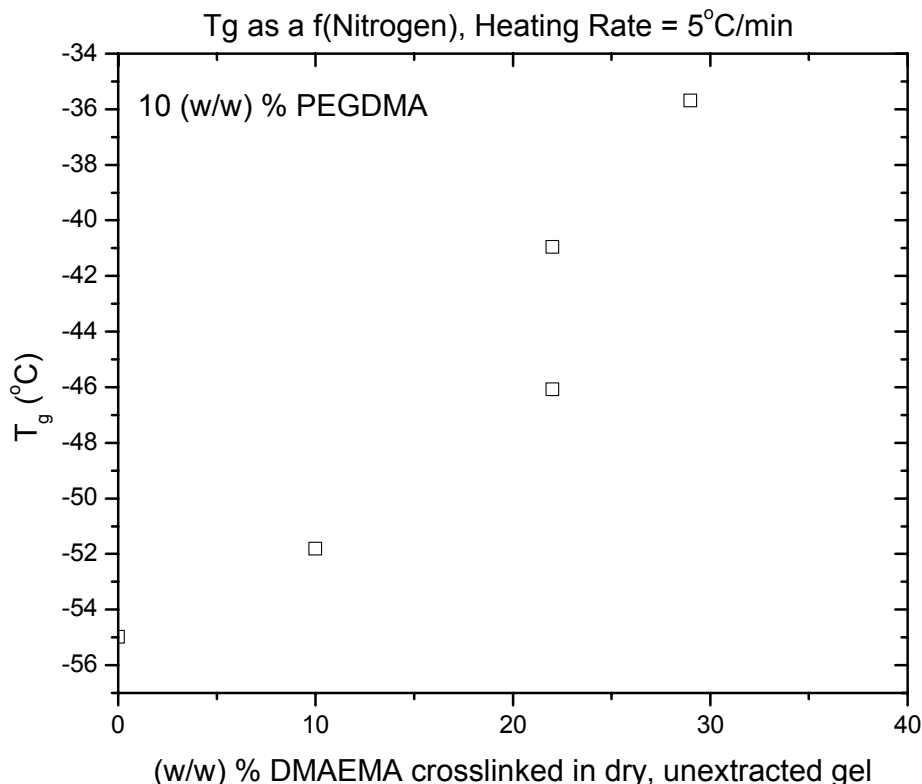


Figure 3.16 Glass transition temperatures of films compared to actual DMAEMA concentration incorporated.

The dependence of T_g still increases monotonically (within the experimental uncertainty of the elemental analysis) and nearly linearly with DMAEMA concentration, so the explanation offered above for the variation in T_g in terms of hydrogen bonding remains plausible. The DMAEMA-induced reduction in elastic modulus cannot, therefore, reflect a plasticizing effect due to residual unreacted monomer. A scenario that is consistent with both datasets is a heterogeneous network composed of hydrogen-bonded molecular bundles. Both dynamic rheology and rheometry probe supramolecular length scales and

detect the defects (voids) introduced by these bundles. Thermal calorimetry probes molecular length scales and reveals that the molecules comprising the bundles become less mobile as the DMAEMA content increases, which is consistent with enhanced intermolecular interaction (due, for instance, to hydrogen bonding). Clearly, this scenario is speculative, but it is consistent with the data and amenable to experimental verification using either microscopy (surface probe or transmission electron) or small-angle scattering (x-ray or neutron).

3.4 CONCLUSIONS

Poly(ethylene glycol) elastomers with covalently bound tertiary amines have been synthesized by bulk free-radical copolymerization. The resultant hydrogel networks swell in aqueous environments, but exhibit limited flexibility and strength in their dry state. At 10 wt% crosslinker, incorporation of the tertiary amine monomer is limited to 30 wt%, as demonstrated by elemental analysis after extraction. This lack of reactivity and chemical incorporation of DMAEMA into the PEG network is believed to create defects in the network, leading to measurable reductions in rigidity and strength. Increasing the crosslinker concentration to 30 wt% or more minimizes these deleterious effects of DMAEMA, suggesting that amine incorporation is increased. The hydrogel formulation exhibiting the most promise in terms of elasticity, strength, amine incorporation and swelling consists of 30 wt% crosslinker. On the basis of these results, other free-radical polymerization strategies, such as solution polymerization and UV-initiated photopolymerization, may also help to improve the copolymerization of DMAEMA in PEG hydrogels.

3.5 REFERENCES

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4 QUATERNIZATION AND ANTIBACTERIAL EFFICACY OF PEG HYDROGELS

4.1 INTRODUCTION

With the increasing resistance of bacteria to antibiotics, the need to prevent bacterial infections in hospitals and in everyday life is growing at an alarming rate.^{1,2,3,4} Preventing infection of implanted and medical devices is becoming increasingly important as it is estimated that 45% of hospital infections are associated with these devices.⁵ Examples of biomedical devices affected include vascular prostheses, orthopedic implants, contact lenses, and urinary tract catheters.⁶ Bacterial infections of these devices are often unresponsive to antibiotics and necessitate removal of the implant.^{6,7}

Bacterial infection of a medical device can be prevented by physical modification of the material constituting the device including *(i)* alteration of surface properties to prevent protein and bacterial adhesion^{5,6}, *(ii)* impregnation with antibiotics or biocides intended to initially kill bacteria⁸⁻¹³, and *(iii)* immobilization of antibiotics or biocides to permanently kill bacteria.¹⁴⁻¹⁹ Each of these antifouling strategies has been applied to soluble polymers^{17,20} and solid surfaces^{14,21}, but little research has been performed on elastomeric polymer systems²²⁻²⁵ such as hydrogels. Hydrogels are hydrophilic macromolecular networks that swell in aqueous media.²⁶ Because of their high water content and surface properties, hydrogels can be used to simulate natural tissue and often exhibit good biocompatibility, allowing them to be used in numerous biomedical

applications such as implanted drug delivery devices²⁷ and biosensor coatings²⁸. In these applications, hydrogels are likewise susceptible to bacterial infection.

In this work, we investigate the covalent attachment of antibacterial groups to hydrogels to produce a hydrogel with permanent antibacterial properties. Our goal is to prevent bacterial infection and potentially improve hydrogel performance in implant applications. Specifically, we employ poly(ethylene glycol) (PEG) hydrogels because of their known biocompatibility and hydrophilicity with quaternary ammonium compounds, which possess known antibacterial properties and have been previously used in antifouling materials. Quaternized hydrogel films are evaluated by elemental analysis, swelling, differential scanning calorimetry (DSC), and an antibacterial efficacy assay.

4.2 EXPERIMENTAL METHODS

4.2.1 MATERIALS

The macromonomer poly(ethylene glycol) methacrylate (PEGMA, $M_n \sim 526$ g/mol), amine monomer dimethylaminoethyl methacrylate (DMAEMA), and crosslinker poly(ethylene glycol) dimethacrylate (PEGDMA, $M_n \sim 800$ g/mol) were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used as received. The initiator 2,2'-azobisisobutyronitrile (AIBN) was used as received from Sigma Chemical Co. (St. Louis, MO). All polymer reactants were stored at 4°C until use.

Dimethylsulfoxide (DMSO) was purchased from Alfa Aesar (Ward Hill, MA) and used as received. Bromooctane (C_8Br), 1-bromododecane ($C_{12}Br$), and 1-bromohexadecane ($C_{16}Br$) were purchased from Acros Organics (Morris Plains, NJ) and used as received. Hexane was purchased from Aldrich Chemical Co. (Milwaukee, WI)

and used as received. Monofilament polyester mesh (20.3 x 20.3) was purchased from McMaster-Carr (Atlanta, GA) and cut into 4-inch and 9-inch diameter circles. Large 500-mL wide-mouth jars were purchased from Fisher Scientific (Pittsburgh, PA).

Tryptic soy agar, tryptic soy broth, pipette tips (1-100 μ l and 200-1000 μ L), and sterile plastic petri dishes (100-mm diameter) were purchased from Fisher Scientific (Pittsburgh, PA). Phosphate buffered saline powder (PBS, pH 7.4) was purchased from Sigma Chemical Co. (St. Louis, MO). A TLC reagent sprayer, 50 mL with screw thread ground joint, was purchase from Kimble/Kontes (Vineland, NJ).

4.2.1.1 Hydrogel Synthesis

Hydrogels of PEG-co-poly(DMAEMA) were prepared as described in Chapter 3. Briefly, transparent, homogenous PEG-based hydrogels were synthesized by free-radical copolymerization of PEGMA, DMAEMA, and PEGDMA within rubber molds bound between glass plates (0.15 x 6 x 5 cm³) at 80°C for 34 hours. Films with increasing weight fraction of DMAEMA were prepared (0, 0.1, 0.3, 0.5, 0.7, 0.9) by varying DMAEMA and PEGMA while holding the crosslinker PEGDMA constant at 0.1 wt. fraction and the initiator AIBN constant at 0.005 wt. fraction. After polymerization, molds were slowly cooled to room temperature. Hydrogels were removed from molds, rinsed with DI water, dried at room temperature for 24 h, dried under vacuum for 24 h, and stored in a desiccator until subsequent use. Note that hydrogel films here and in all subsequent processing steps were always laid flat to prevent film curling.

4.2.1.2 Quaternization

Dry, unextracted hydrogels of each monomer composition were placed in 500-mL wide-mouth jars, one jar for each alkyl bromide. A 4-inch polyester mesh circle separated each layer of hydrogel films. Each alkyl bromide was separately mixed with DMSO for a concentration of 15 vol %. Each reactant solution was gently poured into its respective jar containing the hydrogel films. Each jar was capped and sealed with parafilm. The jars were held at 60°C in a water bath for 4 days, tightening lids and replacing parafilm daily. The jars were slowly cooled to room temperature before opening in a fume hood. Reactant solution was removed from the jars and replaced with fresh DMSO. Jars were recapped, and films were soaked for 3 days for diffusion of unreacted alkyl bromides, monomer, and initiator out of the films. The used DMSO was removed from jars and replaced with hexane to further extract unreacted alkyl bromides and oligomers. The jars were recapped and left for 3 days after which the hexane was removed. The films were removed from jars with tweezers and each film composition was placed on polyester mesh on top of a wire rack in a 9-inch aluminum pan. Each pan was covered and sealed with aluminum foil to slow diffusion of solvent out of the films. Pans were completely sealed for 4 days, vented with holes in foil for 2 days, covered but not sealed with foil for 10 days, and placed in fume hood for 4 days to slowly remove all solvent. Films were subsequently stored in a desiccator until use.

4.2.2 HYDROGEL PROPERTY CHARACTERIZATION

4.2.2.1 Elemental Analysis

Carbon, hydrogen, nitrogen, and bromine elemental microanalyses (Atlantic Microlab, Inc., Norcross, GA) were performed on dried hydrogel samples prior to and after the quaternization reaction.

4.2.2.2 Swelling Experiments

Quaternized, dry hydrogel samples were weighed, placed in 10 or 50-mL beakers, and swelled in DI water in triplicate. DI water was replaced daily for 3 days to remove any residual reactants. Each sample was removed from water, pressed between Kimwipes with a 100-g weight for 30 seconds, and weighed. Samples were subsequently dried under vacuum for 24 hours and reweighed.

4.2.2.3 Differential Scanning Calorimetry

Pieces of dry quaternized hydrogel were cut to weigh approximately 15 mg. A sample was placed in the bottom of an aluminum DSC pan, and a lid was crimped on top. Each sample was tested on a TA Instruments DSC Q100 (New Castle, DE): cooling from 40°C to -80°C at 60°C/min, equilibrating at 80°C, and heating from -80°C to 40°C at 5°C/min. Resulting plots of heat flow versus temperature were used to determine the glass transition temperature (T_g) of each material. Dry hydrogel and dry quaternized hydrogel were tested.

4.2.3 ANTIBACTERIAL EFFICACY CHARACTERIZATION

4.2.3.1 Stock Supply Preparation

Soy agar gel petri dishes and broth centrifuge tubes were prepared. Briefly, forty grams of tryptic soy agar powder and 30 grams tryptic soy broth powder were weighed to make 1 L of solution. Tryptic soy agar powder, tryptic soy broth powder, and PBS powder mix were each poured into a 1-L Pyrex bottle. DI water was added to each bottle up to the 1-L mark, a large stir bar was added, and the bottles were loosely capped. Solutions were stirred at room temperature until powders were completely dissolved, approximately 30 minutes, and stir bars were removed with a magnetic stick. Broth was poured into centrifuge tubes, approximately 10 mL in each tube, and the tubes were loosely capped. All bottles of liquid were autoclaved on the liquid sterilization cycle. Once slightly cooled all bottles were tightly capped. Warm liquid agar was poured into sterile plastic petri dishes, approximately 5 mL to cover the bottom of each dish. The perimeter of each dish was wrapped with parafilm. Once the agar solidified, sealed dishes were turned upside down to limit contamination and wrapped in 12-dish stacks with aluminum foil. All medium was stored at 4°C until use.

4.2.3.2 Antibacterial Testing

4.2.3.2.1 *Bacteria Preparation*

Bacteria solutions were prepared as described elsewhere.^{29,30} Briefly, *Staphylococcus aureus* (ATCC #25923) colonies were grown overnight on an agar plate. This stock plate was used for up to 1 month. One *S. aureus* colony was selected from the

stock plate with a sterile wooden inoculating stick and incubated in 10 mL of broth at 37°C overnight. The broth/bacteria solution was centrifuged for 12 minutes at 3000 rpm, and the broth was subsequently removed, leaving a bacteria pellet in the bottom of tube. The bacteria pellet was washed by adding 5-mL of PBS and mixing with a glass pipette. Centrifugation and washing were repeated two more times. The washed bacteria solution was serially diluted 5 times by powers of 10.

4.2.3.2.2 Surface Testing

The surface-testing protocol of Klivanov et al^{3,14} was used but modified to accommodate hydrogel materials. Hydrogel samples placed in DI water and allowed to swell for 3 days, replacing water daily to remove any reactant contaminants. Cold agar plates were warmed in a 40°C incubator. The dilute bacteria solution described in 4.2.3.2.1 was plated with a 10-μL inoculating loop in triplicate to determine the average number of bacteria cells per volume. The rest of the diluted bacteria solution was poured into a sterile 50-mL TLC reagent sprayer. A large pipette bulb was connected to the glass sprayer with plastic tubing. The bacteria solution was sprayed on to 3 agar plates, 1 spray per plate at a height 15-cm from the plate, as a control to determine the CFU per spray per cm². Triplicates of each hydrogel composition were exposed in the same way. First, a hydrated hydrogel sample was pressed between Kimwipes for 30 seconds to remove excess water. The sample was sprayed with bacteria solution, and the solution was allowed to dry for approximately 2 minutes. Soy agar at 40°C was lifted with a sterile metal spatula from the petri dish, and the treated hydrogel sample was placed bacteria-side up under the agar. Each petri dish was re-sealed with parafilm and incubated at 40°C

overnight. Bacteria colonies were counted by hand using a Colony Counter (Fisher Scientific, Pittsburgh, PA), assuming each colony represented a bacteria cell, or colony forming units (CFU), that survived treatment. Since film size varied, the surface area of each film was measured by analyzing digital pictures of each sample, as described below. The antibacterial efficacy of each film was then quantified by dividing the CFU counted by hand by the digitally measured surface area.

A photograph of each sample backlit by the Colony Counter was taken at a constant distance and zoom without flash. A photo of a ruler was taken in the same way. In Adobe Photoshop 7.0, the “Measuring Tool” was used to determine the number of pixels per cm of the ruler at this constant distance and zoom. Each individual sample was then analyzed. From a photo of the whole plate, the lasso tool was used to select the hydrogel sample, taking care to get the exact shape. A new image was then opened, and the selected sample copied and pasted into the new image. The sample was then converted to only black using the “Threshold” function and setting it to 255. A histogram of the image was then taken. Solid black in the image shows up under “Count 0”, and the number of black pixels reported there was divided by the total number of pixels in the image, giving the fraction of picture area taken up by the sample. The total image size in pixels² was converted to cm² using the scale determined with the ruler, and this number was subsequently multiplied by the area fraction of the sample to get the area of the sample in cm².

4.3 RESULTS AND DISCUSSION

4.3.1 QUATERNIZATION

The hydrogel films exhibited a color change from the quaternization reaction. Prior to quaternization, all films were homogeneous, transparent, and clear to light yellow-brown color. The film color comes from the red-brown color of the PEGMA used. The color thus decreases with decreasing PEGMA, or increasing DMAEMA, content. Films exposed to C_8Br changed to light orange-brown, $C_{12}Br$ dark orange-brown, and $C_{16}Br$ light yellow-brown (not very different from before quaternization). This color change indicated that bromide ions had been formed and that the quaternization reaction was to some degree successful. The reaction fluid was the same color as the films, indicating that free tertiary amine monomer was quaternized and had diffused out of the films.

When the reaction fluid was replaced with fresh DMSO, the fresh solvent also became colored, exhibiting further release of quaternized monomer. A small amount of reacted quaternized monomer, a film-colored liquid, was also observed in as a separate phase in the hexane extraction. The films were highly swollen but maintained their original crosslinked shape. The films generally did not break except when they expanded into the walls or lid of the jar. The exception to this was the tensile testing samples, which all broke at the tab ends during quaternization. Small internal cracks were visible within the 50 wt% and 70 wt% DMAEMA monomer films prior to de-swelling. This phenomenon will be discussed in conjunction with elemental analysis results. Elemental

analysis was required to determine the DMAEMA content and degree of quaternization after extraction. Photographs of the quaternized films are shown in Figure 4.1.

Alkyl Bromide	10 wt% DMAEMA	30 wt% DMAEMA	50 wt% DMAEMA	70 wt% DMAEMA
None				
C ₈ Br				
C ₁₂ Br				
C ₁₆ Br				

Figure 4.1 Quaternized hydrogels with constant 10 wt% PEGDMA exhibit color change.

Upon de-swelling and drying, all films had minimal cracking. Most cracks appeared to propagate from the perimeter of the films. This suggests that edge microcracks cause most of the large cracks in the films. Molding of samples may reduce edge microcracks but does not completely prevent them. Samples dried flat on elevated racks, allowing solvent to drip into the aluminum pan, remained flat. A few samples that were dried flat on filter paper exhibited curling, which had been seen in previous experiments as solvent evaporates from the top of the film but remains pooled around or trapped in the bottom of the film. The lack of curling in samples dried on the elevated rack shows that uniform drying allows slow, uniform contraction of the films and eliminates curling, shown in Figure 4.2. Overall, large flat samples can be produced by this quaternization method. Investigation of improved molding procedures could eliminate cracking altogether.



Figure 4.2 Films dried on an elevated rack in aluminum pans (a) did not curl while films dried on flat filter paper (b) did curl during drying. Uniform drying thus eliminates film curling.

4.3.2 ELEMENTAL ANALYSIS

Dry hydrogel films after crosslinking and after quaternization followed by extraction were sent for elemental analysis. All films had a constant 10 wt% PEGDMA concentration with varying DMAEMA concentration and alkyl bromide length. As seen in Table 4.1, the amount of nitrogen, which is 1:1 with DMAEMA, is less after quaternization and extraction. As the percent DMAEMA in the monomer solution increases, the amount of DMAEMA incorporated into the film decreases.

Table 4.1 Degree of Quaternization of Films

DMAEMA (w/w) %	Alkyl Bromide Chain Length	Extracted	Nitrogen (mol N/g film) %	Bromine (mol Br/g film) %	% Quaternization
0	none	No	0.000	0.000	-
10	none	No	0.064	0.000	0
10	C8	Yes	0.066	0.064	97
10	C12	Yes	0.063	0.053	85
10	C16	Yes	0.064	0.051	79
30	none	No	0.183	0.000	0
30	C8	Yes	0.141	0.141	100
30	C12	Yes	0.131	0.125	96
30	C16	Yes	0.160	0.144	90
50	none	No	0.313	0.000	0
50	C8	Yes	0.125	0.123	98
50	C12	Yes	0.166	0.166	100
50	C16	Yes	0.140	0.144	103
70	none	No	0.428	0.000	0
70	C8	Yes	0.193	0.197	102
70	C12	Yes	0.187	0.176	94
70	C16	Yes	0.168	0.168	100

This lack of incorporation of DMAEMA is most likely due to the low reaction rate of DMAEMA with PEGDMA and PEGMA as compared to the reaction rate of PEGDMA and PEDMA with each other. DMAEMA monomer is less reactive, becomes trapped due to diffusion limitations, and remains unreacted in the bulk of the network. This increased amount of trapped monomer may explain the internal cracking seen in 50

wt% and 70 wt% DMAEMA films. Once the unreacted DMAEMA is extracted, the network is left with significant amount of voids and internal cracks.

Quaternization of all films was significant as shown in Table 4.1, where percent quaternization is the ratio of bromine to nitrogen times 100. All films except for 10 wt% DMAEMA have greater than 90 % quaternization. Films with 50 wt% and 70 wt% DMAEMA have almost 100 % quaternization for all alkyl bromide chain lengths. Films with 10 wt% and 30 wt% have decreased quaternization with increasing alkyl bromide chain length. This decreased reactivity may be due to the tightness of the network limiting diffusion of longer alkyl chains, since these film compositions would have fewer voids for reasons explained above. It could also be due to more complex kinetics than can be explained here. Perhaps excess DMAEMA monomer acts as a catalyst for the nucleophilic substitution reaction. A third hypothesis is that lower DMAEMA, higher PEG concentration films are more hydrophilic, limiting the uptake and interaction with the hydrophobic alkyl bromide.

4.3.3 SWELLING EXPERIMENTS

Degree of swelling prior to quaternization was not dependent on DMAEMA, or nitrogen, content. After quaternization, films exhibited dependence on DMAEMA, or quaternized nitrogen, content, shown in Figure 4.3. As DMAEMA content increases, swelling decreases. Also, as alkyl bromide chain length increases, swelling decreases. These two observations together indicate that swelling decreases with increased hydrophobicity. Hydrophobicity of the films should increase with increasing quaternized nitrogen concentration and increasing alkyl bromide chain length.

An interesting observation from these swelling experiments is film cracking. Quaternized films were re-swelled in water after removal of extraction solvents. Existing cracks in the films propagated during this re-swelling, but generally no new cracks formed. The exception to this was 10 wt% DMAEMA with 10 wt% PEGDMA. This film began cracking into small pieces within 30 seconds of contact with water. Popping sounds of the films breaking were loud enough to be heard from 5 feet away. A few small pieces of hydrogel were left, but the rest of the film was reduced to sand-sized pieces. This extreme cracking may be due to incompatibility of the high PEG-content film, approximately 90 % PEG, with the hydrophobic unit.

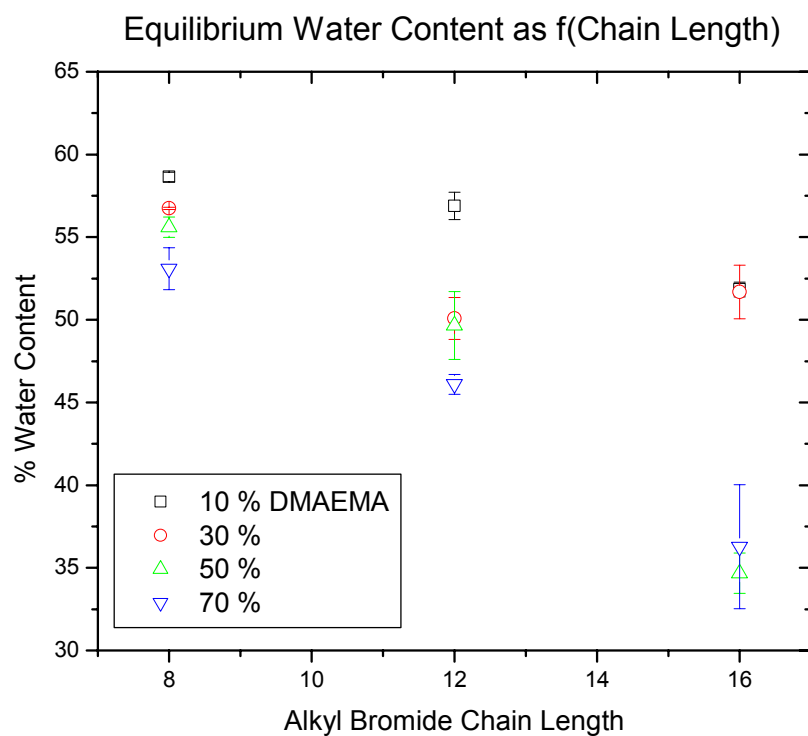
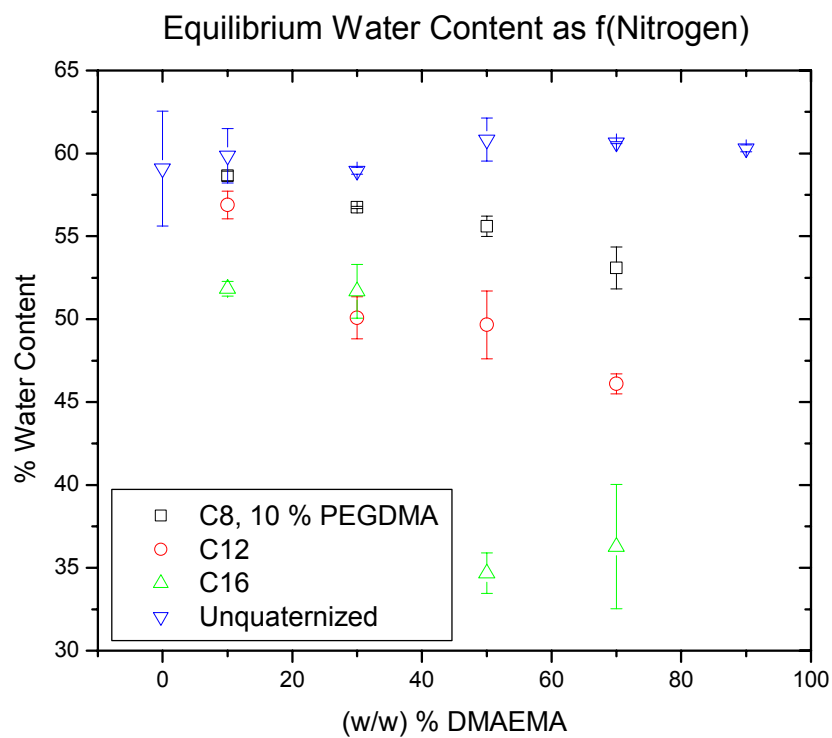


Figure 4.3 Equilibrium water content of hydrogels decreases with increasing quaternized DMAEMA concentration and alkyl halide chain length. Note that DMAEMA concentration of films prior to extraction is used for comparison to unquaternized swelling.

4.3.4 DIFFERENTIAL SCANNING CALORIMETRY

The glass transition temperatures of dry, unextracted films with constant PEGDMA concentration of 10 wt% were measured with DSC. The glass transition temperatures of these films showed a linear dependence on DMAEMA concentration. After quaternization with C₁₂Br followed by extraction, the dry film compositions were again measured. The measured values are shown in Figure 4.4.

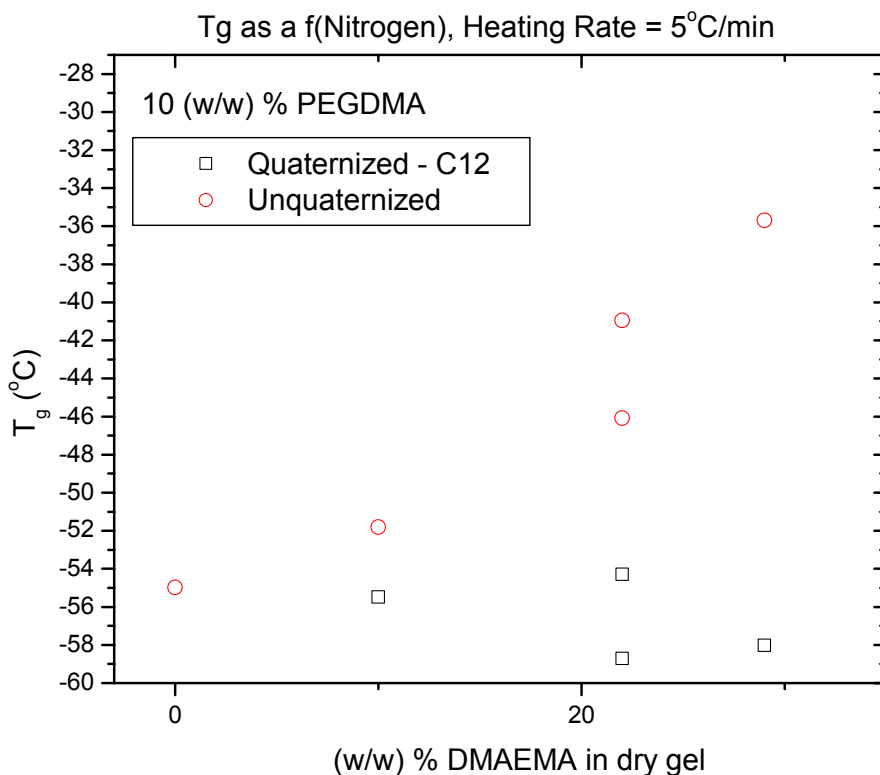


Figure 4.4 Quaternization eliminates T_g dependence on DMAEMA concentration. Note that both quaternized and unquaternized films are plotted against DMAEMA concentration of extracted films, assuming that the T_g measured would mostly depend on DMAEMA bound to the network rather than unbound DMAEMA.

From Figure 4.4, the glass transition temperature only shows dependence on nitrogen prior to quaternization. The increase in T_g with increased nitrogen may be due to hydrogen bonding between nitrogen and PEG limiting rotation of chains in the network.

Quaternization of the nitrogen groups would eliminate hydrogen bonding and thus the dependence of T_g on nitrogen concentration. Alternatively, alkyl chains attached during quaternization have previously been observed to act as plasticizers, lowering the T_g , which is consistent with the trend observed here. If this is the case, the T_g would also depend on length of the attached alkyl chain. Further investigation could test this theory. Last, the T_g observed in the unextracted films may be due to excess unbound DMAEMA monomer acting as a plasticizer. Removal of this plasticizer by quaternization followed by extraction would also eliminate T_g dependence on nitrogen content. This interpretation could be tested by solvent extraction of unquaternized films followed by T_g measurement. If the DMAEMA monomer were acting as a plasticizer, however, T_g would be expected to decrease with increasing DMAEMA. Since the opposite phenomenon is observed, DMAEMA may not be acting as a plasticizer.

4.3.5 ANTIBACTERIAL EFFICACY

Hydrogel samples were sprayed with a bacteria solution diluted to 21.5 ± 4.77 CFU/ μ L. The undiluted bacteria suspension originally contained $1.08 \pm 0.24 \times 10^{10}$ CFU. Samples exposed to bacteria were covered with agar and incubated overnight. *S. aureus* colonies on each hydrogel composition with constant 10 wt% PEGDMA were counted. The number of colony forming units (CFU) per cm^2 was calculated assuming each colony on the surface represented one CFU that survived biocide exposure and measuring the surface area of each film with Adobe Photoshop, as described in 4.2.3.2.2. The antibacterial efficacy of the quaternized films is shown in Figure 4.5 in plots of CFU/ cm^2 as a function of quaternized nitrogen content and alkyl bromide chain length.

First, note that the unquaternized films show a decrease CFU/cm² with increasing DMAEMA concentration. These unquaternized films should show no correlation with DMAEMA concentration. Since these films were not extracted with solvents, only with water, all unreacted DMAEMA monomer was not removed, as shown in the elemental analysis results of Chapter 3, and must have leached out during the antibacterial testing, affecting the bacteria growth and making the unquaternized films an unrepresentative control. Instead the CFU/cm² from 1 spray of bacteria solution on agar will be used as the control. On the plots, the control is represented by an open black square, and a dashed line extends it horizontally for easy comparison to all samples. Also, note that error bars represent standard error intervals, essentially 1 standard deviation.

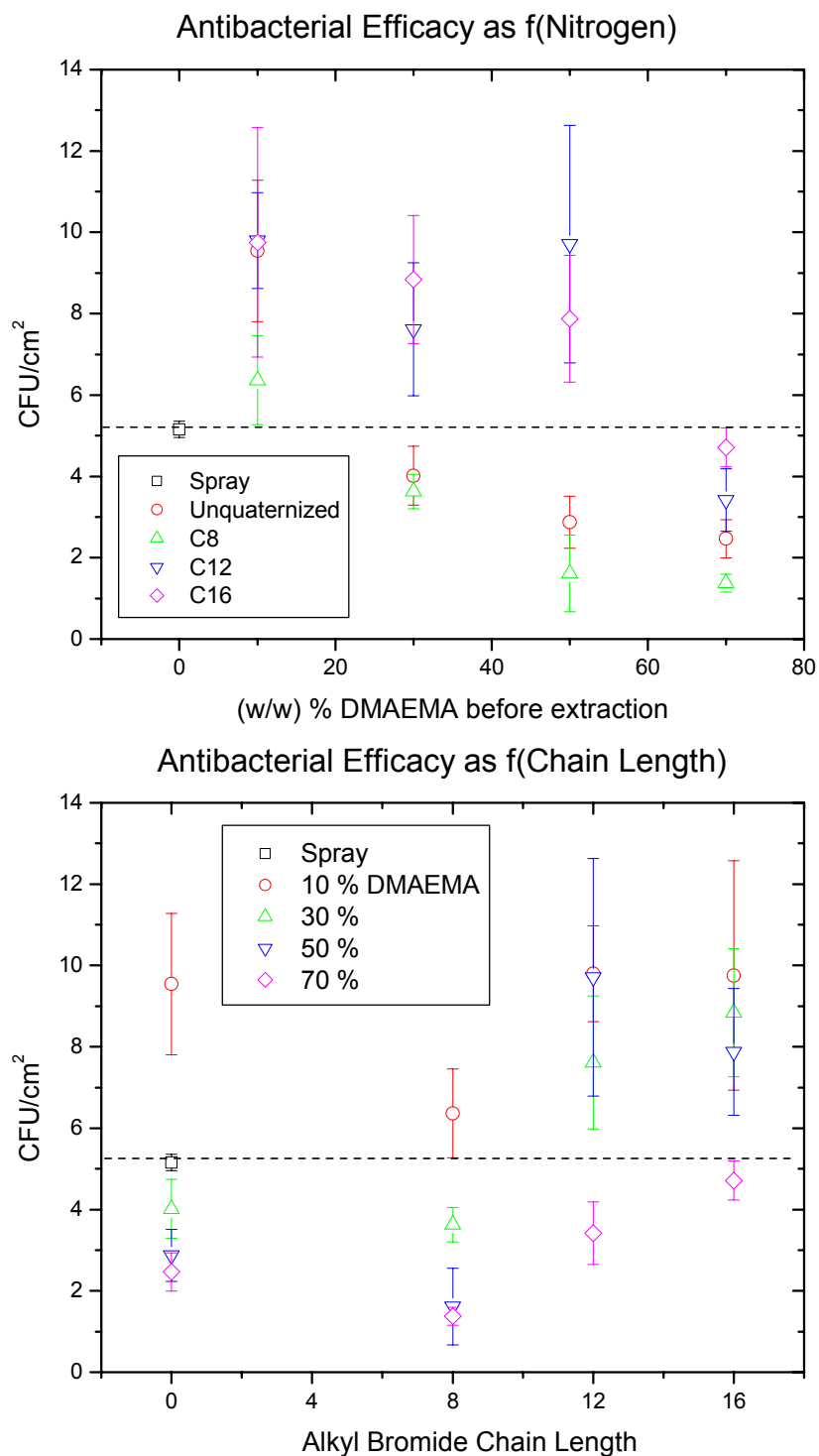


Figure 4.5 Antibacterial efficacy of hydrogels increases with increasing quaternized nitrogen content while it decreases with increasing alkyl halide chain length (C₈-C₁₆). Note that the plots are versus DMAEMA concentration prior to extraction for comparison with unquaternized films. The dashed line extends the spray CFU/cm² and is included to help guide the eye.

In Figure 4.5, CFU/cm² decrease with increasing DMAEMA, or quaternized nitrogen, content. This dependence was expected if quaternization imparted films with antibacterial properties. Films quaternized with C8Br reduced the CFU/cm² below the control with 22 and 30 wt% DMAEMA (30-70 wt% prior to extraction), exhibiting bactericidal properties. Films quaternized with C₁₂Br and C₁₆Br reduced CFU/cm² below the control with 30 wt% DMAEMA (70 wt% prior to extraction), also exhibiting bactericidal properties. Bactericidal behavior is a significant finding. Reports in the literature, as discussed in Chapter 2, state that most elastomers synthesized with quaternary ammonium compounds (QACs) exhibit only bacteriostatic activity.^{23,24,31} These elastomers were not swollen hydrogels, however, and swelling of the elastomeric network may improve exposure to and interaction of the QACs with bacteria cells.

When comparing alkyl bromides, the most effective chain length was C₈ followed by C₁₂ and C₁₆, respectively. The optimum alkyl chain length for other polymer systems has previously been reported between 10 and 12 carbons¹⁶, as discussed in Ch. 2. Most of those systems were soluble. Since these films are insoluble and elastomeric, however, the exposure of bacteria to the quaternized nitrogen may be more limited or hindered by network chains. Increasing alkyl chain length attached to the nitrogen may decrease the mobility of the quaternized functional group in the network, further decreasing bacteria exposure and decreasing antibacterial efficacy. Antibacterial elastomers may thus have a different optimum alkyl chain length than soluble polymers. For this PEG system, C₈ is the optimum chain length with a minimum quaternary ammonium concentration of 22 wt%.

Photographs of bacteria-infected films incubated overnight are shown in Figure 4.6. Each small white dot on the films is a bacteria colony. The colonies are somewhat difficult to see due to similarity between the colors of the colonies and of the films. The view is also obstructed as the bacteria are between agar and the film, leaving no direct view or camera shot of the bacteria. In future work, fluorescent labeling of the bacteria may allow a more quantitative assessment of bactericidal behavior.

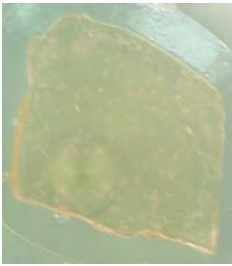
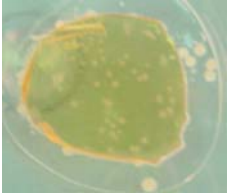
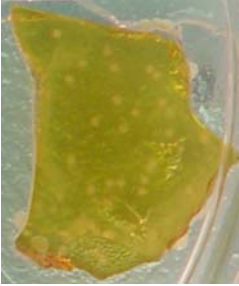
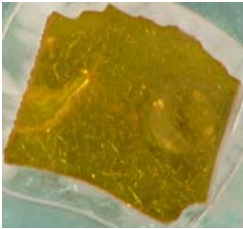
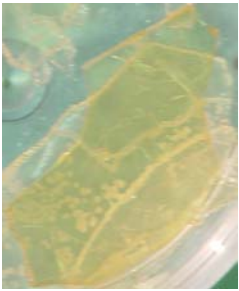
Alkyl Bromide	10 wt% DMAEMA	30 wt% DMAEMA	50 wt% DMAEMA	70 wt% DMAEMA
None				
C8Br				
C12Br				
C16Br				

Figure 4.6 Quaternized hydrogels with constant 10 wt% PEGDMA exposed to *S. aureus* exhibit bactericidal behavior.

4.4 CONCLUSIONS

Tertiary amines were successfully covalently bound to a PEG hydrogel and quaternized with alkyl bromides of varying chain length, C₈-C₁₆. Quaternization of the immobilized tertiary amines reduced the swelling of the hydrogels and decreased the T_g of the dry hydrogels to a constant $\sim$ -56°C. The swollen hydrogel films exhibited bactericidal behavior against *S. aureus* sprayed on the films and incubated overnight. Hydrogel films quaternized with C₈Br were the most antibacterial followed by C₁₂Br and C₁₆Br. The minimum effective bactericidal concentration of QACs was 22 wt% for C₈Br and 30 wt% for C₁₂Br and C₁₆Br.

Further experimentation is needed to improve incorporation of DMAEMA into the network and thus increase the maximum QAC concentration. To develop a more consistent film for mechanical characterization, reduction of film cracking should also be explored.

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5 CONCLUSIONS AND FUTURE WORK

5.1 SUMMARY

5.1.1 HYDROGEL SYNTHESIS

Hydrogels derived from PEG have been synthesized by thermally-initiated bulk free-radical copolymerization of PEGMA, PEGDMA, and DMAEMA to incorporate tertiary amines into a crosslinked PEG network. Mass fractions of PEGDMA and DMAEMA have been varied from 0 to ~1 with the balance consisting of PEGMA. The tertiary amine monomer (DMAEMA) is incorporated into the PEG network up to about 30 wt%. The polymerization at the hydrogel surface has been monitored by ATR-FTIR, and bulk film compositions have been measured by elemental analysis. The thermo-mechanical properties of the hydrogel films have been investigated by a combination of dynamic mechanical analysis, rheometry, swelling, and thermal calorimetry.

According to ATR-FTIR, the surface polymerization of a formulation with 10 wt% PEGDMA, 30 wt% DMAEMA, and 60% PEGMA is found to be complete after only 40 min at 80°C. Comparable polymerization kinetics in the bulk could not be monitored by transmission FTIR due to experimental limitations. Solidification of films measuring 1.5 mm thick occurs between 18 and 20 h. Elemental analysis of films containing 10 wt% PEGDMA reveals that the copolymerization of DMAEMA with PEGDMA and PEGMA is not efficient. As the concentration of DMAEMA is increased, the fraction of DMAEMA incorporated into the network decreases, with a maximum of

30 wt% DMAEMA incorporated into the network from a monomer mixture containing 70 wt% DMAEMA.

Increasing the crosslinker (PEGDMA) concentration increases the modulus, ultimate tensile stress, and yield stress, but decreases the extent of swelling in water. These trends are expected as increasing the crosslinker concentration serves to increase the crosslink density, which promotes increases in rigidity and strength. Increasing the tertiary amine concentration surprisingly increases T_g at 10 wt% PEGDMA, but decreases the modulus, ultimate tensile stress and yield stress at this and other PEGDMA concentrations. The percent elongation and toughness display no conclusive dependence on hydrogel composition. The reduced strength of 10 wt% PEGDMA with increasing DMAEMA concentration may be due to incomplete incorporation of DMAEMA into the network, as determined from elemental analysis. The concurrent increase in T_g precludes the possibility that the residual unreacted monomer acts as a plasticizer, in which case we surmise that the network may not be homogeneous. A heterogeneous network composed of molecular bundles held together by hydrogen bonds and separated by voids would, for instance, be consistent with the observed modulus reduction and T_g increase. Relatively good physical properties are obtained with formulations containing 30 wt% PEGDMA. The swelling behavior of this system is inferior only to the one with 10 wt% PEGDMA, while the mechanical properties (such as strength and rigidity) are better and more consistent than those measured for the formulation with 10 wt% PEGDMA. Since the mechanical properties show little dependence on DMAEMA content, the concentration of DMAEMA incorporated in the hydrogel with 30 wt% PEGDMA may be higher than that with 10 wt% PEGDMA, but this has not been verified.

5.1.2 HYDROGEL QUATERNIZATION

Films containing 10 wt% PEGDMA have been successfully quaternized with alkyl bromides of varying chain length. Greater than 90% quaternization is achieved in most films. The extent of quaternization decreases with increasing alkyl bromide chain length in films with less than 20 wt% DMAEMA. Equilibrium swelling is observed to decrease with increasing quaternized DMAEMA content presumably due to an increase in hydrophobicity (a consequence of the quaternized moieties). The T_g of these samples is about -56°C and shows no dependence on quaternized DMAEMA content. Prior to quaternization, recall that the T_g increased with DMAEMA content. Such invariance in T_g with DMAEMA composition in quaternized samples may reflect a reduction in (or elimination of) hydrogen bonding upon quaternization or removal of unreacted monomers during extraction.

Hydrogels with greater than 22 wt% DMAEMA quaternized with C_8Br , as well as with 30 wt% DMAEMA with C_{12}Br and C_{16}Br , exhibit bactericidal behavior against *S. aureus*. Antibacterial efficacy is observed to increase with increasing quaternized DMAEMA concentration and decrease with increasing alkyl bromide chain length. The former dependence is expected if the quaternized moieties possess antibacterial properties, and suggests that an increase in the concentration of these groups may further improve antibacterial efficacy. The latter dependence is somewhat unexpected, since C_{10} to C_{12} functionality has been previously reported¹ as the optimum chain length for antibacterial potency. Since these polymer films are insoluble, however, network chains may hinder the exposure of bacteria to the quaternized nitrogen. If this is the case,

increasing alkyl chain length may decrease the mobility of the quaternized moiety, decreasing bacteria exposure and, thus, decreasing antibacterial efficacy.

5.2 FUTURE WORK

5.2.1 POLYMERIZATION

The synthesis protocol employed here is successful at incorporating tertiary amines into a PEG network, but the incomplete incorporation of DMAEMA monomer leaves opportunities for considerable improvement. First, the kinetics of the copolymerization could be studied more thoroughly at varying compositions and reaction temperatures with ATR-FTIR, which, together with elemental analysis, would help identify copolymerization conditions that improve DMAEMA incorporation and thus network properties. Also, the mold thickness could be decreased to reduce heat transfer limitations and improve network homogeneity during polymerization. The molding conditions should be optimized to reduce edge cracks. A machined all-Teflon mold might improve film uniformity and overall material production efficiency.

Alternatively, other copolymerization strategies could be used to generate formulations of interest. Such strategies include photoinitiated and solution free-radical copolymerization. Depending on the availability of a UV radiation source, the protocol proposed by Peppas et al.², discussed in Chapter 2, could be used in conjunction with this polymer system to determine if photopolymerization improves the reaction kinetics, incorporation of DMAEMA and ultimate network properties. If thermal initiation is still preferred, the solution polymerization protocol reported by Prausnitz and co-workers^{3,4} could be used with this polymer system to decrease diffusion limitations and again

improve DMAEMA incorporation and network properties. Real- and reciprocal-space methods such as microscopy and small-angle scattering, respectively, could be used to elucidate the topology of the networks generated by these methods and could be used to ascertain if the conflicting results reported herein can, in fact, be attributed to a heterogeneous network composed of hydrogen-bonded molecular bundles.

5.2.2 QUATERNIZATION AND ANTIBACTERIAL TESTING

All of the hydrogels generated for antibacterial testing, including the unquaternized films, should be thoroughly extracted with solvents at elevated temperature or in water for several weeks to remove unreacted monomer and initiator. Unquaternized films that are not extracted leach monomer and cannot be used as controls. Although the hydrogel quaternization reaction employed here was successful, the reaction protocol could be improved. An improved reaction setup is currently under development in the Genzer Group for a similar surface modification reaction. It includes the use of a glass reactor, a nitrogen purge, and mechanical agitation. The hydrogel films could still be stacked and separated by polyester mesh within the more robust reaction setup.

The modified bacteria spraying protocol developed by Tiller et al.⁵ is effective at uniformly dispersing cells on the hydrogel surface. Since bacteria cells are sandwiched between a film being tested and agar gel, however, colonies are relatively small and often difficult to detect. Fluorescent labeling of bacteria followed by observation under a fluorescent microscope may improve antibacterial analysis, as well as provide more data about film antibacterial efficacy as a function of exposure time. Also, thin film samples

should be synthesized, quaternized, and tested using a bacteria flow cell and automated video microscopy to discern the interaction of the hydrogel with bacteria cells. The quaternized networks produced here should also be tested against other bacteria strains, such as *E. coli* and *V. harveyi*, to determine if the hydrogels would be suitable for marine or water purification applications.⁶

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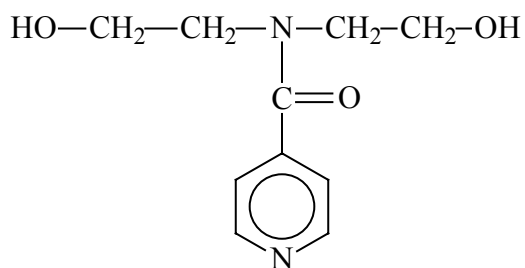
APPENDIX A: QUATERNARY AMMONIUM COMPOUND SYNTHESIS AND CHARACTERIZATION

In this study, we have synthesized quaternary ammonium biocides and characterized their properties for future incorporation into polyurethane-based hydrogels. The biocides were found to be effective against *S. aureus*. We have specifically researched synthetic routes for addition of these biocides to poly(ethylene glycol)-polyurethane hydrogels for future experimentation.

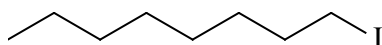
Materials

Quaternary ammonium biocides were synthesized from N,N-bis(2-hydroxyethyl) isonicotinamide (BIN), 1-iodooctane (C8I), 1-bromotetradecane (C14Br), and 1-bromooctadecane (C18Br). The reaction was performed in toluene and dimethylacetamide (DMAc). All chemicals were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. The chemical structures of each follow.

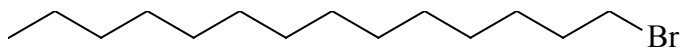
N,N-bis(2-hydroxyethyl) isonicotinamide (BIN)



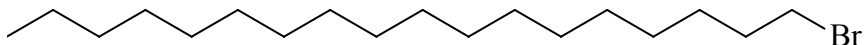
1-iodooctane (C8I)



1-bromotetradecane (C14Br)



1-bromooctadecane (C18Br)



Biocide Synthesis

We synthesized three quaternary ammonium compounds by reacting alkyl halides with different carbon chain lengths, eight carbons (C8I), fourteen carbons (C14Br), and eighteen carbons (C18Br), with the chain extender BIN. The biocides were synthesized with a modified experimental protocol outlined by Grapski in his PhD thesis from the University of Delaware. BIN was dissolved in DMAc at room temperature. Toluene was then added to the mixture to give 5 (w/v) % BIN in a 70% toluene/30% DMAc solution. The BIN solution was stirred and heated to 80°C. Once the mixture temperature was stable at 80°C, thirty-percent excess alkyl halide was added. The reaction mixture was stirred at 80°C for approximately 24 hours.

The reaction mixture was cooled to room temperature and poured into a separatory funnel. Hexane was added to the funnel in a 1:1 ratio with the reaction mixture. The mixture was mixed and then allowed to separate. The more-dense, viscous fluid was the product while the less-dense fluid was hexane containing unreacted alkyl halide and oligomers. This separation was repeated two to three times. The product was poured into

a beaker and dried at 60°C for 48 hours and at 40°C under vacuum for 72 hours. The BIN-C8I product was a viscous liquid, the BIN-C14Br product was an orange soft waxy solid, and the BIN-C18Br product was a yellow-brown hard waxy solid.

Biocide Characterization

The biocides' structures were characterized by Carbon-13 Nuclear Magnetic Resonance Spectroscopy (C13 NMR) and by Fourier Transform Infrared Spectroscopy (FTIR).

For C13 NMR, each biocide was dissolved in 1 mL of deuterated methanol, CD₃OD. The solutions were concentrated to near saturation so that each solution gave a strong signal and thus a clean C13 spectrum. The concentrated biocide solutions were each ran for approximately 13 hours on a Bruker AVANCE 500 MHz Spectrometer.

For FTIR, biocide solutions of 10 (v/v) % in water were prepared. One drop of solution was placed on a BaF₂ crystal, and the water was evaporated in a fume hood. Each sample was analyzed on a Nicolet Magna-IR Spectrometer 750 with 64 scans and resolution of 4.

The antibacterial properties of the biocides were characterized by a microbiology technique of dilution and plating described by Grapski. We will summarize the technique here for completeness.

One colony of bacteria, *Staphylococcus aureus*, was placed in centrifuge tube with 5 mL of broth medium and incubated overnight, approximately 16 hours at 37°C. The bacteria suspension was centrifuged, leaving a pellet of bacteria on the tube bottom.

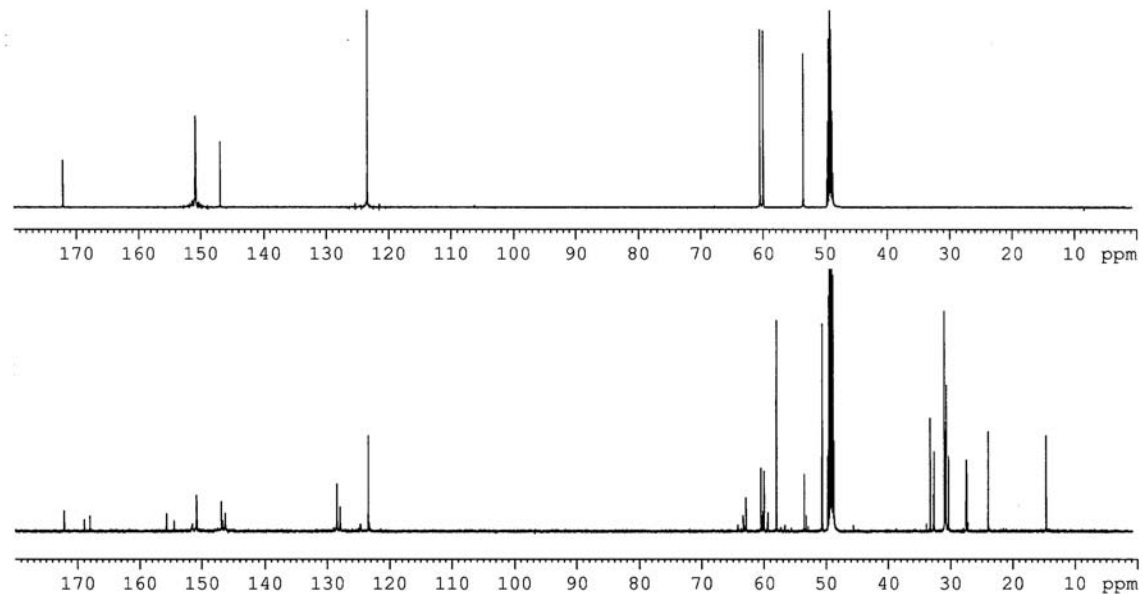
The broth was removed from the tube with a glass pipette, and 5 mL of phosphate buffer saline (PBS), pH 7.4, was added with a Finn timer. The PBS and bacteria pellet were mixed with the glass pipette. This centrifuge and washing procedure was repeated two more times. 100 μ L of bacteria suspension was serially diluted in PBS by powers of 10, 100 μ L of suspension in 900 μ L of PBS. Three of these dilutions, $\times 10^5$ - 10^7 dilutions, were sampled and three plates of each dilution were made. The plating procedure consisted of dipping a 10 μ L sterile inoculating loop in the solution, smearing the solution on a petri dish filled with tryptic soy agar, and wrapping the plate with parafilm to prevent contamination. This first dilution was the time equal to 0 minutes time point and served as the control group. The bacteria suspension was then ready for exposure to the biocide.

Several concentrations of each biocide were tested to determine their killing efficacy. Each biocide was dissolved in water at room temperature. The concentration of this stock solution was set by the desired concentration of biocide in the bacteria suspension. The concentrations of biocide tested in the bacteria suspension were 0.01 M, 0.001 M, and 0.0002 M. For each biocide and concentration, 100 μ L of stock biocide solution was added to the now 4900 μ L of bacteria suspension. The biocide-bacteria mixture was placed in a shaker for 1 hour with 100 μ L samples taken at 5, 30, and 60 minutes. Each 100- μ L sample was serially diluted by powers of 10. Three dilutions were plated with 3 replicates each. All plates were incubated overnight at 37°C. Bacteria colonies were counted and recorded by hand. Each colony is assumed to represent one viable bacteria cell in the bacteria suspension. The number of viable bacteria in the

original biocide-bacteria suspension can thus be calculated taking into account the number of serial dilutions and the solution sample size per plate of 10 μL .

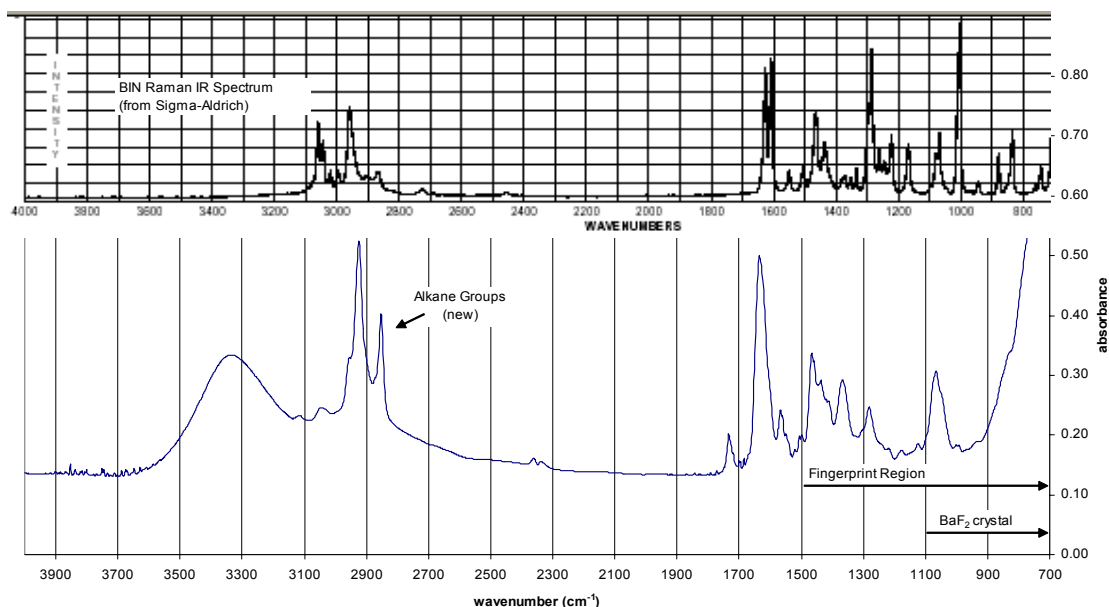
Results

^{13}C NMR spectra analysis shows that each alkyl chain was added to the base chain extender molecule BIN. The additional peaks from approximately 15 to 34 ppm represent the bulk of the alkyl chain. The only alkyl chain carbon not included in these peaks is the carbon attached to the quaternary nitrogen. The C-N carbon is the peak at approximately 58 ppm. See figure below.



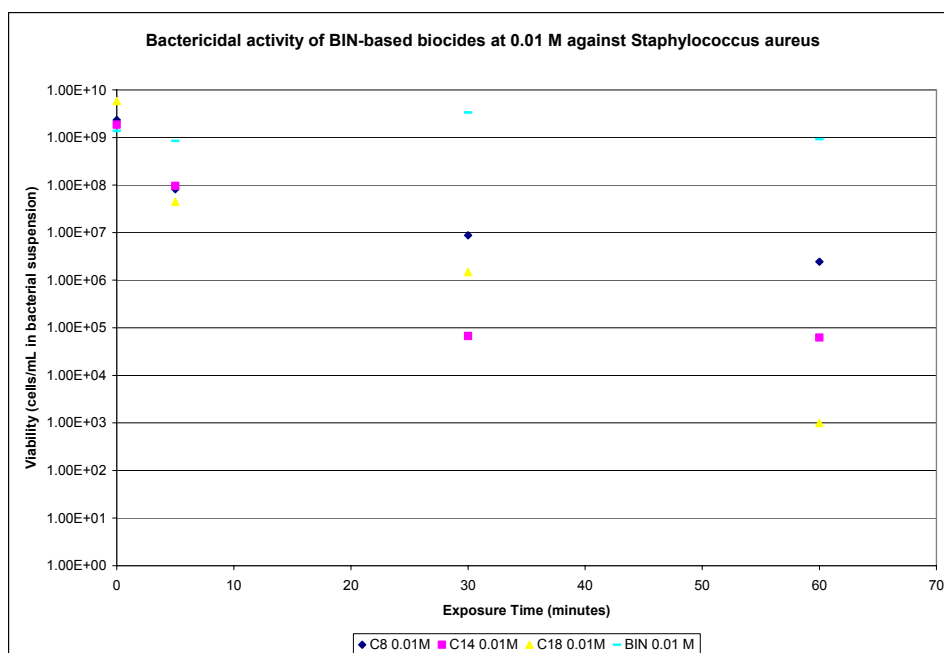
^{13}C NMR spectra of BIN (top) and BIN-C14Br (bottom) show addition of the alkyl chain with new peaks on the BIN-C14Br spectrum between 15-34 ppm and at 58 ppm.

FTIR spectra analysis also shows the addition of the alkyl chain with a new peak between 2800 and 2900 cm^{-1} . See in figure below.



BIN Raman IR (top) and BIN-C14Br FTIR spectra show the addition of the alkyl chain with the new peak on the BIN-C14Br spectrum between 2800 and 2900 cm^{-1} .

Microbiology dilution and plating experiments showed that BIN-C18Br and BIN-C14Br are biocidal and effective at 0.01 M while BIN-C8I was biocidal and partially effective at 0.01 M. BIN-C14Br and BIN-C18Br were partially effective at 0.001 M while BIN-C8I was ineffective at 0.001 M. BIN-C14Br and BIN-C18Br were ineffective at 0.0002 M. The results of 0.01 M experiments are shown in figure below.



BIN-C14Br and BIN-C18Br effectively reduce the bacterial cell viability while BIN-C8I is partially effective.

Discussion and Conclusions

From the NMR and FTIR spectra, we have shown successful synthesis of the BIN-C8I, BIN-C14Br, and BIN-C18Br compounds. Tests of the biocides reduction in cell viability of *S. aureus* show the BIN-C14Br and BIN-C18Br are potent biocides. The difference in efficacy of the medium/long chain biocides (C14 and C18) and the short chain biocides (C8) is expected. The short chains are hypothesized to interact less with the bacteria cell membrane than the medium/long chains, and the short chain biocides thus cause less cell membrane lyses than the medium/long chain biocides. BIN-C14Br and BIN-C18Br are good candidates for attachment to a hydrogel polymer backbone.

We researched several hydrogels as potential candidates for attachment of these biocides. We looked for a versatile hydrogel that has been used in many applications as well as for a hydrogel whose chemistry lends itself to the attachment of the biocides' via

their hydroxyl end groups. Chemically-crosslinked poly(ethylene glycol) (PEG) hydrogels are used in drug delivery and biosensor coating applications. These applications are susceptible to biofouling and bacterial infection, and the hydrogels performance in these applications would benefit from the addition of antimicrobial groups. Several research groups have chemical-crosslinked the hydroxyl end groups of PEG chains with diisocyanates. This synthetic route lends itself to addition of the biocides in the crosslinking step, substituting the biocide for some of the triol that is added to promote branching.

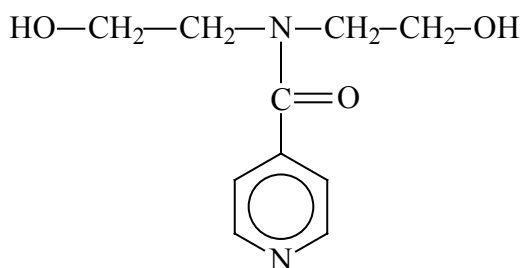
APPENDIX B: POLY(ETHYLENE GLYCOL)-POLYURETHANE HYDROGELS

Several different synthesis strategies were tried before deciding on free radical copolymerization. Specifically we tried incorporation of a tertiary amine into a polyethylene glycol (PEG) network using polyurethane chemistry. The experiments related to this alternative strategy are document here.

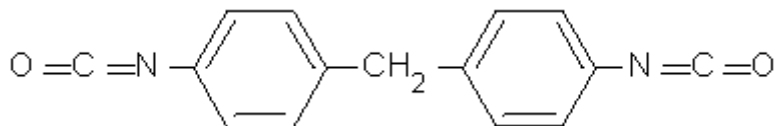
Materials

Poly(ethylene glycol) polyurethanes were synthesized from N,N-bis(2-hydroxyethyl) isonicotinamide (BIN), 4,4'-methylenebis(phenyl isocyanate) (MDI), PEG ($M_n \sim 600$ g/mol). Hydrogels were also synthesized with these reactants plus a triol as a crosslinker and benzyl chloride as an inhibitor. The reaction was performed in dimethylacetamide (DMAc). In polyurethane reactions, dibutyltin dilaurate was used as a catalyst. All chemicals were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. The chemical structures of each follow.

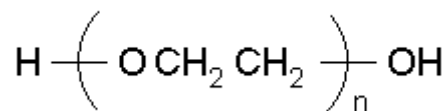
N,N-bis(2-hydroxyethyl) isonicotinamide (BIN)



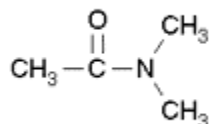
4,4'-methylenebis(phenyl isocyanate) (MDI)



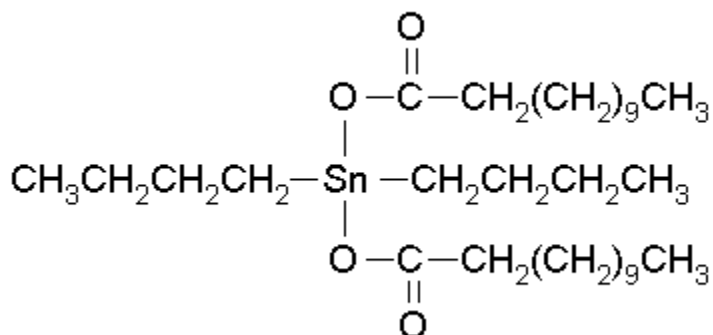
Poly(ethylene glycol) (PEG)



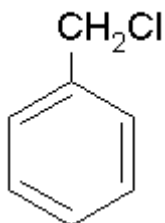
Dimethylacetamide (DMAc)



Dibutyltin Dilaurate



Benzyl Chloride



Synthesis (Each run is represented by a bullet point)

- PEG-polyurethane hydrogels were synthesized by an all-in-one pot method. Used appropriate molar ratios of each reactant: PEG:Triol:BIN:MDI of 3:1:1:5.5. PEG

and triol were mixed and dried at 100°C under vacuum for 24 h to remove water. BIN was dissolved in DMAc at 70°C. PEG and triol mixture were heated to 70°C in scintillation vial in oil bath. Added BIN in DMAc. Added solid MDI. As MDI dissolved and mixed, network began to form. Gelation happened in 10 seconds. Solid after 1 minute. Tried the same setup, but added 2 drops of benzyl chloride as an inhibitor. Gelation occurred in 1 min. Spread gel into Teflon mold, and put mold in vacuum oven. Bubbles formed and gel foamed under vacuum, probably due to water in the reactants. Solid gel never formed, most likely due to too much inhibitor and too much water in the reactants.

- Try splitting reaction up into two steps: addition of BIN to PEG with MDI to make a polyurethane and subsequent crosslinking of the polyurethane. This was a series of reactions where individual variables were changed to tweak the synthesis. Here is the general protocol that was developed. PEG:BIN:MDI is 1:1:2. PEG and BIN were separately dried at 70-80°C under vacuum for 24 h. PEG was dissolved in anhydrous DMAc at 5% w/v at room temperature in a 3-neck round bottom flask with condenser, nitrogen purge, heating mantle, and mechanical agitator. Polymer mixture was heated to 40°C. MDI was dissolved in a small amount of DMAc (10 mL) and injected through rubber septum into reaction vessel. Catalyst was dissolved in DMAc to give 300 ppm catalyst based on amount of PEG. Catalyst was injected. Temperature was raised to 60°C and reacted for 1 h to attach MDI to PEG. Temperature was reduced to 40°C. BIN dissolved in DMAc (10 mL) was added drop-wise over approximately 2 h. If gelation of the reaction mixture began, additional DMAc was added, and addition

of BIN to the reaction vessel was slowed. (Gelation was the biggest problem I had. Nina Lambda gave some insight into this. Below is an email from her for future reference.) Once all BIN was added the reaction temperature was raised 60°C for 2 h and 80°C for overnight. Polymer was precipitated in water (water = 10 x amount of polymer solution). Bulk of water was evaporated in fumehood. Once a small amount of water was left, water and polymer were poured into a Teflon evaporation dish and dried under vacuum. A solid light yellow translucent polymer film was left. In future experimentation, I would suggest stripping water out of the reaction mixture with toluene and then removing toluene prior to addition of MDI. I would also suggest more robust water removal from BIN, perhaps via re-crystallization and vacuum drying. I think these two things would further reduce gelation problems.

- From Dr. Nina Lambda (2/12/04):

Gelation is the biggest problem you will face when using amines in polyurethane synthesis. ...try:

- 1) Increase the dilution of reactants in your flask - to 5% or lower if need be.
- 2) Lower the temperature even further - say 50-55°C.

You are on the right track to add the chain extender slowly.

One thing to be careful of though is the amount of moisture in the reaction. Although your polyol may be vacuum dried, the solvents usually contain small amounts of water, which can react with the isocyanate, and alter the ratio. For this reason, we usually dried the polyol and solvent together by stripping with toluene, rather than vacuum dry. Polymer properties are usually optimal when there is a slight excess of isocyanate (say 1-2%).

If your MDI is clear in DMAc, that's fine. If it is cloudy in DMAc, then it has dimerized, and this will also affect your reaction.