Effects of swelling on the mechanical properties of a pH-sensitive hydrogel for use in microfluidic devices

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Available online 24 December 2003

Abstract

Tensile tests were conducted on a 2-hydroxyethyl methacrylate (HEMA)–acrylic acid (AA) comonomer gel sensitive to variations in environmental pH. Mechanical properties (Young’s modulus, Poisson’s ratio, ultimate tensile strength, and percent elongation at break) were determined at different levels of swelling equilibrium. Shifts in ultimate tensile strength (from 300 to 60 kPa) and percent elongation at break (from 150% to 30%) were observed between samples tested in the swollen and unswollen states. Additional tests were conducted to determine the effects of crosslinking and polymerization conditions on mechanical properties. Increasing the crosslinker component from 0.2% to 1% resulted in an increase in Young’s modulus (34 to 380 kPa) by an order of magnitude. It is found that adjustment of the crosslinker component of the hydrogel is an effective method of controlling mechanical properties.

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Keywords: Hydrogel; Smart materials; Mechanical properties

1. Introduction

There is a growing interest in developing engineered actuation systems that have properties more in common with soft biological materials, such as muscles and tendons, than with traditional engineering materials. In an aqueous environment, stimuli-responsive hydrogels undergo a reversible phase transformation that results in dramatic volumetric swelling and shrinking upon exposure and removal of a stimulus. Thus, these materials can be used as muscle-like actuators [1], fluid pumps [2], and valves [3]. Interest in hydrogels has gained momentum recently because these materials can be actuated by a variety of stimuli such as pH [4], salinity [5], electrical current [6], temperature [7], and antigens [8].

Since the rate of swelling and shrinking in a hydrogel is diffusion-limited, the temporal response of macroscale hydrogel structures to a given stimulus is typically measured on time scales of hours or days. However, by reducing the size of hydrogel structures to the microscale, volumetric transitions occur within minutes or even seconds [9]. The favorable scaling of hydrogel dynamics has been the essential element in the development of microfluidic devices that employ hydrogel valves for flow control. One benefit of these devices is that they are autonomous and require no external power source.

Our research considers a 2-hydroxyethyl methacrylate (HEMA)–acrylic acid (AA) comonomer gel applied to the autonomous control of flow in microfluidic devices. HEMA–AA-based hydrogels are sensitive to the pH of their aqueous environment; expanding at high pH and shrinking at low pH. Microfluidic devices, designed to sense specific characteristics or components of the fluid within their channel networks, contain “intelligent” valves and actuators constructed from hydrogels that exhibit swelling phenomena in response to changes in local conditions. Generally, hydrogel structures must withstand forces imposed by the flow of aqueous solution and the constraint of device walls during swelling. However, specific actuation applications utilize a thin (30–50 \( \mu \text{m} \)) elastomeric membrane deflected by the hydrogel to control fluid flow in adjacent channels [10, 11]. Therefore, structures of this type must also endure the restoring force of...
the elastic membrane as well. Optimizing the design of these devices demands an understanding of how the mechanical properties of the gel change with its surrounding solution.

To assist device designers, material properties must be known in order to ensure functional and reliable structures. Additionally, there is a strong desire to better understand the underlying mechanisms for swelling in hydrogels so that more predictive models can be developed to guide the design process [12,13]. De et al. [12] have developed a model that is able to predict the degree of equilibrium swelling as well as the dynamic swelling behavior of cylindrical hydrogels. Their model utilizes coupled chemical diffusion and mechanical equations that require material properties like Young’s modulus and Poisson’s ratio to calculate the osmotic pressure within the gel that is created by the diffusion of ions. Therefore, determining to what extent, if any, the mechanical properties change as the hydrogel structures swell is of critical importance to the effectiveness of predictive models.

A number of mechanical testing techniques have been employed to study the behavior of hydrogels [1,14–19]. However, due to the specialized nature of hydrogel applications (e.g. artificial tissue replacements for ligaments [19], muscles [1], and cartilage [14]), it is often the functional behavior and not the fundamental material properties that are determined. When developing hydrogels intended to replace biological tissues, it is necessary to design test protocols that mimic the natural loading conditions that occur in the body. For comparative purposes, these protocols are often dictated by previously developed techniques for testing biomaterials. For example, Stammen et al. [14] used shear and unconfined compression tests to determine the functional compatibility of a poly(vinyl alcohol) (PVA) hydrogel with human articular cartilage. Tests of this nature are critical for compatibility studies, but are not necessarily ideal for determining material constants. Compression tests, in particular, are not a reliable method for measuring Young’s modulus due to specimen end-effects that must be considered [20].

Previous hydrogel studies have explored how variations in polymer concentration affect mechanical properties [21–24]. However, there is limited experimental information available regarding the effects of swelling on fundamental material properties like Young’s modulus and Poisson’s ratio for hydrogels [1,16,25]. Poisson’s ratio, in particular, has only been measured directly by a few researchers [16,26–28]. In 1980, Geissler and Hecht [29] measured the elastic modulus and shear modulus of a polyacrylamide (PAAm) gel to determine the microscopic Poisson’s ratio, where only the deformation of single polymer chains is considered. This is beyond the scope of our study since the behavior of the entire hydrogel structure, not its microstructure, is of interest. Li et al. [26] demonstrated a method for measuring Poisson’s ratio in thin PAAm films; however, precise thickness measurements (to within 5 μm) are required to obtain consistent results. Hirotsu [27] and Urayama et al. [28] employed optical methods to measure the transverse strain of the gel sample boundaries while Marra et al. [16] used ink markers to track gel deformations (a technique similar to the one used in our previous study [30]).

Experimental results obtained from tensile tests conducted on a 2-hydroxyethyl methacrylate (HEMA)–acrylic acid (AA) coemonomer gel sensitive to variations in environmental pH are reported below. These results are compared to experimental data obtained for similar gels and existing theoretical models below.

2. Materials and methods

2.1. Hydrogel composition

The hydrogel prepolymer mixture consists of 2-hydroxyethyl methacrylate (HEMA) monomer, acrylic acid (AA) monomer, ethyleneglycol-dimethacrylate (EGDMA) cross-linker, and 2,2-dimethoxy-2-phenyl-acetophenone (DMPA) photoinitiator. All chemicals were purchased from Sigma-Aldrich. A vacuum distillation process was used to remove inhibitor from the HEMA monomer while the remaining chemicals were used as received. The constituent amounts were determined by weight in an analytical balance and were mixed ultrasonically for 20 min. The prepolymer mixture was stored (under refrigeration) in an amber bottle to protect against unwanted exposure to ultraviolet (UV) light.

Experiments designed to investigate the effects of swelling on mechanical properties employed a hydrogel composed of 84 wt.% HEMA monomer, 11 wt.% AA monomer, 3 wt.% DMPA photoinitiator, and 1 wt.% crosslinker. This composition produced a pH-sensitive hydrogel that swells in basic solutions.

A second set of experiments, designed to determine the influence of crosslinking on hydrogel properties, used a modified form of the hydrogel chemistry mentioned above. Weight quantities of HEMA, AA, and DMPA were held constant while the quantity of crosslinker (EGDMA) added to the mixture was varied to produce hydrogel chemistries composed of 0.2, 0.5, 1, 2, 3, 4, 5, 10, and 20 wt.% crosslinker.

2.2. pH buffer solutions

Phosphate buffer solutions of 0.2 M ionic strength were used to induce swelling in the hydrogel. Solutions ranging from pH 2 to 4 were made from NaH₂PO₄, HCl, and NaCl; pH 5 through 9 solutions consisted of NaH₂PO₄, Na₂HPO₄, and NaCl; solutions of pH 10 and 11 were made from Na₂HPO₄, NaOH, and NaCl; and pH 12 buffer was made from Na₂HPO₄ and NaOH. All
2.3. Sample fabrication

Recent developments in micro-fabrication techniques have proven to be an efficient and cost-effective method of creating complex three-dimensional microchannels in microfluidic devices [31]. Similar techniques were employed in the fabrication of tensile specimens for this study. A compression molding process, as described by Jo et al. [31], was used to produce a polydimethylsiloxane (PDMS) stencil which acted as a mold for the prepolymer solution during polymerization. Because the polymerization process is sensitive to oxygen, the polymerization must be performed in a sealed mold. Polymerization took place under a Novacure UV source that delivered ultraviolet light at a wavelength of 365 nm and an intensity of 15 mW/cm² for 2 min. The samples were washed with methanol and deionized water and were stored in the appropriate pH solution prior to testing. The resulting samples had a 5.5-mm gage length, 1-mm neck width, and an approximate thickness of 500 μm (data presented in Fig. 1 resulted from 200-μm-thick samples).

2.4. Experimental setup

An Instron Model 5548 MicroTester, equipped with a 10 N load cell, was used for measuring the mechanical properties of the hydrogel. The position of the screw-driven actuator could be determined to within ±6.0 μm over 100 mm of travel, while loads could be measured to within ±0.25% of the indicated force. An environmental chamber, capable of holding a phosphate buffer solution at a constant temperature, was added to the MicroTester such that experiments could be conducted in an aqueous environment. Custom grips were constructed to handle the potentially corrosive buffer solutions used in the experiments. Self-aligning grip faces were clamped to the sample by a screw-tightened mechanism. Further details of sample fabrication and experimental techniques are published elsewhere [30].

3. Experimental results

In accordance with ASTM D 638-99 [32], a set of at least five samples was tested for each parameter of interest. The resulting experimental data was averaged and standard deviations were calculated for each sample set. Unless otherwise specified, all experimental results are presented in graphical form as follows: data points signify the average value of five samples while error bars represent the standard deviation of the measurement. Due to grip effects, some samples did not fracture within the gage section leading to unusable results. Thus, certain data points in ultimate strength and percent elongation at break graphs may con-
tain as few as two samples. The temperature of the test solution was held at 24 ± 1 °C for all experiments.

3.1. Influence of swelling on mechanical properties

A constant crosshead displacement rate of 5 mm/min was used for all tests reported to determine the tensile properties of the hydrogel as a function of solution pH. The rate of displacement produced fracture in the specimen within 1/2 to 5 min of test initiation, as suggested by ASTM D 638-99 [32]. Tensile samples were allowed 24 h to reach equilibrium with their respective pH solution prior to testing.

Under fully hydrated conditions, both the unswollen and swollen gels exhibit linear stress/strain behavior to failure (to within $R^2$ of 0.94). Young’s modulus was calculated from the slope of the engineering stress–strain curve within the region of 10% strain, where axial strains were determined from crosshead position. Cross-sectional areas for stress calculations were measured with an optical microscope and image analysis software (Metamorph version 4.6r6) after each test was completed. All strain measurements were calculated from the initial, stress-free state the gel assumes after reaching equilibrium in its respective pH solution. Therefore, only strains due to mechanical deformation, and not swelling, were considered in the determination of Young’s modulus. Fig. 1 shows how the modulus of the hydrogel changes for samples tested in a higher pH environment.

Swelling had a more pronounced affect on the amount of stress and strain the hydrogel specimen could withstand up to the point of fracture. Figs. 2 and 3 depict a significant reduction in both ultimate tensile strength and percent elongation at break as the pH of the surrounding solution increases. Because polymer chain disentanglement occurs with gel swelling and strains were measured after swelling, less mechanical strain was required to elongate the swollen gel to the point of failure. These results are summarized in Table 1.

3.2. Influence of crosslinking on mechanical properties

Young’s modulus, ultimate strength, and percent elongation at break were also determined experimentally for
hydrogel chemistries with varying quantities of crosslinker (EGDMA) component. The experimental protocol was identical to that used in the aforementioned Young’s modulus tests with the exception of the sample thickness, which was 500 μm. All crosslinking experiments were conducted on swollen hydrogels tested in pH 7 solution.

Figs. 4 and 5 show how both Young’s modulus and ultimate strength increase with increasing crosslinker concentration, respectively. For clarity, inset graphs reveal the initial portion of each data set in detail. Percent elongation at break, however, decays markedly as polymer crosslinks increase (Fig. 6). These results are summarized in Table 2.

### 3.3. Influence of polymerization time on mechanical properties

An additional study was undertaken to investigate the affect of polymerization time on the elastic modulus. Representative data are shown in Fig. 7 and Table 3 for samples that were exposed to UV light for durations varying from 1

<table>
<thead>
<tr>
<th>Wt. % crosslinker</th>
<th>Average cross-sectional area (mm²)</th>
<th>E (MPa)</th>
<th>UTS (MPa)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>1.92</td>
<td>0.03 ± 0.004</td>
<td>0.05 ± 0.03</td>
<td>146 ± 63</td>
</tr>
<tr>
<td>0.5</td>
<td>1.54</td>
<td>0.11 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>147 ± 13</td>
</tr>
<tr>
<td>1</td>
<td>1.25</td>
<td>0.38 ± 0.04</td>
<td>0.33 ± 0.14</td>
<td>85 ± 30</td>
</tr>
<tr>
<td>2</td>
<td>1.09</td>
<td>0.83 ± 0.05</td>
<td>0.73 ± 0.17</td>
<td>95 ± 25</td>
</tr>
<tr>
<td>3</td>
<td>0.98</td>
<td>1.48 ± 0.10</td>
<td>0.82 ± 0.31</td>
<td>60 ± 23</td>
</tr>
<tr>
<td>4</td>
<td>0.86</td>
<td>2.16 ± 0.08</td>
<td>0.79 ± 0.05</td>
<td>37 ± 1</td>
</tr>
<tr>
<td>5</td>
<td>0.71</td>
<td>4.17 ± 0.48</td>
<td>1.26 ± 0.47</td>
<td>30 ± 12</td>
</tr>
<tr>
<td>10</td>
<td>0.61</td>
<td>10.7 ± 0.1</td>
<td>1.55 ± 0.64</td>
<td>15 ± 6</td>
</tr>
<tr>
<td>20</td>
<td>0.44</td>
<td>124 ± 15</td>
<td>14 ± 5</td>
<td>22 ± 10</td>
</tr>
</tbody>
</table>

All samples were tested in pH 7 buffer solution.

Results presented in Fig. 1 for the HEMA-based gel studied here show a slight decrease in modulus with increased swelling. It has also been shown experimentally that Young’s modulus tends to decrease with increased swelling for both polyacrylonitrile (PAN) [1] and PAAm [25] gels.

The largest span in average modulus occurred between samples tested in pH 4 and pH 12 solutions. An unswollen sample tested in pH 4 exhibited a modulus of 0.31 MPa while a swollen sample tested in pH 12 had an average modulus of 0.18 MPa, which translates to a maximum decrease of 42%. In the unswollen state, polymer chains within the gel structure are highly entangled and resistant to deformation; whereas, in the swollen gel structure, the polymer chains have become somewhat disentangled due to the influx of buffer ions and water. From a microstructural viewpoint, one would expect the stiffness of individual polymer chains to remain constant regardless of the degree of swelling. However, additional mechanical work is required to deform the entangled network of polymer chains in unswollen gels where frictional and electrostatic effects are more pronounced.

The significance of the decay in stiffness with swelling is dependent on the application. It is likely that a hydrogel of this type would not be ideal for structural applications (e.g. artificial tissue replacements) due to its compliance and phase transformation ability. However, this stiffness/swelling relationship may be an important design advantage for microfluidic and drug-delivery applications where hydrogels actuate upon exposure to a stimulus.

Our prior experiments reveal an insignificant change in the average Poisson’s ratio measured in unswollen and swollen hydrogels with an average value of 0.43 for the equilibrium testing conducted [12]. Urayama et al. [28] measured Poisson’s ratio in a PVA hydrogel at four distinct polymer concentrations. Their resulting values ranged from 0.426 to 0.447, which agree well with values measured in our previous study [30]. This particular HEMA–AA gel has constant crosslinking with only the fixed charge changing as
pH changes, therefore, it is reasonable to conclude that Poisson’s ratio would have a similar value in both the swollen and unswollen states. Because water is the dominant constituent in hydrogel structures, one would expect the Poisson’s ratio of hydrogels to approach the theoretical limit of 0.5, implying material incompressibility.

Extreme shifts in Poisson’s ratio have been observed in temperature sensitive N-isopropylacrylamide (NIPA) gels over a small temperature range (approximately 2 °C) [27,34]. Li et al. [34] and Hirotsu [27] both demonstrated a dramatic shift in Poisson’s ratio to negative values in NIPA gels near the phase transition point. Li et al. [34] also showed that the shift did not occur in PAAm gels and Poisson’s ratio remained positive throughout the phase transition region. Whether or not a pronounced variation in material properties near the point of phase transition occurs is still in question however. Experiments to date have been at equilibrium, thus, additional mechanical testing focused on the transition region would be recommended to determine the existence of a phenomenon of this sort.

A considerable decrease in both ultimate strength (Fig. 2) and percent elongation at break (Fig. 3) was demonstrated in swollen hydrogels as compared to unswollen gels. Both figures provide information that contributes to the definition of a domain for the failure properties of the hydrogel. Figs. 2 and 3 also show distinct differences in experimental error between unswollen and swollen hydrogels. A preconditioning regimen is often prescribed when testing hydrogels [16] and biomaterials [35] to allow the internal structure of the material to reach a steady state prior to testing. Therefore, if the unswollen hydrogel were preconditioned such that the internal polymer chain entanglements achieved a steady state, experimental error may be favorably reduced.

Figs. 4–6 illustrate a nonlinear relationship between the degree of crosslinking in the hydrogel and Young’s modulus, ultimate tensile strength, and percent elongation at break, respectively. Increasing the number of crosslinking sites in the polymer network makes the hydrogel more resistant to deformation thereby inhibiting the swelling ability of gel. The increased resistance to deformation translates into increased Young’s modulus and ultimate strength, as well as decreased percent elongation at break. Only the initial portion (0.2–5%) of the curves is of interest for actuator applications since hydrogel swelling diminishes with increased crosslinking. When used in conjunction with equilibrium swelling data, this information will contribute to the beginnings of a library of design parameters that will allow microfluidic device designers to choose the optimal hydrogel chemistry for specific material function within a component application.

As demonstrated by the results presented in Fig. 7, the elastic modulus of a hydrogel is also affected by the polymerization process. Photo Differential Scanning Calorimetry (Photo-DSC) experiments on a HEMA gel of similar composition showed that there is a peak time during polymerization when the reaction rate is maximum and the reaction reaches saturation [33]. As the reaction rate increases, an increase in the conversion of monomer to polymer is expected. This would also imply an increase in cross-linking and thus modulus. Such a trend is represented in Fig. 7 for values of Young’s modulus corresponding to polymerization times of 1, 2, and 4 min. The modulus appears to reach a maximum at a polymerization time of four minutes, after which it begins to decay. Photo-DSC experiments have also reported a peak in the reaction rate between 2 and 4 min of UV exposure [33].

After peak cross-linking is achieved, an apparent decay in Young’s modulus is observed for polymerization times of 6 and 10 min. Because the conversion of monomer to polymer should cease once the reaction stalls, the modulus would be expected to maintain its peak value upon prolonged exposure to UV. However, in the presence of UV, oxygen forms ozone that can subsequently form oxygen radicals. The oxygen radicals have the ability to break bonds, thereby causing a decrease in the modulus.

5. Conclusions

The extent to which the mechanical properties of a pH-sensitive hydrogel change with increasing pH (swelling) and crosslinking was presented. It was demonstrated that the mechanical properties of this hydrogel are strongly dependent on the degree of equilibrium swelling. Changes in polymerization conditions (i.e. varying polymerization time while maintaining constant UV intensity) were also shown to affect the elastic modulus of the HEMA–AA gel. Adjusting the crosslinker component of the hydrogel is an effective method of controlling mechanical properties. The influence of monomer components should be studied as well. With precise control over mechanical and swelling characteristics, microfluidic device designers will have the knowledge required to produce optimal hydrogel valves and sensors.

Acknowledgements

This research was sponsored by DARPA-MTO (#F33615-98-1-2853) and DARPA, AFRL, Air Force Command, and USAF under agreement #F30602-00-1-0570. The authors would also like to thank Sudipto K. De, Narayan Aluru, David Eddington, Glennys Mensing, and Jaisree Moorthy for the helpful discussions and technical assistance.

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