Polymer 51 (2010) 2207–2214

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Tailoring the mechanical properties of polyacrylamide-based hydrogels

Bryan A. Baker[a], Rebecca L. Murff[b], Valeria T. Milam[a,b,c,*]

[a] *School of Materials Science & Engineering, Georgia Institute of Technology, 771 Ferst Dr. NW, Atlanta, GA 30332-0245, USA*
[b] *Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA*
[c] *Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, GA 30332, USA*

ARTICLE INFO

Article history:
Received 14 December 2009
Received in revised form
10 February 2010
Accepted 13 February 2010
Available online 18 February 2010

Keywords:
Hydrogel
Rheology
Polyacrylamide

ABSTRACT

Here, we studied the mechanical properties of polyacrylic acid-co-polyacrylamide (pAAc/pAAm) and polyacrylate-co-polyacrylamide (pNaAc/pAAm)-based hydrogels as a function of composition and total polymer content. While both polyacrylate and polyacrylic acid are weakly-charged polyelectrolytes with carboxylate side groups, they exhibit different behavior as copolymers with polyacrylamide. In swelling studies the highest degree of volumetric expansion occurs in the polyacrylate-rich hydrogels. Rheological measurements indicate that the shear storage modulus of the hydrogels typically increases with the percentage of polyacrylamide for a given polymer volume fraction; however, the simultaneous strengthening and embrittling effects of polyacrylamide as a copolymer are more dramatic for the pNaAc/pAAm hydrogels.

1. Introduction

Hydrogels are used in bio-related applications ranging from cellular supports [1–3] to matrices for electrophoretic separation of biological macromolecules [4]. Hydrogels are typically water-swollen networks of hydrophilic polymer [5] and thus exhibit viscoelastic behavior of both a liquid and a solid. Polyacrylamide (pAAm) is a particularly popular hydrogel material for cell-based studies [6–11]. The hydrophilic and semipermeable nature of pAAm is advantageous for mimicking the extracellular matrix (ECM) surrounding cells and tissue; however, the application of pAAM as an ECM mimic is limited by the relatively compliant mechanical properties of this viscoelastic material. While reported values for the elastic (Young's) modulus of biological tissue and matrices can range from 0.6 MPa for elastin to 10 GPa for bone [12], polyacrylamide, on the other hand, has a relatively low shear elastic modulus value of ~10 kPa or less [13]. In both acrylamide-based as well as other hydrogel systems, a number of variables have been explored to alter the modulus including cross-linker concentration [13], chemical composition [14], polymer concentration [15,16], and temperature processing conditions [13]. Variations in the hydrogel composition can be achieved through the simultaneous copolymerization of different monomers or by forming interpenetrating networks of different homopolymer or copolymer chains [2,17]. While techniques such as AFM can provide local measures of stiffness [18,19], rheology is a well known characterization tool that has been applied to a diverse array of polymer systems [20,21] and that allows for careful control of experimental variables such as temperature, shear stress, and frequency. Additionally, one can load the initial liquid phase of a monomer solution and monitor the changes in mechanical properties before, during, and after polymerization [13,22,23].

Here, we present a systematic study of the mechanical properties of acrylamide-based hydrogels as a function of polymer composition and volume fraction using oscillatory rheology as our primary characterization tool. Acrylamide was co-polymerized with either acrylic acid or sodium acrylate to impart a net negative charge on the hydrogel. The goal of this study was to explore the range of stiffness values possible with hydrogel material alone. In separate studies, we have employed these hydrogels as the matrix for colloid-hydrogel composites in which positively-charged colloidal particles act as an interactive filler to increase stiffness of the hydrogels [24]. Additionally, the presence of carboxylate groups on the anionic copolymer can serve as convenient functional groups for coupling other molecules or macromolecules to the hydrogel network. Since the hydrogel is polymerized *in situ* on the rheometer stage prior to rheological characterization, separate swelling and pH studies were also undertaken to better understand the role that, for example, residual monomer may play in the behavior of these hydrogels.

---

* Corresponding author. School of Materials Science & Engineering, Georgia Institute of Technology, 771 Ferst Dr. NW, Atlanta, GA 30332-0245, USA. Tel.: +1 404 894 2845; fax: +1 404 385 3734.
E-mail address: valeria.milam@mse.gatech.edu (V.T. Milam).

0032-3861/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2010.02.022

2. Experimental methods

2.1. Materials

All chemicals for hydrogel preparation were purchased from Sigma (St. Louis, MO) unless otherwise noted. *N*,*N*′-methylenebisacrylamide (BIS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were used as-received as the cross-linker and accelerator species, respectively. DIamond nanopure water (Barnstead International) was used to prepare all initiator and buffer solutions. Phosphate buffered saline (PBS) was diluted from a 10× concentration to a 1× concentration. Sodium acrylate and acrylamide were dissolved in PBS to prepare either 30 or 40 wt% monomer solutions. Acrylic acid was vacuum distilled (17 mbar) in an oil bath at 60–70 °C to remove the inhibitor, monomethyl ether hydroquinone (MEHQ). Ammonium persulfate (initiator) was prepared as a 10 wt % solution. For pH studies, 7 M NaOH buffer solution was prepared using sodium hydroxide pellets from JT Baker (Phillipsburg, NJ).

For swelling studies, cell culture media was prepared using bovine calf serum, trypsin/EDTA, penicillin/streptomycin, Dulbecco's Modified Eagle Medium (DMEM), and PBS purchased from American Type Culture Collection (ATCC) (Manassas, VA). The serum preparation is based on ATCC's “General Protocols for Serum Use.” The bovine calf serum was heat inactivated by submersion into a 56 °C water bath for 30 min and then separated into aliquots for storage at −20 °C. The cell culture media was then prepared with a composition of 90 vol% DMEM, 10 vol% bovine calf serum, and 10 mL penicillin/streptomycin. The mixture was vacuum filtered and stored at 4 °C.

2.2. Hydrogel synthesis

Polyacrylic acid-*co*-polyacrylamide (pAAc/pAAm) and polyacrylate-*co*-polyacrylamide (pNaAc/pAAm) copolymers were prepared from either distilled acrylic acid or monomer solutions of sodium acrylate and acrylamide at a fixed hydrogel volume. While the ratio of monomers and the total polymer volume fraction were varied in this study, the BIS cross-linker was fixed at a value of 0.24 vol% (based on total hydrogel volume and volume of pure BIS liquid). For the polymerization step, 50 μL of 10 wt% ammonium persulfate solution and 0.2 vol% TEMED were used per mL in the acrylic acid-based hydrogels. For pure pAAm as well as pNaAc/pAAm hydrogels, 10 μL of ammonium persulfate solution was used per mL of pre-polymerized solution. After mixing the monomer solutions with BIS cross-linker, the monomer and initiator solutions were degassed separately via nitrogen bubbling (1 min and 5 min, respectively) to reduce oxygen content in the solutions. It has been previously reported that oxygen scavenges the primary radicals generated by the initiator reaction, but their inhibitory effects on polymerization can be decreased by evacuating oxygen from the system [25]. While still in liquid form, 0.140 mL of the mixed solution was loaded onto the sample stage of the rheometer. The cone was then lowered to a separation distance or gap setting of 0.052 mm and the sample was then enclosed by a Peltier thermal hood with an evaporation blocker to minimize water loss. The loaded sample was first heated to 50 °C for 20 min and then cooled to 37 °C for another 15 min prior to measurement. Previous studies have reported heating solutions to 50 °C in order to expedite acrylic acid polymerization [26]. The polyacrylate-based hydrogels polymerized more readily at room temperature but were also heated in the same manner for direct comparison. For rheology measurements of pAAc/pAAm hydrogels of varying pH values, the synthesis was carried out as described above with the exception that a pH measurement of the mixture was taken before initiating polymerization.

2.3. Rheology

Oscillatory rheology was used to characterize the mechanical properties of the hydrogels. Frequency and strain amplitude sweeps were performed on an Anton-Paar MCR 301 rheometer (Anton-Paar USA). Frequency sweeps were conducted for a range of 0.1 rad/s to 100 rad/s at a fixed strain of 0.5%. Strain sweeps were conducted at a frequency of 6 rad/s and a strain range of 0.001–3000% or 0.001–4500%, depending on the sample. Measurements for each composition were done three times using freshly-prepared samples and a cone and plate geometry of 25 mm in diameter (CP-25).

2.4. pH and swelling studies

To determine the effects of polymerized hydrogels on surrounding solution, hydrogels consisting of 100/0, 75/25, 50/50, 25/75, 15/85, and 0/100 monomer volume ratios were prepared for both polyacrylic acid-based and polyacrylate-based hydrogels. Samples for these studies consisted of a total hydrogel volume of 5 mL prepared in 50 mL centrifuge tubes. Prior to initiation, the pH of the monomer mixture was measured with an Orion 3 Star pH Meter (Thermo, Beverly, MA). Following the pH measurement, initiator and TEMED were added and the solution was immediately split into two tubes for a hydrogel volume in each tube of 2.5 mL. The polyacrylic acid-based hydrogels were then allowed to polymerize for 30 min in a hybridizing oven set at 50 °C. Polyacrylate hydrogels were polymerized in a hybridizing oven at a temperature of 37 °C for 20 min. After polymerization, 10 mL of either cell media or PBS was added on top of the hydrogel. The pH of either the cell media or PBS supernatant above the hydrogel was measured once every 24 h over a period of 10 days. After each daily measurement, the supernatant was removed and replaced with 10 mL of cell media or PBS.

For the swelling studies, 1 mL polyacrylic acid and polyacrylate hydrogels were prepared in 10 mL graduated cylinders with 0.1 mL graduations. The same compositions indicated above for the pH studies were also used for this study. Polymerization was carried out at 50 °C for 30 min for polyacrylic acid-based hydrogels and at 37 °C for 20 min for polyacrylate-based hydrogels. The pNaAc/pAAm hydrogels polymerized more readily allowing for lower temperatures to be used. Following polymerization, 1 mL of either cell media or PBS was added to the hydrogels. Every 24 h, any excess cell media or PBS supernatant was removed and a hydrogel volume was recorded. Following each daily measurement, 1 mL of cell media or PBS was then added.

3. Results and discussion

3.1. pAAc/pAAm and pNaAc/pAAm: pH studies

Hydrogel systems are popular as cellular substrate materials. One concern with any synthetic polymer system in a biological environment is the effect of residual reactants and polymerization products on the physiological solution. Unreacted monomers are often cytotoxic and can alter the pH of physiological solutions. Conversely, the effects of the physiological solution on the properties of polymer networks are also an important consideration, especially with regard to swelling in salt solutions [27]. To elucidate these effects, a series of pH and swelling studies was carried out for both the polyacrylic acid-*co*-polyacrylamide and polyacrylate-*co*-polyacrylamide hydrogels using either cell culture media or PBS as the solution media. Since the supernatant was changed daily following each measurement, we expect the effects of leftover monomer and soluble by-products to become less pronounced over time with each wash step.

Plots of the supernatant pH as a function of time are shown in Fig. 1a and b for the polyacrylic acid-*co*-polyacrylamide system at varying compositions or ratios of the copolymers. The initial pH of each mixture was taken prior to polymerization at day 0 while all subsequent measurements were taken from the supernatant. As shown in Fig. 1a and b for cell media and PBS respectively, all mixtures containing polyacrylic acid initially exhibited a low pH of 2–3 compared to the pH of cell media alone (7.6 ± 0.1), PBS alone (7.5 ± 0.1), or any supernatant derived from pure polyacrylamide hydrogel (7.4). While the pH values of supernatant derived from pure polyacrylamide (0/100 pAAc/pAAm) remained relatively constant for the ten-day study, the pH of all the copolymer systems increased steadily until day six when a plateau in pH values was reached. This slow rise in pH is attributed to the gradual removal of residual acidic monomer (acrylic acid) from the hydrogel network and supernatant. Notably, for any given time hydrogels with a higher percentage of pAAc had lower pH values, even at the end of the study when the presence of residual monomer is less likely. A separate, focused rheological study of the effects of monomer solution pH on the mechanical properties of pAAm/pAAc hydrogels showed no significant difference in the elastic storage modulus as a function of pH (see Supplementary Information, Fig. S1).

The supernatant pH studies were also carried out for polyacrylate-based hydrogels as shown in Fig. 1c and d. Unlike the relatively acidic polyacrylic acid-based hydrogels with high hydronium ion content, the initial pH of polyacrylate mixture at day 0 is approximately 10.3. This higher pH value is attributed to Na^+ counterions from acrylate groups that effectively reduce the concentration of H^+ ions in the supernatant. As residual sodium acrylate monomer is removed during each wash and the system approaches equilibrium, more hydronium ions are formed and thus the pH is lowered. In contrast to the acrylic acid system, each exchange of supernatant with either fresh cell media (Fig. 1c) or PBS (Fig. 1d) in the acrylate system results in a gradual drop in pH over the first six days until a plateau is reached at pH 8 for cell media and pH 7.5 for PBS. The initial pH values of hydrogels with higher percentages of pNaAc are further displaced from neutral pH conditions, similar to the pAAc system. This trend for both pAAc and pNaAc-based hydrogels to steadily shift towards neutral pH solution conditions indicates that unincorporated monomers are gradually being leached from the polymer network and removed from the system each time the supernatant is replaced. Compared to the polyacrylic acid-based hydrogels, the initial pH values, however, of the polyacrylate system more closely match that of the cell media.

3.2. pAAc/pAAm and pNaAc/pAAm: swelling studies

Hydrogels used in bio-related applications are typically surrounded by excess liquid such as cell media. As shown by previous studies [15,16], the mechanical properties of hydrogels are largely dependent on the concentration of polymer or polymer volume fraction of the hydrogel network. The polymer volume fraction in a hydrogel, however, may be altered if the hydrophilic polymer

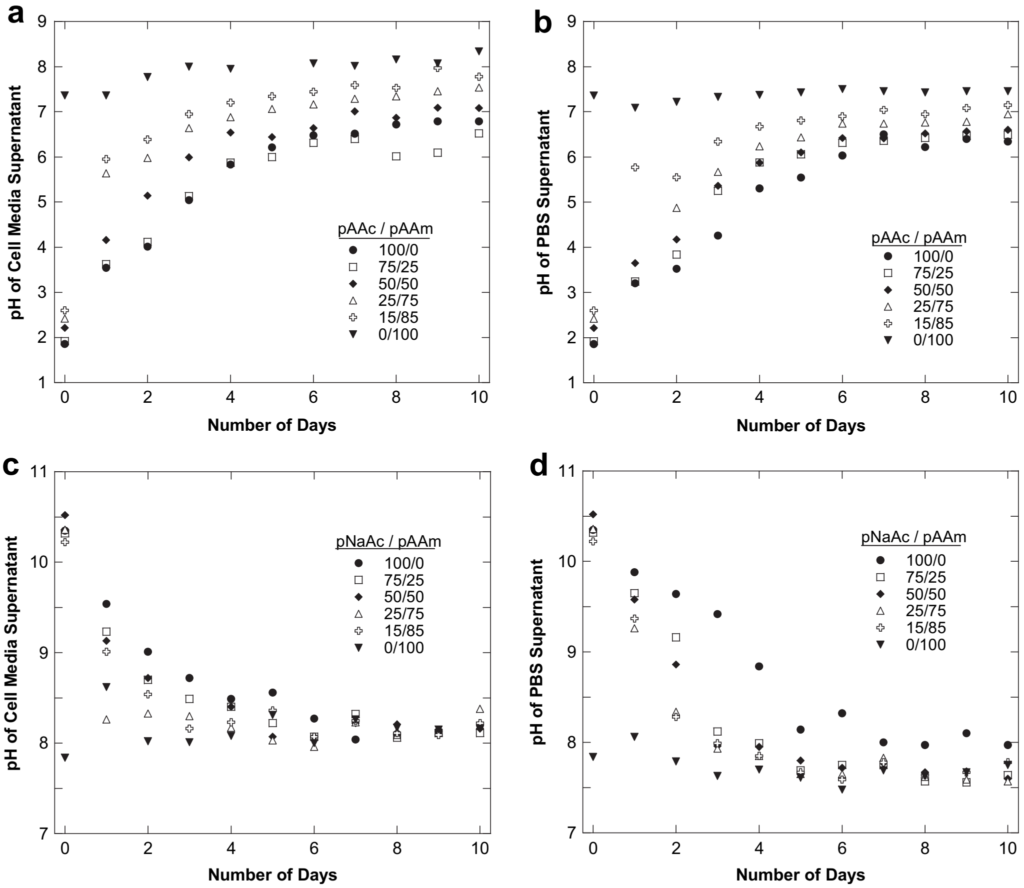

Fig. 1. pH of supernatants as a function of time following polymerization for pAAc/pAAm hydrogels (top) or pNaAc/pAAm hydrogels (bottom) in either cell media (a, c) or PBS (b, d). Hydrogel compositions varied as shown, but total polymer volume fraction remained fixed at 0.1.

network imbibes liquid over time. To examine this effect in pAAc/pAAm and pNaAc/pAAm hydrogels, swelling studies were performed in both PBS and cell media as shown in Fig. 2. With the exception of the pure polyacrylamide hydrogel (0/100 pAAc/pAAm and 0/100 pNaAc/pAAm), both systems exhibited noticeable increases in hydrogel volume over time. Hydrogels with high percentages of polyacrylamide generally showed the least amount of swelling. For polyacrylic acid-based hydogels, there was not any significant swelling for any composition until after day 2 as shown in Fig. 2a and b for cell media and PBS, respectively. Even after several days of supernatant exchange, hydrogel swelling of the pAAc/pAAm system in PBS was relatively modest and did not exhibit a strong dependence on hydrogel composition. At day 10, the extent of swelling was more dramatic in cell media as shown by the 1.4-fold volumetric increase in hydrogels comprised of 15% pAAc or higher. Insight into this delay in swelling is gained by considering the time-dependent changes in the supernatant pH data shown in Fig. 1a and b for pAAc/pAAm systems since days 2 and 3 marked the onset of pH values meeting or exceeding the pK_a of ∼5.2 for polyacrylic acid in salt solutions [28] for all compositions tested. Thus, as solution pH increases, the percentage of dissociated carboxylate groups (COO^-) increases resulting in more electrostatic repulsion along the polymer chains as well as a more hydrophilic hydrogel network that embibes more water.

Compared to the polyacrylic system, the polyacrylate-based hydrogels generally showed more extensive swelling over time as shown in Fig. 2c and d. The most dramatic swelling was observed for the pure polyacrylate (100/0 pNaAc/pAAm) hydrogel with the ∼2.5-fold increase in volume by day 10 in PBS. Swelling for pNaAc hydrogels in either PBS or cell media occurred earlier starting on day 1 (i.e. prior to the first wash) and continued to increase the next two to nine days, depending on composition. A constant value in hydrogel volume was reached more quickly as the percentage of sodium polyacrylate decreased. Unlike the initially acidic conditions of the polyacrylic acid, the relatively higher supernatant pH values of the polyacrylate system are well above the reported pK_a of ∼4.8 for polyacrylate [29] indicating that largely ionized, hydrophilic polyelectrolyte chains are consistently present under the conditions examined here.

In order to facilitate comparison of swelling behavior of the two copolymer systems, the percentage of hydrogel swelling in cell media between days 0 and 10 is plotted as a function of percentage polyacrylamide in Fig. 3. In comparing the polyacrylic and polyacrylate systems, it is evident that the polyacrylate-based hydrogels exhibited more extensive swelling and a stronger dependence on composition as shown by the continual decrease in percent swelling with increasing percentage polyacrylamide. While previous studies have investigated swelling of polyacrylate-based hydrogels [30,31], Okay et al. specifically examined the role of the sodium counterion in the swelling behavior of poly(acrylamide-*co*-sodium acrylate) hydrogels cross-linked with BIS [30]. Na^+ counterions serve to screen the negative charges along polyacrylate and reduce the range of electrostatic repulsion between chains. Results by Okay indicate that increasing the concentration of pNaAc in these hydrogels results in an increase of mobile Na^+ ions inside the hydrogel relative to the supernatant. The ion concentration difference may create an osmotic driving force to dilute the effective Na^+ concentration in the hydrogel by imbibing more

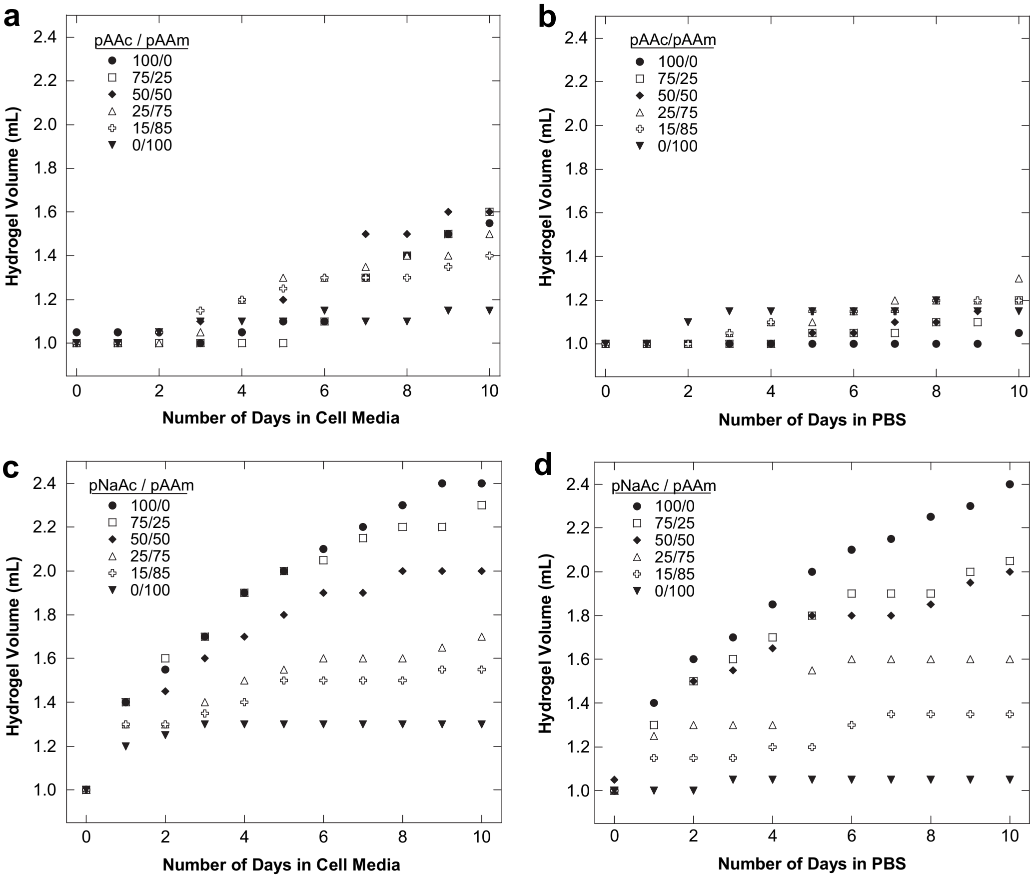

Fig. 2. Total hydrogel volume as a function of time following polymerization for pAAc/pAAm hydrogels (top) or pNaAc/pAAm hydrogels (bottom) in either cell media (a, c) or PBS (b, d). Hydrogel compositions varied as shown, but total polymer volume fraction remained fixed at 0.1.

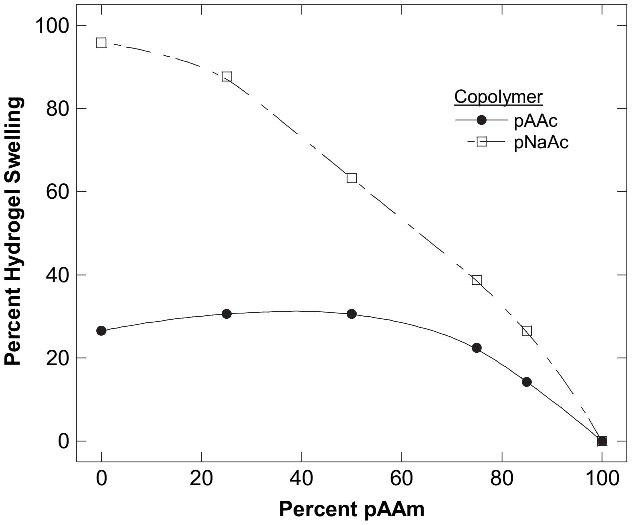

Fig. 3. Percent hydrogel swelling as a function of percent pAAm taken on day 10 for polyacrylic acid-*co*-polyacrylamide and polyacrylate-*co*-polyacrylamide hydrogels in cell media. The data point for the 100% pAAm case represents the average of two measurements. All other data represents a single measurement.

liquid resulting in network swelling [30]. The swelling properties of hydrogels are also dependent on the charge state of the polymer chains [31] which, in turn, can be affected by salt conditions [32]. Subsequently, the ready dissociation of the acrylate into negatively-charged side groups on polymer chains must also contribute to the enhanced swelling behavior observed. In fact, the most important contributor to the swelling may arise from the highly basic conditions of the polyacrylate-*co*-polyacrylamide hydrogels. Tanaka and coworkers used highly basic conditions (pH 12) and 0.4% by volume of TEMED to hydrolyze acrylamide side groups into acrylic acid [33]. Okay et al. also used basic solution conditions and TEMED during their hydrogel preparation [30]. Thus, in the current study acrylamide hydrolysis may have provided additional ionic groups within the hydrogel network resulting in the extensive swelling observed. Unlike the polyacrylate system, the polyacrylic acid-based hydrogels are less likely to be highly charged immediately after polymerization due to the initial acidic conditions. As the supernatant is replaced, however, the pH eventually surpasses the pK_a of polyacrylic acid. Thus, over time the polyacrylic acid groups are likely to dissociate causing repulsive interactions between polymer chains and promoting the swelling of the hydrogel network.

3.3. Rheological studies

As a first step in characterizing the hydrogel stiffness, frequency sweeps were conducted for a range of hydrogel compositions to determine any frequency dependence of the shear modulus values. For all compositions studies the elastic modulus was significantly higher than the loss modulus (see Supplementary Information, Fig. S2 and Fig. S3). Our results for the one component hydrogels also agree with previous reports for pure polyacrylamide and pure polyacrylate hydrogels using BIS as the cross-linking agent [16]. Since the shear storage modulus, G', is the dominant contribution to the complex modulus, G^*, for both the pAAc/pAAm and the pNaAc/pAAm systems in these frequency sweep studies as well as in all our strain amplitude sweep studies, the loss modulus data will not be included in subsequent figures. For strain amplitude sweep studies that followed, the angular frequency was set at an intermediate value of 6 rad/s.

In order to further examine the effects of hydrogel composition on shear storage modulus values, strain amplitude sweeps were performed for hydrogels with a total polymer volume fraction of 0.1, but varying volume percentage ratios of polyacrylic acid to polyacrylamide (pAAc/pAAm) and polyacrylate to polyacrylamide (pNaAc/pAAm). Fig. 4 shows representative strain sweeps for polyacrylic acid and polyacrylate-based hydrogels. Both systems exhibited an increase in G' values in the linear viscoelastic regime with increasing volume percentages of polyacrylamide; however, the polyacrylate-based hydrogels exhibited stronger composition dependence as indicated by the wider range in G' values. For all hydrogels co-polymerized with acrylic acid in Fig. 4a, a slight rise in G' followed by a sudden drop occurs at 10^2 percent strain, independent of the volume percentage of polyacrylic acid. Past studies with stiffer hydrogel systems have attributed this rise in G' values near the upper limit of the linear viscoelastic regime to strain hardening [14]. In sharp contrast, as shown in Fig. 4b for hydrogels co-polymerized with acrylate, the steady drop in G' values at intermediate strain values is not preceded by a rise in G'. Additionally, this drop in G' occurs at progressively lower strain values as the percentage of polyacrylamide in the hydrogels increases. Thus, while polyacrylamide has a more dramatic strengthening effect in pNaAc-based hydrogels, it also makes these hydrogels more brittle causing them to fracture at lower strain values.

To directly compare composition effects for these two hydrogel systems, G' values are plotted as a function of percentage polyacrylamide in Fig. 5. The average storage modulus values from at least three measurements were taken from the linear viscoelastic regime at strains of approximately 0.40% for the polyacrylic acid-based system and 0.34% for the polyacrylate-based system. In comparing the one component hydrogels first, pure polyacrylate hydrogels have the lowest G' value of $\sim$1 kPa which is about an order of magnitude less than that of the pure polyacrylic acid hydrogels ($G' \sim 8$ kPa). Pure polyacrylamide has a relatively high storage modulus value ($\sim$20 kPa), though the error bars span the widest range of all hydrogel compositions studied here.

Prior studies [15,16] on the polymerization process and structure of these three pure (i.e. single polymer component) hydrogel systems reveal reasons for the differences in mechanical properties observed in the current work. Polyacrylamide reportedly forms networks with dense regions of acrylamide and cross-linker spanned by more open chain regions due to the more favorable reaction between acrylamide monomers and BIS cross-linker than between acrylamide monomers. Initially, cross-linking competes with polymerization to generate regions rich in BIS. Once depleted of BIS, the remaining polymerization reactions form either links between the denser microgel domains or result in dangling ends [13,15,22]. More open network structures, on the other hand, have been reported in pure polyacrylic acid due to the slower cross-linking reactions [15]. Once polymerized, repulsive Coulombic interactions can affect the mechanical properties of polyelectrolyte-based hydrogels as demonstrated in previous studies involving the adjustment of the charge density of polyacrylic acid gels [34]. Though the acidic pH values for supernatant studies in Fig. 1a and b suggest that the degree of ionization is small in the polyacrylic acid system, even small variations in the degree of ionization can affect properties in these weakly-charged polyelectrolytes. The extended conformation reported for polyacrylate chains is also attributed to repulsive charge interactions [16]. Copolymerization with acrylamide alters the mechanical behavior of these hydrogels.

For both copolymer systems, increasing the concentration of pAAm generally resulted in a continual increase in storage modulus until a plateau occurs at $\sim$75% pAAm. Of the two hydrogel systems, however, the polyacrylate system showed a more dramatic increase

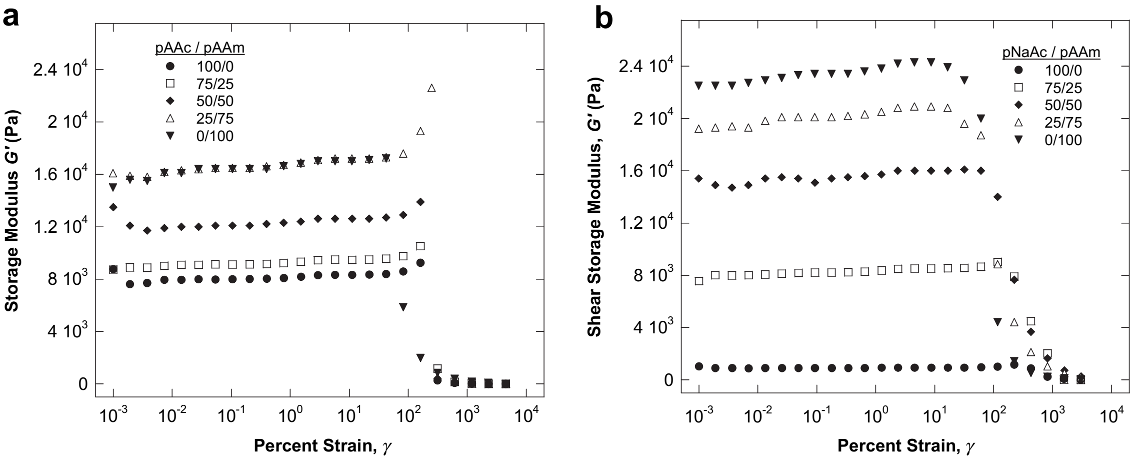

Fig. 4. Representative strain sweeps showing shear storage modulus as a function of percent strain for hydrogels with a fixed total polymer volume fraction of 0.1 and varying volume percentage ratios of a) polyacrylic acid to polyacrylamide (pAAc/pAAm) and b) polyacrylate to polyacrylamide (pNaAc/pAAm).

in storage modulus with percentage polyacrylamide. Since pure polyacrylate and polyacrylamide hydrogels represent the weakest and strongest hydrogels respectively, the marked change in modulus is attributed to copolymerizing acrylate with acrylamide. Here, including polyacrylamide as a copolymer may allow for more spatial separation of charged acrylate groups between stretches of uncharged chains resulting in a denser hydrogel which is also less subject to swelling compared to pure polyacrylate. The stiffening effects of acrylamide on polyacrylic acid are also apparent though less dramatic than for the polyacrylate system. Based on supernatant analysis shown in Fig. 1a and b, the polyacrylic acid chains are already partially neutralized under the relatively acidic polymerization conditions. Thus, spatial separation of the fewer charged acrylic acid groups is less pronounced with the addition of uncharged acrylamide copolymer and results in more modest stiffening effects for the pAAc/pAAM system compared to the pNaAc/pAAm system.

We next examined the effects of the total hydrogel volume fraction on the shear elastic modulus for a fixed composition. Three hydrogel compositions were chosen for the polyacrylic acid-based system, namely, 25/75, 50/50 and 100/0. These compositions were tested across a range of polymer volume fractions (ϕ_p) from 0.02 to 0.25 using strain amplitude sweeps ranging from 0.001% to 4500% at a fixed frequency of 6 rad/s. Fig. 6 shows representative strain sweeps of hydrogels with either a 50/50 pAAc/pAAm or pNaAc/pAAm composition. For pAAc/pAAm hydrogels shown in Fig. 6a, an increase in total polymer volume fraction results in a higher solids content and a corresponding increase in the shear storage modulus. It is also important to note that similar to the G' behavior shown in Fig. 4, all polymer volume fractions except the lowest ($\phi_p = 0.02$) in Fig. 6a exhibit nearly identical critical strain values marking the transition from a constant G' value to a sharp decrease in G'. Thus, for the pAAc/pAAm hydrogels, one can effectively stiffen the hydrogels by increasing either the polyacrylamide content or the total polymer volume fraction without embrittling the hydrogels.

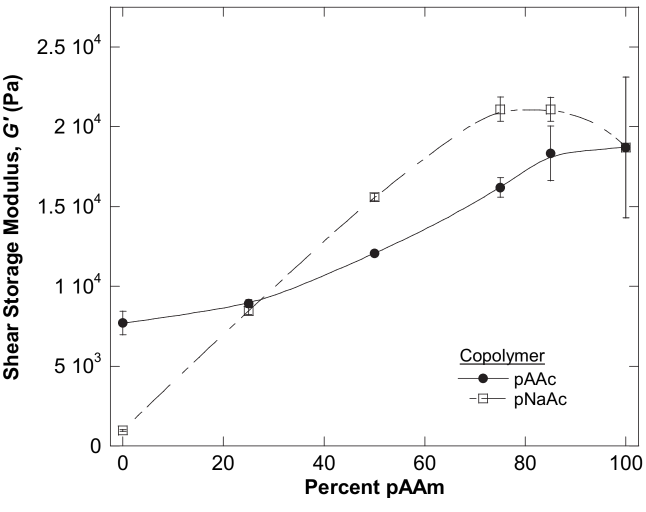

Fig. 5. Average shear storage modulus as a function of the percent polyacrylamide (pAAm) for hydrogels with either polyacrylic acid or polyacrylate serving as the copolymer. The modulus values were taken at a strain of approximately 0.40% and 0.34% for pAAc/pAAm and pNaAc/pAAm hydrogels, respectively from strain sweep measurements. The total polymer volume fraction for each hydrogel was 0.1.

Similarly, five compositions of the polyacrylate-based hydrogels were chosen for volume fraction studies: 0/100, 15/85, 25/75, 50/50, and 100/0 and the total polymer volume fractions ranged from 0.02 to 0.25. Strain amplitude sweeps were used to measure the storage modulus and ranged from 0.001 to 3000%. Fig. 6b depicts a representative strain sweep of a 50/50 pNaAc/pAAm composition. As expected, an increase in total polymer volume fraction, corresponding to an increase of elastically active polymer chains, results in an increase in shear storage modulus. Unlike pAAc-based hydrogels, however, the critical strain values (preceding the steep downturn in G') decreased as the polymer volume fraction increased. This trend in critical strain values is similar to the behavior observed in Fig. 4b for the polyacrylate-based hydrogels in which copolymer ratios were varied, but the total polymer volume fraction was fixed. Collectively, Figs. 4 and 6 demonstrate that increasing either the polyacrylamide content or the polymer volume fraction results in a simultaneous stiffening and embrittlement of pNaAc/pAAm hydrogels.

To facilitate comparison of composition effects on the shear storage modulus, the average modulus values at a strain of ~0.34% from strain sweeps are plotted in Fig. 7a as a function of volume fraction for both polyacrylic acid and polyacrylate-based hydrogel systems. For all compositions, an increase in polymer volume fraction results in a continuous increase in shear storage modulus, but the pure polyacrylate hydrogels are consistently the most mechanically compliant hydrogels at all polymer volume fractions. Consistent with results shown in Fig. 5 for one fixed polymer volume fraction of 0.1, increasing the concentration of

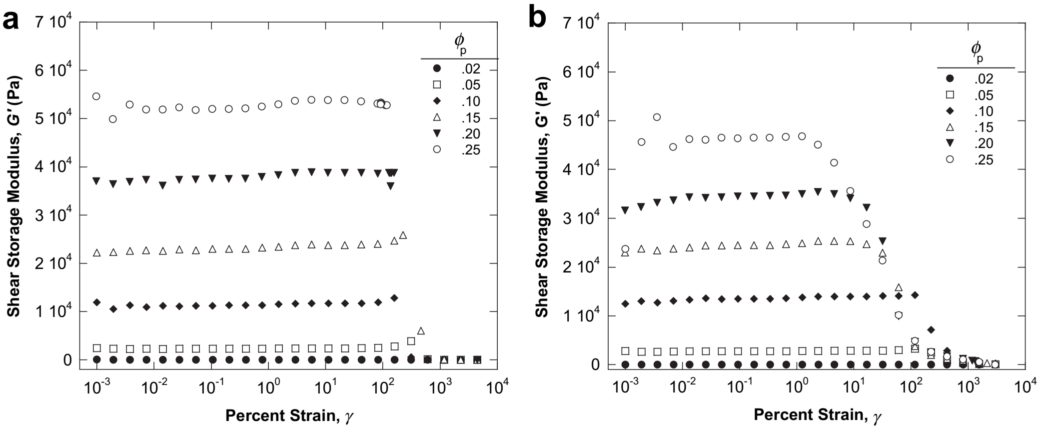

Fig. 6. Representative strain sweeps showing shear storage modulus as a function of percent strain for hydrogels with a 50/50 fixed composition of a) pAAc/pAAm or b) pNaAc/pAAm, but varying total polymer volume fractions, ϕ_p.

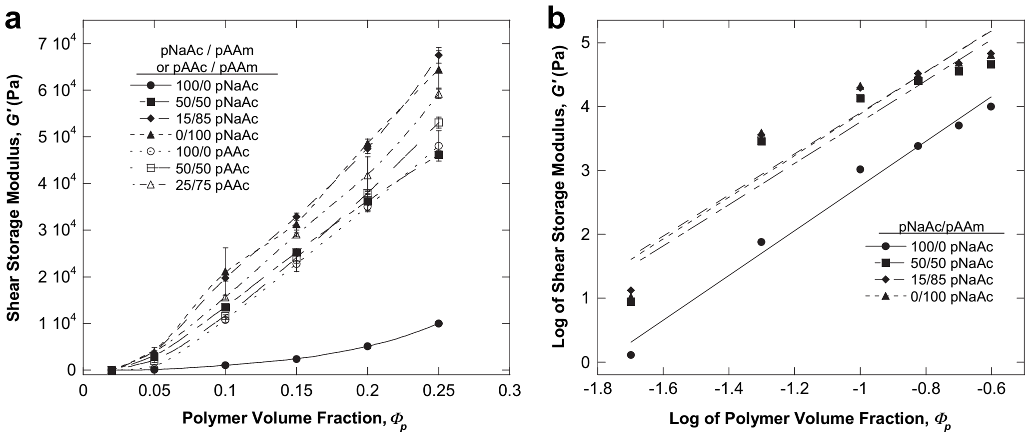

Fig. 7. a) Linear plot of average shear storage modulus values as a function of total polymer volume fraction for polyacrylic acid/polyacrylamide (pAAc/pAAm) and polyacrylate/polyacrylamide (pNaAc/pAAm) hydrogels with varying volume percentage ratios. b) Log–log plot of average shear storage modulus values as a function of total polymer volume fraction for select compositions of polyacrylate/polyacrylamide (pNaAc/pAAm) hydrogels with varying volume percentage ratios. Each modulus value shown was taken from strain sweeps at approximately 0.34% strain.

polyacrylamide in the hydrogel generally results in higher storage modulus values at any given hydrogel volume fraction. Moreover, the strengthening effects of copolymerizing with pAAm become more pronounced with volume fraction as shown by the increasing divergence in modulus values with hydrogel volume fraction, particularly for the polyacrylate system.

Similar to previous studies [35,36] we then explored correlating the elastic modulus with volume fraction for the polyacrylate-based hydrogels since these hydrogels exhibited the widest range in composition-dependent modulus values. To elucidate scaling behavior, data from Fig. 7a was plotted as a log–log plot in Fig. 7b. Slopes of 3.2 and 3.5 were determined for the pNaAc/pAAm copolymer hydrogels and for pure pNaAc, respectively. These values deviate from de Gennes' power law of 2.25 for elastic modulus as a function of concentration [37,38]. This deviation may stem from the fact that in real polymer systems, such as our hydrogels, dangling polymer chain ends contribute to the polymer volume fraction, but not to the elasticity of the hydrogels [36,37,39]. Our scaling values do, however, agree closely with the more recent experimental work of Meyvis et al. who report a scaling relationship of 3.4 for hydrogels that reach a state of equilibrium swelling [36]. Notably, the *in situ* polymerization conditions within the confined gap geometry on the rheometer stage would not allow the hydrogels to reach swelling equilibrium prior to modulus measurements in our system. Despite these differences in experimental approaches, good agreement in scaling relationships exists between our current system and that of Meyvis.

4. Conclusions

In summary, we have investigated the effects of composition and polymer volume fraction on the stiffness of pAAc/pAAm and pNaAc/pAAm-based hydrogels. We found that the stiffening effects of polyacrylamide as a copolymer were more dramatic for the polyacrylate-based hydrogels. While both swelling and stiffening effects were more modest in polyacrylic-based hydrogels, these hydrogels did not exhibit the embrittlement evident in polyacrylate-based hydrogels with either increasing polyacrylamide content or polymer volume fraction.

Acknowledgments

The authors thank Richard Seeger for implementing Serum Culture Protocols and Dr. Niren Murthy for helpful discussions. We are grateful for funding support from Georgia Tech for start-up funds and from the American Chemical Society Petroleum Research

Funds (ACS-PRF 43683-G7). R. Murff acknowledges support as a participant in the REU program sponsored by the National Science Foundation (DMR-0453293).

Appendix. Supplementary information

Supplementary data associated with this article can be found in the on-line version, at doi:10.1016/j.polymer.2010.02.022.

References

[1] Schneider GB, English A, Abraham M, Zaharias R, Stanford C, Keller J. Biomaterials 2004;25:3023–8.

[2] Stile RA, Healy KE. Biomacromolecules 2001;2:185–94.

[3] Woerly S, Pinet E, Robertis Ld, Diep DV, Bousmina M. Biomaterials 2001; 22:1095–111.

[4] Voet D, Voet JG, Pratt CW. Fundamentals of Biochemistry. New York: John Wiley & Sons, Inc; 2002. p. 52–56.

[5] Peppas NA. Hydrogels. In: Ratner BD, Hoffman AS, Schoenand FJ, Lemons JE, editors. Biomaterials science: an introduction to materials in medicine. San Diego: Academic Press; 1996. p. 60–4.

[6] Pelham RJ, Wang YL. Proc Natl Acad Sci USA 1997;94:13661–5.

[7] Wang YL, Pelham RJ. Preparation of a flexible, porous polyacrylamide substrate for mechanical studies of cultured cells. In: Vallee RB, editor. Molecular motors and the cytoskeleton part B. San Diego: Academic Press; 1998. p. 489–96.

[8] Gray DS, Tien J, Chen CS. J Biomed Mater Res 2003;66A:605–14.

[9] Wang HB, Dembo M, Wang YL. Am J Physiol Cell Physiol 2000;279:C1345–50.

[10] Semler EJ, Lancin PA, Dasgupta A, Moghe PV. Biotechnol Bioeng 2004;89: 296–307.

[11] Reinhart-King CA, Dembo M, Hammer DA. Langmuir 2003;19:1573–9.

[12] Fung YC. Biomechanics: mechanical properties of living tissues. New York: Springer-Verlag; 1993. p. 251.

[13] Calvet D, Wong JY, Giasson S. Macromolecules 2004;37:7762–71.

[14] Breedveld V, Nowak AP, Sato J, Deming TJ, Pine DJ. Macromolecules 2004; 37:3943–53.

[15] Benguigui L. J Phys 1995;5:437–43.

[16] Trompette JL, Fabregue E, Cassanas G. J Polym Sci B Polym Phys 1997;35:2535–41.

[17] Gong JP, Katsuyama Y, Kurokawa T, Osada Y. Adv Mater 2003;15:1155–8.

[18] Engler A, Bacakova L, Newman C, Hategan A, Griffin M, Discher D. Biophys J 2004;86:617–28.

[19] Domke J, Radmacher M. Langmuir 1998;14:3320–5.

[20] Jiang H, Su W, Mather PT, Bunning TJ. Polymer 1999;40:4593–602.

[21] Ng WK, Tam KC, Jenkins RD. Polymer 2001;42:249–59.

[22] Thevenot C, Khoukh A, Reynaud S, Desbrieres J, Grassl B. Soft Matter 2007;3: 437–47.

[23] Haines-Butterick L, Rajagopal K, Branco M, Salick D, Rughani R, Pilarz M, et al. Proc Natl Acad Sci USA 2007;104:7791–6.

[24] Baker BA, Murff R, Milam VT. Mater Res Soc Symp Proc; 2009. 1190:1190-NN1103-1107.

[25] Benda D, Snuparek J, Cermak V. Eur Polym J 2001;37:1247–53.

[26] Katime I, Novoa R, Apodaca ED, Mendizabal E, Puig J. Polym Test 1999;18: 559–66.

[27] Horkay F, Tasaki I, Basser PJ. Biomacromolecules 2000;1:84–90.

[28] Gregor HP, Frederick M. J Polym Sci 1957;23:451–65.

[29] Meier S, Haussinger D, Grzesiek S. J Biomol NMR 2002;24:351–6.

[30] Okay O, Sariisik SB. Eur Polym J 2000;36:393–9.

[31] Karadag E, Saraydin D. Turk J Chem 2002;26:863–75.

[32] Zhang YQ, Tanaka T, Shibayama M. Nature 1992;360:142–4.

[33] Tanaka T, Fillmore D, Sun ST, Nishio I, Swislow G, Shah A. Phys Rev Lett 1980;45:1636–9.

[34] Schosseler F, Ilmain F, Candau SJ. Macromolecules 1991;24:225–34.

[35] Horkay F, Hecht AM, Geissler E. J Chem Phys 1989;91:2706–11.

[36] Meyvis TKL, Smedt SCD, Demeester J. J Rheol 1999;43:933–50.

[37] Gennes PG. Scaling concepts in polymer physics. Ithaca: Cornell University Press; 1979. p. 141, 156–158.

[38] Lo YM, Robbins KL, Argin-Soysal S, Sadar LN. J Food Sci 2003;68:2057–63.

[39] Candau SJ, Bastide J, Delsanti M. Structural, elastic, and dynamic properties of swollen networks. In: Dusek K, editor. Advances in polymer science. Berlin: Springer; 1982. p. 27–71.