Swelling Kinetics of Disulfide Cross-Linked Microgels

Kyle N. Plunkett, Mary L. Kraft, Qing Yu, and Jeffrey S. Moore*

The Departments of Chemistry and Materials Science & Engineering and The Beckman Institute for Advanced Science and Technology, The University of Illinois at Urbana–Champaign, Urbana, Illinois 61801

ABSTRACT: Hydrogels of 2-hydroxyethyl methacrylate (HEMA) cross-linked with *N,N*-cystaminebis-(acrylamide) (CBA) were prepared inside microchannels using an in situ photopolymerization for the study of chemical-responsive microgels. By chemically reducing disulfide bonds with dithiothreitol (DTT), the cross-link density of the hydrogel network decreased, leading to an observable swelling of the hydrogel. To maximize swelling response, acrylic acid (AA) was copolymerized with HEMA and CBA to afford a pH/chemically-responsive hydrogel. The combination of a decrease in cross-link density and a driving force for swelling (deprotonation of AA) led to a fast swelling response. Hydrogel swelling switched between half- and first-order kinetics depending on hydrogel composition and de-cross-linking conditions. A direct relationship between the hydrogel swelling rate and the rate of cross-link cleavage was found. A linear dependence between the square of the swelling rate and DTT concentration suggests the possibility of using responsive hydrogels as a quantitative chemical sensor.

Introduction

Stimuli-responsive hydrogels have earned the reputation of "smart materials" due to their unique ability to change volume or shape in response to environmental signals. Hydrogels have been developed that display sensitivity toward a broad range of chemical or physical stimuli including pH,[1] solvent composition,[2] temperature,[3] light,[4] electric field,[5] glucose,[6] and, more recently, biological pertinent agents such as nucleotides[7] and antibodies.[8] Because the stimuli-triggered volume change can be harnessed to perform mechanical work, stimuli-responsive hydrogels have potential applications in controlled drug delivery devices,[9,10] biomaterials,[11] actuators,[12] and biosensors.[7,8]

The stimuli-induced volume change usually arises from one of three major mechanisms: (1) changes in osmotic pressure or charge density (i.e., pH-responsive hydrogels); (2) changes in solvent affinity of the polymer backbone (i.e., temperature-sensitive hydrogels); or (3) changes in the polymer cross-link density. Of these systems, pH-responsive hydrogels have been most extensively studied. These materials usually contain acidic or basic pendent groups, such as carboxylic acids or tertiary amines. The pH-dependent ionization of the pendent groups generates fixed charges on the polymer backbone. Water diffuses into the hydrogel to lower the internal osmotic pressure caused by the fixed charges, which is the basis for hydrogel expansion.

Volume transitions can also arise from changes in hydrogel cross-link density. Several systems have been developed to demonstrate the effects of cross-link density on hydrogel expansion. Biodegradable hydrogels based on unsaturated polyesters demonstrated that macromolecules physically entrapped within the hydrogel matrix could be released as ester linkages were broken.[13] Subsequent investigations led to the discovery that azo-cross-linked, pH-responsive hydrogels could be developed for colon specific targeting.[14,15] Recently, the disruption of noncovalent interactions was exploited for inducing a volume change in an antibody-responsive hydrogel.[8] An antibody and its antigen were independently grafted to a polymer network so that a noncovalent cross-link would form once binding occurred. Introduction of free antigen into the system initiated competitive binding with the antibody, and thus a disruption of the noncovalent cross-links and an expansion of the hydrogel. Of relevance to the present study, hydrogels cross-linked with disulfide bonds that can be cleaved and re-cross-linked have been reported. These hydrogels provide a straightforward method to control cross-link density and to study its effects on the hydrogel's properties.[16–18] Disulfide-cross-linked hydrogels swell or completely dissolve once the disulfide bonds are cleaved by reducing reagents such as dithiothreitol (DTT) and have found use in the controlled release of islets[19] or determination of average molecular weight between cross-links of polymerized hydrogels.[18]

These studies suggest that such cross-linking-volume relationships can be harnessed for potential applications such as controlled-release devices and biosensors. The long response times characteristic of macroscopic bulk-polymerized hydrogels (or "slab gels"), however, is unsuitable for many of these anticipated applications. The rate-limiting step for hydrogel swelling has been found to be the diffusion of the polymer backbone in the aqueous solution.[20] Due to this limitation, the swelling rates can be improved by reducing the hydrogel dimensions, as the response time is proportional to the inverse square of the gel dimension.[20,21] This scaling behavior makes hydrogels well suited for applications in microfluidic devices.[12] Micrometer-scale ionic hydrogels have been synthesized by various research groups, and fast time responses have been reported.[22,23] These systems demonstrate the utility of microscopic hydrogels for applications where fast response times are required.

Recently we developed a method to fabricate microscopic hydrogel structures (microgels) inside microchannels via a liquid-phase photopolymerization process.[12,24] The liquid-phase photopolymerization not only allows the construction of numerous hydrogel structures with

* To whom correspondence should be addressed. Telephone: (217) 244-4024. Fax: (217) 244-8068. E-mail: moore@scs.uiuc.edu.

varying properties quickly and easily, but also provides the capabilities to make various shapes and sizes by simply choosing the proper photomask during the polymerization process. Because these microscopic hydrogels are stationary inside microchannels, flowing a suitable buffer solution through the microchannel can control the chemical environment around the hydrogels without disrupting or moving the hydrogel. These microchannel-confined hydrogels demonstrate fast volume responses and provide a convenient way to study hydrogel kinetics and other properties. Real time monitoring of the hydrogels volume changes can be achieved by videotaping the entire volume transition process through an optical microscope.

Here we report the polymerization and characterization of disulfide-cross-linked microgels. This configuration not only provides a convenient means to investigate the relationship between hydrogel volume and cross-link density, but also serves as a model for potential hydrogel-based biosensors. To this end, hydrogels prepared with tailored cross-linkers may be used to detect biologically induced de-cross-linking, such as peptide cleavage by a protease enzyme.

Experimental Section

Materials. Dithiothreitol (DTT, Aldrich), *N,N*-methylenebis(acrylamide) (NMBA, Aldrich), 2,2-dimethoxy-2-phenyl-acetophenone (DMPA, Aldrich), rhodamine-B (Aldrich), *N,N*-cystaminebis(acrylamide) (CBA, PolySciences, Inc.), NaH_2PO_4 (analytical reagent grade, Mallinckrodt Inc.), Na_2HPO_4 (analytical reagent grade, Mallinckrodt Inc.), and NaCl (Fisher Scientific) were used as received without further purification. 2-Hydroxyethyl methacrylate (HEMA, Aldrich) and acrylic acid (AA, Aldrich) were distilled under reduced pressure in the presence of the radical inhibitor 4-methoxyphenol (Aldrich) prior to use. UV-curable adhesive (No. 61) was purchased from Norland Optical Adhesive. Fisherbrand microscope slides (75 × 50 × 1 mm), Corning cover glass slides (22 × 50 mm No. 1) and United Laboratory Plastics pipet tips (201−1000 μL) were purchased from Fisher Scientific. Photomasks were prepared by printing patterns on transparency films using a high-resolution commercial printer system (Linotype Herkules Imagesetter) with a resolution of 5080 dpi. Phosphate buffers were prepared from 0.05 M NaH_2PO_4 solution or 0.05 M Na_2HPO_4 solution, and HCl or NaOH solution was added to obtain the desired pH. The ionic strength of all buffers was adjusted to a constant value of 0.20 M with NaCl.

Fabrication of Microchannels. The microchannels used in this study were constructed by bonding microscope cover glass to a glass slide using UV curable adhesive. UV light (365 nm) from a handheld UV lamp (Spectroline ENF-240C) was used as the light source for curing the adhesives. Two pieces of 22 × 50 mm No. 1 cover glass were first bonded to a 75 × 50 × 1 mm glass slide (Fisher Scientific) with their edges parallel to each other and separated by the desired channel width (5000 μm). A third piece of 22 × 50 mm No. 1 cover glass was then bonded as the channel top, producing microchannels 150 to 180 μm deep. Plastic pipet tips (United Laboratory) were cut to desired length with a razor blade and glued to both ends of the microchannel with Loctite Quick Set Epoxy to serve as the inlet and outlet for the microchannel. Silicone tubing (Helix Medical, Inc.) with an inner diameter of 1.57 mm was used to connect these pipet tips with the liquid source.

Photopolymerization of Hydrogels. Photopolymerizations were carried out with the UV source from an Olympus Epi-Fluorescent microscope (BX-60) through a near-UV filter cube (U-MNUA, type BP 360−370) with a 360−370 nm bandpass. Light intensity was adjusted by changing the magnification of the lens. In a typical procedure, HEMA (1.041 g, 8 mmol), AA (144 mg, 2 mmol), CBA (26.0 mg, 0.1 mmol), DMPA (33.2 mg), and a small amount of rhodamine-B (for visualiza-
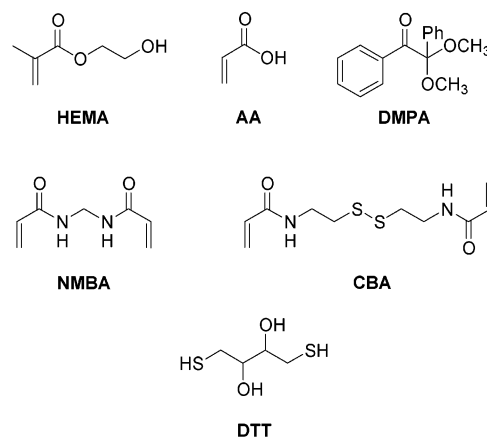
Figure 1. Chemical structures of hydrogel components and reducing agents. Monomers: 2-hydroxyethyl methacrylate (HEMA) and acrylic acid (AA). Photoinitiator: 2,2-dimethoxy-2-phenyl-acetophenone (DMPA). Inert cross-linker: *N,N*-methylenebis(acrylamide) (NMBA). Reducible cross-linker: *N,N*-cystaminebis(acrylamide) (CBA). Reducing agent: dithiothreitol (DTT).
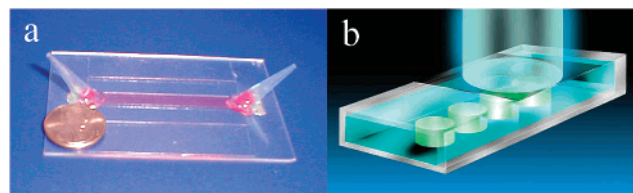
Figure 2. Preparation of hydrogels. Hydrogels were prepared by first introducing a prepolymer mixture (monomers, cross-linker, and a photoinitiator) into a microchannel (a). Exposure to UV light through a photomask resulted in the desired hydrogel structures (b).

tion purpose) were mixed to produce a liquid prepolymer mixture (Figure 1). This prepolymer mixture was injected into a microchannel with a syringe and allowed to reach a quiescent state (3 min). A proper photomask was then placed on top of the microchannel at the desired location, followed by exposure of the unmasked region to UV light to produce hydrogels with a 4:1 molar ratio of HEMA-*co*-AA and 1 mol % CBA cross-linking (Figure 2). The standard polymerization time for this prepolymer mixture was 1 min 40 s under a 2× magnification lens. Unpolymerized prepolymer mixture was removed by flushing the channel with deionized water and methanol and the resulting hydrogel structures were stored in deionized water. Hydrogels containing NMBA as cross-linker were prepared in a similar manner.

Characterization. Shape and size characterization of the microgels was carried out with either an Olympus Epi-Fluorescent microscope (BX60) equipped with a Sony CCD-IRIS/RGB color video camera and Panasonic AG-1980 video-cassette recorder with monitor or a Zeiss optical microscope. The diameter of the hydrogels was determined by measuring the microgel on the microscope monitor and calibrating to a known standard. For each prepolymer mixture, four cylindrical hydrogel structures were simultaneously created in the same microchannel using a photomask patterned for multiple structures. The dimensions in the horizontal and the vertical directions were measured and averaged for each hydrogel to eliminate the effect of slight irregularity in shape during expansion.

De-Cross-Linking of Disulfide Cross-Linked Hydrogels in Methanol. Microchannels containing hydrogels were filled with methanol and allowed to sit overnight. Equilibrium diameters of the hydrogels were measured and recorded as "swelling in methanol". A 10 mM DTT solution was prepared by dissolving DTT (30.8 mg) in methanol (20 mL). The 10 mM DTT solution was pumped through the microchannel at a flow rate of 6.5 mL·h^{-1} using a Harvard Apparatus PHD 2000

syringe pump, and the hydrogels were monitored in real time through a microscope.
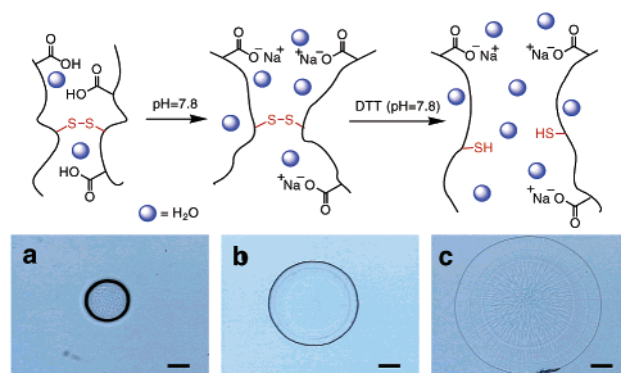
De-cross-linking of Disulfide Cross-Linked pH-Sensitive Hydrogels in Aqueous Buffer Solution. Microchannels containing hydrogels were filled with phosphate buffer (pH 7.8) and allowed to sit overnight. Equilibrium diameters of the hydrogels were measured and recorded as "swelling in base". A 10 mM DTT solution was prepared by dissolving DTT (30.8 mg) in phosphate buffer (20 mL, pH 7.8). The 10 mM DTT solution was pumped through the microchannel at a flow rate of 6.5 mL·h⁻¹ using a Harvard Apparatus PHD 2000 syringe pump, and the hydrogels were monitored in real time through a microscope. Diameter measurements of the hydrogels were taken at 3-min intervals until the maximum swelling was reached, as evidenced by the diameter remaining constant for a period of 20 min. Hydrogels subjected to different DTT concentrations or buffer pHs were de-cross-linked in a similar manner.

Results and Discussion

Photopolymerization. The polymerization of neat HEMA-based hydrogels was successfully achieved within 2 min using DMPA as a photoinitiator, which was previously reported to be suitable for the photopolymerization of HEMA.[25] Under these conditions, a considerable amount of cross-linking occurred during the polymerization of a mixture consisting solely of HEMA and DMPA in the absence of a difunctional cross-linker. We refer to this background cross-linking as "intrinsic cross-linking" of the hydrogel, and it is presumed to arise from chain transfer processes during polymerization.[26] Because the chain transfer process is monomer-dependent, it cannot be avoided completely. It was observed that the intrinsic cross-link density was high enough to prevent the poly(HEMA) hydrogel from dissolving.

De-Cross-Linking of Hydrogels by DTT. Two types of poly(HEMA) hydrogels were examined. One hydrogel was cross-linked with CBA which contained a cleavable disulfide bond. The second hydrogel, for control experiments, was cross-linked with NMBA which was inert to chemical reduction. DTT was chosen as the reducing agent for disulfide bond cleavage since it rapidly reduces disulfide bonds to free sulfhydryl groups under slightly basic conditions.[27] It was expected that the DTT could diffuse into the hydrogel network and cleave the disulfide bond cross-links and the reduction in cross-link density would lead to hydrogel expansion. In our initial studies, CBA cross-linked hydrogels showed no obvious expansion compared to those cross-linked with NMBA when exposed to a basic DTT buffer solution. However, after flushing of the channel with methanol, hydrogels cross-linked by CBA swelled to a significantly larger degree than hydrogels cross-linked by NMBA. This enlarged swelling suggests that the disulfide bonds in the cross-linker were in fact cleaved by the DTT. Since methanol is a better solvent for poly(HEMA) than water, this observation suggested that a driving force was necessary to induce the hydrogel to swell quickly as the cross-links were cleaved.

Two differing strategies were pursued to provide a driving force for hydrogel expansion. In the first approach, acrylic acid (AA) was copolymerized into the hydrogel network to generate a pH-responsive hydrogel matrix. Since the disulfide cross-link reduction was carried out under basic conditions, the deprotonation of AA created an osmotic pressure gradient that triggered hydrogel expansion. In this approach, the hydrogels were first allowed to reach equilibrium swelling in

Figure 3. Schematic representation of disulfide-cross-linked, pH-responsive hydrogels: (a) micrograph of a disulfide-cross-linked hydrogel after photopolymerization; (b) micrograph of the hydrogel after it has reached equilibrium swelling in pH 7.8 phosphate buffer, where the hydrogel expanded moderately due to the deprotonation of the pendent pH-sensitive carboxylic acid groups; (c) micrograph of the hydrogel after disulfide cross-links were cleaved by exposure to a solution of 10 mM DTT in pH 7.8 phosphate buffer. The hydrogel in part c expanded significantly, but did not dissolve completely due to intrinsic cross-linking. Scale bar = 200 μm.

a basic phosphate buffer. Exposure to a 10 mM DTT solution in the basic phosphate buffer induced de-cross-linking that led to further swelling of the hydrogels (Figure 3).

In the second approach, a 10 mM DTT in methanol solution was employed instead of aqueous buffer. The disulfide-cross-linked hydrogels were soaked in methanol and allowed to reach an equilibrium swelling diameter. Similar to the pH-responsive hydrogels, the methanol soaked hydrogels began to swell immediately once exposed to the DTT solution. Both approaches were sufficient to produce hydrogel expansion while undergoing de-cross-linking, and the rate of swelling could be used as a measure of de-cross-linking kinetics. The pH-responsive hydrogels were studied in detail as the de-cross-linking occurred in aqueous buffer solutions, which may be utilized for models of biologically induced de-cross-linking.

Swelling Kinetics. To investigate the effects of AA on hydrogels cross-linked via CBA, we first examined the swelling rates and maximum swelling volume as a function of AA content. Kinetic rate equations were derived from a simplified model for hydrogel swelling. The model considers hydrogel expansion to be controlled by two nonrelated steps, disulfide bond breaking and polymer diffusion (eq 1). Although these steps will occur

$$A_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} 2A \overset{k_2}{\rightarrow} 2P \qquad (1)$$

in unison in a real hydrogel, the separation is required to simplify the rate equations. In this analysis, the disulfide bond which acts as the hydrogel cross-linker is represented by A_2. Once exposed to DTT, the disulfide is reduced into two sulfhydryl groups (2A) which de-cross-links the hydrogel network. After de-cross-linking, the hydrogel can expand from an initial, shrunken state (2A) to a swollen state (2P). The rate constants k_1, k_{-1}, and k_2 represent the disulfide cleavage, disulfide formation, and polymer swelling rates, respectfully. Using the derived rate law equation (eq 2) and a steady-state

$$-\frac{d[A_2]}{dt} = \frac{1}{2}\frac{d[P]}{dt} = k_1[A_2] - k_{-1}[A]^2 = \frac{k_2}{2}[A] \qquad (2)$$

approximation we can obtain the change in disulfide concentration as a function of time (eq 3).[28] When this equation is analyzed in terms of $a[A_2]$ we see that two outcomes are possible. When $a[A_2] \ll 1$, a first-order rate

$$-\frac{d[A_2]}{dt} = \frac{2k_1[A_2]}{\sqrt{1 + a[A_2]} + 1} \tag{3}$$

$$a = \frac{16k_1k_{-1}}{k_2^2} \tag{4}$$

law is found (eq 5) that after subsequent solving of the differential equation and integration gives the familiar concentration dependence on time (eq 6). This scenario

$$-\frac{d[A_2]}{dt} = k_1[A_2]; \quad \text{if } a[A_2] \ll 1 \tag{5}$$

$$\ln\left(\frac{[A_2]}{[A_0]}\right) = -kt \tag{6}$$

would coincide with a process that is rate limiting in the first step of the model (i.e., disulfide breakage). However, if $a[A_2] \gg 1$, a half-order rate law is found (eq 7), which gives eq 8 after solving the differential

$$-\frac{d[A_2]}{dt} = \frac{1}{2}\left(\frac{k_1}{k_{-1}}\right)^{1/2} k_2[A_2]^{1/2}; \quad \text{if } a[A_2] \gg 1 \tag{7}$$

$$[A_2]^{1/2} = [A_0]^{1/2} - \frac{k}{2}t \tag{8}$$

equation and integration. This scenario would be indicative of a process where expansion of the polymer network is rate limiting. Using the hydrogel diameter change as an indicator of the concentration of A_2 at time t (eq 9)[28] and the total diameter change as A_0 (eq 10), we can then find best order fits for the hydrogel swelling data.

$$A_2 = D_t - D_\infty \tag{9}$$

$$A_0 = D_0 - D_\infty \tag{10}$$

In the de-cross-linking study, hydrogels with various ratios of HEMA-*co*-AA were polymerized inside a microchannel with either CBA or NMBA as a cross-linker. After the hydrogels equilibrated to a partially expanded initial diameter (D_0) in pH 7.8 phosphate buffer, a 10 mM DTT solution in the same buffer was pumped through the microchannel at a flow rate of 6.5 mL·h⁻¹. The swelling profile of a 3:1 HEMA-*co*-AA hydrogel cross-linked with 1 mol % CBA (◆) is shown in Figure 4. After rapid initial expansion, the swelling rate slowed and asymptotically approached the equilibrium diameter. In comparison, the control hydrogel (3:1 HEMA-*co*-AA cross-linked with 1 mol % NMBA, ■) which contained an inert cross-linker did not swell in the presence of DTT, demonstrating that swelling indeed resulted from a decrease in the cross-linking density caused by the breaking of disulfide linkages. The swelling data for the CBA-cross-linked hydrogel were plotted both as first- and half-order processes (Figure 5) according to eqs 6 and 8, respectively. The quality of linear regression for the first-order process suggests that under these de-cross-linking conditions, the breaking of the disulfide cross-linker is the rate-limiting step.[29] The
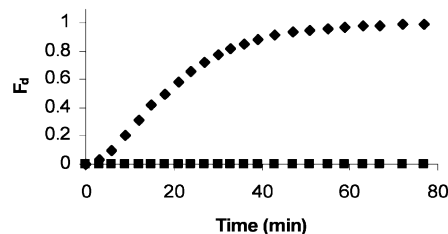
Figure 4. Swelling profile of a 3:1 HEMA-*co*-AA hydrogel cross-linked with 1 mol % CBA (◆) or 1 mol % NMBA (■) exposed to a 10 mM DTT/pH 7.8 phosphate buffer flowed at 6.5 mL·min⁻¹. Swelling of the disulfide cross-linked hydrogel occurred quickly when exposed to the DTT reductant, while the chemically inert NMBA did not swell. F_d is the fractional change in diameter of the hydrogel defined as $(D - D_0)/(D_\infty - D_0)$, where D is the diameter at a given time, D_0 is the initial diameter, and D_∞ is the final diameter of the hydrogel.
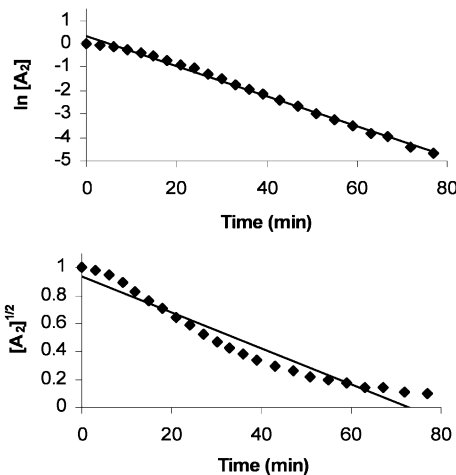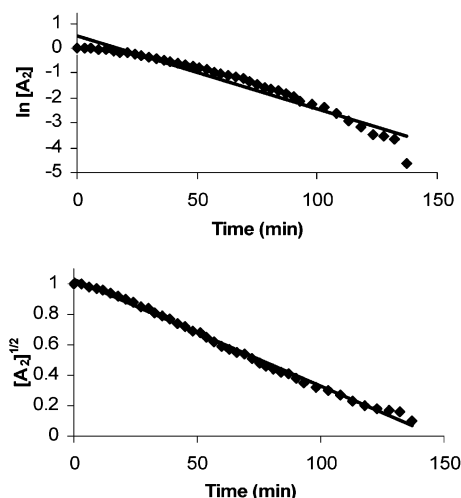

Figure 5. Plot of the first-order (top, eq 6) and half-order (bottom, eq 8) kinetic data for the swelling of a 3:1 HEMA-*co*-AA hydrogel cross-linked with 1 mol % CBA and exposed to a 10 mM DTT/pH 7.8 phosphate buffer. $R^2 = 0.982$ for first-order plot.
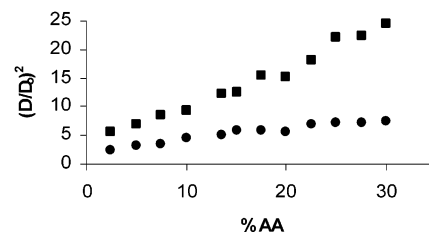
Figure 6. Effect of AA composition on the swelling of hydrogels in pH 7.8 phosphate buffer prior to addition of DTT (●) and the maximum swelling of hydrogels after de-cross-linking by 10 mM DTT in pH 7.8 phosphate buffer (■). Hydrogels contain 1 mol % CBA and varying HEMA-*co*-AA ratios. $(D/D_0)^2$ is the volumetric swelling ratio of the hydrogel.

following experiments show that the reaction order actually switches between half- and first-order kinetics depending on the hydrogel composition and de-cross-linking conditions.

Effect of Comonomer Composition on Swelling Kinetics. The effect of AA composition on the degree of swelling is shown in Figure 6. Hydrogels with 1 mol % CBA and a variable ratio of HEMA-*co*-AA were allowed to reach an initial equilibrium swelling volume (●) in pH 7.8 phosphate buffer. The volumetric swelling ratio, defined as the square of the diameter change compared to the square of the initial diameter, increased with increasing AA composition up to 15 mol % and then reached a limiting value. Although the decrease in

Figure 7. Plot of the first-order (top) and half-order (bottom) kinetic data for the swelling of a 97.5:2.5 HEMA-*co*-AA hydrogel cross-linked with 1 mol % CBA and exposed to a 10 mM DTT/pH 7.8 phosphate buffer. $R^2 = 0.995$ for the half-order plot.

maximum swelling may be due to increased intrinsic cross-linking arising from a change in the monomer ratios, we suggest this effect is due to a change in the osmotic pressure. This assumption is reasonable since, in contrast to the plateau seen for the maximum hydrogel swelling in base, the maximum swelling of DTT de-cross-linked CBA hydrogels (■) increased linearly with increasing AA percentage. If the intrinsic cross-linking was the dominant factor, a similar plateau in swelling would be found for de-cross-linked hydrogels. Instead, we suggest that for the cross-linked hydrogel the osmotic pressure difference between the inside and outside of the hydrogel is the dominant factor up to 15 mol % AA, with an increase in expansion due to an increased net charge in the hydrogel matrix. Above 15 mol % AA, a limit is reached where the osmotic pressure difference is balanced by an inability of the cross-linked polymer network to further expand. In the de-cross-linked hydrogel, however, the polymer chains have more freedom and can swell further to lower the osmotic pressure difference. Therefore, hydrogels (1 mol % CBA) with AA content above the critical value (15 mol %) are restrained by the amount of cross-linking and thus unable to reach an reduced osmotic pressure value. This pressure imbalance provides a "loaded spring" which drives water uptake and expansion through de-cross-linking.

The de-cross-linking kinetics of hydrogels with varying HEMA-*co*-AA compositions was examined by monitoring the gel dimensions while flowing a 10 mM DTT in pH 7.8 buffer through the microchannel. Although all hydrogels, regardless of AA content, displayed similar swelling profiles (see Figure 4) during the de-cross-linking process, a change in swelling rate order was found at 15 mol % AA. Below 15 mol % the hydrogels swelled with a half-order fit (Figure 7), which suggested that the polymer expansion was rate limiting. The swelling rate constants (k), obtained from the slope of the best fit line, increased with increasing AA content (Figure 8) as would be expected for a hydrogel with a larger driving force (i.e., higher osmotic pressure). Above 15 mol % however, the first-order plot gave a better line fit, indicating that the disulfide cleavage was rate limiting. Though disulfide cleavage is the rate limiting step, which gives rise to the overall order of unity for
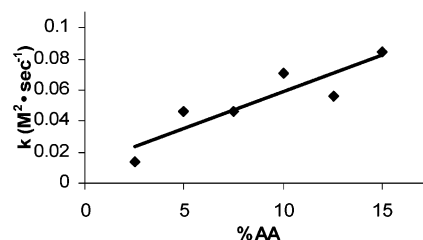
Figure 8. Half-order swelling rate constant (k) as a function of acrylic acid composition in the hydrogel. The rate constant increased linearly with increasing AA content up to 15 mol %. Above 15 mol % the data are better fit to the first-order kinetics model.
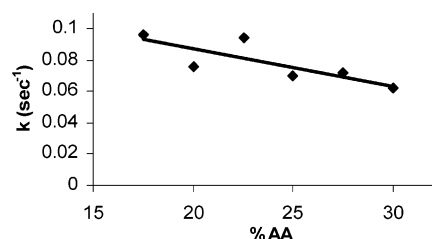

Figure 9. First-order swelling rate constant (k) as a function of acrylic acid composition in the hydrogel. The rate constant decreased with increasing AA content.

the process, the influence of the second step, outward diffusion of the polymer chains, is evidenced in a plot of the apparent first-order rate constant (Figure 9). The decrease in rate with increasing AA content above 15 mol % is likely due to the increase in the final gel volume for gels with increased AA content (Figure 6). This trend has also been observed in similar microgels with noncleavable cross-linked hydrogels.[29] As described above, we suggest the driving force for hydrogel expansion occurs due to an imbalance in osmotic pressure created by the inability of hydrogels with >15 mol % AA to expand further in its cross-linked state (Figure 6). Once de-cross-linking of a hydrogel with >15 mol % AA occurs, the polymer swells rapidly and is dependent only on the rate of disulfide cleavage and size of hydrogel.
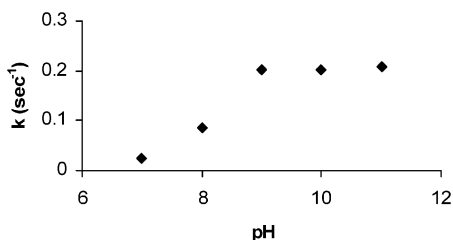
Effect of Buffer on Swelling Kinetics. In addition to varying the amount of acrylic acid in the hydrogel, the buffer pH and DTT concentrations were systematically varied to explore the influence of the buffer composition on the hydrogel swelling rate. Since cross-

$$k_1 = k'[\text{DTT}][\text{disulfide}] \qquad (11)$$

link cleavage is known to be first-order with respect to both DTT and cross-linker concentrations (eq 11),[31] we tested the hypothesis that the hydrogel swelling rate constant (k) could be directly dependent on the rate of cross-link cleavage (k_1). The rate of the cross-link cleavage reaction can be controlled in two ways. First, the chemical reduction rate constant (k') can be increased through optimizing reaction conditions (i.e., pH).[31] Second, the reaction can be accelerated by increasing the concentrations of reactants. The chemical rate constant (k') for disulfide cleavage can be increased by raising the pH of the buffer to a more alkaline solution. This speeds the reaction by facilitating the formation of the thiolate anion (DTT, pK_a 9.2)[30] which is directly involved in DTT mediated disulfide cleavage. To demonstrate the influence of pH on swelling kinetics, 4:1 HEMA-*co*-AA hydrogels cross-linked with 1 mol % CBA were de-cross-linked with a 10 mM DTT solution
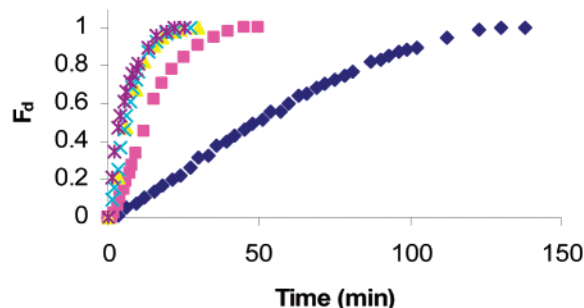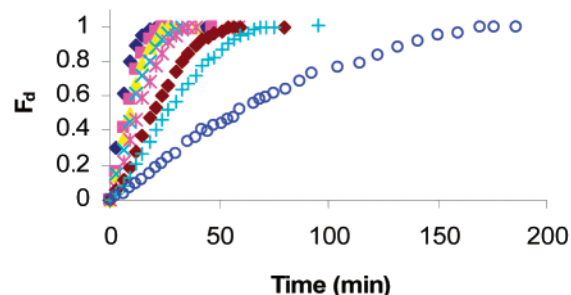
Figure 10. Swelling profiles of 4:1 HEMA-*co*-AA hydrogels cross-linked with 1 mol percent CBA subjected to 10 mM DTT in varying buffer pHs. Increasing the pH of the buffer provided a faster swelling response that reached a maximum at pH 9. Key: pH 7 (blue ◆), pH 8 (pink ■), pH 9 (yellow ▲), pH 10 (blue ×), and pH 11 (purple ∗).

Figure 12. Swelling profiles of 4:1 HEMA-*co*-AA hydrogels cross-linked with 1 mol percent CBA subjected to varying DTT concentrations in pH 7.8 buffer. Increasing the concentration of DTT leads to faster swelling profiles with the maximum swelling volume occurring earlier. Key: 100 (blue ◆), 50 (pink ■), 40 (yellow ▲), 30 (blue ×), 20 (purple ∗), 10 (brown ◆), 5 (blue +), and 1 mM (blue ○).

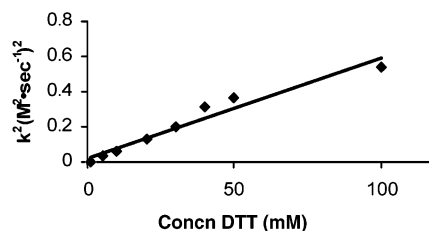**Figure 11.** First-order swelling rate constant (k) as a function of buffer pH (Figure 7). Increasing the pH of the buffer facilitated the reduction of the disulfide cross-linker by increasing the amount of the nucleophilic thiolate anion in solution.

Figure 13. Half-order swelling rate constant (k^2) as a function of DTT concentration (from Figure 10). Increasing the concentration of DTT led to a linear increase in the swelling rate constant suggesting the possible utility of cross-link-cleavable hydrogels as a quantitative measure of chemicals. $R^2 = 0.95$.

in buffers of varying pH (Figure 10). Similar to the hydrogels containing varying concentration of AA, the swelling kinetics appeared to switch between half- and first-order kinetics. Above pH 8, the hydrogels swelling data fit well to the first-order kinetics model, indicating cross-link cleavage was rate limiting. However, at pH 7 and pH 8, the hydrogel showed a more linear half-order plot and a less linear first-order plot. Oddly, the change in swelling order suggested that the expansion of the polymer network was becoming rate limiting at low pH. Not only did the swelling rate order change at pH 8, but the swelling rate leveled off as well (Figure 11). When plotting all first-order swelling rates over the pH range, an increase in swelling rate was visible when increasing from pH 7 to pH 9; however, above pH 9 no swelling rate increase was observed. The maximum swelling rate corresponds with the pK_a of the DTT,[31] which implied the hydrogel swelling was limited by the rate of the polymer diffusion below pH 8 (half-order), while above this value the reduction of disulfide bonds is the limiting factor (first-order). These data are consistent with reports showing a similar plateau in DTT reactivity at high pH.[31] These results may initially be counterintuitive since the rate of disulfide cleavage is slower in this region. One must also consider the rate of gel expansion in this pH range. At low pH, gel expansion is also slower than at elevated pH, and the data indicate that the rate of gel expansion at low pH is more dramatically affected by the pH than disulfide cleavage, producing the observed overall order.

To further support the hypothesis that the hydrogel swelling rate was directly dependent on the rate of cross-link cleavage, DTT concentrations ranging from 1 to 100 mM in a pH 7.8 phosphate buffer were used to reduce 4:1 HEMA-*co*-AA hydrogels cross-linked with 1 mol % CBA. Increasing the concentration of DTT led to

a visible change in the rate of swelling (Figure 12), and the swelling kinetics appeared to follow half order kinetics; however, at the highest concentration (i.e., 100 mM DTT), swelling was also well-equally approximated by a first-order fit. A plot of the square of the half-order swelling rate constants (k^2) showed a linear dependence on the DTT concentration (Figure 13).

These observations suggest that the swelling of the hydrogel depends on both the amount of driving force provided by the hydrogel, as demonstrated by AA composition studies, as well as the chemical makeup of the buffer. Increasing the reductant's concentration or increasing the pH can be used to speed up the disulfide cleavage rate and, consequently, the hydrogel swelling kinetics. The dependence of hydrogel swelling rate (k) on the DTT concentration suggests that cross-link-cleavable hydrogels could be used to detect not only the presence of a chemical but also its concentration by measuring the swelling rate of the hydrogel.

Conclusions

Stimuli-responsive hydrogels based on changes in cross-link density provide new opportunities for potential applications such as biosensors, drug-delivery devices, or biodegradable materials. By incorporating chemical liable cross-links into a hydrogel network, the hydrogel can be de-cross-linked by specific chemical events, providing a mechanism for the detection and quantification of chemical or biological compounds. In addition, hydrogels with cleavable cross-linkers can respond to a specific signal and give rise to a macroscopic event (swelling) as a result of a microscopic event (cross-link cleavage), therefore providing a direct read out mechanism.

We have demonstrated the rapid and facile preparation and characterization of stimuli-responsive micro-

gels. A driving force in the form of an internal (AA) or an external source (methanol) was a prerequisite for quick hydrogel expansion. Variations in swelling rate and the maximum swelling volume were accomplished by modifying the hydrogels composition. The swelling rate of these hydrogels was directly related to the kinetics of de-cross-linking and thus can be controlled through factors such as reductant concentration or buffer pH. Finally, the linear dependence of square swelling rate on the DTT concentration may be exploited for agent detection and quantitative measurements. The fabrication of hydrogels with an internal driving force for hydrogel expansion (i.e., AA) and new cross-linkable functionalities that participate in known recognition events (i.e., protease−peptide cleavage) may find application in robust detection devices. Through further miniaturization of the hydrogels, it may be possible to lower the detection limits to submillimolar concentrations.

References and Notes

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