Chemoenzymatic Synthesis and Characterization of Poly(α-methyl galactoside 6-acrylate) Hydrogels

Xiaomao Chen, Jonathan S. Dordick,* and David G. Rethwisch*

Department of Chemical and Biochemical Engineering and Center for Biocatalysis and Bioprocessing, University of Iowa, Iowa City, Iowa 52242

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ABSTRACT: Novel sugar-based hydrogels were synthesized from an enzymatically prepared monomer, α-methyl galactoside 6-acrylate, in the presence of *N,N'*-methylenebis(acrylamide) (BIS) as the cross-linker. The swelling ratio and elastic modulus of this hydrogel were comparable to those of polyacrylamide hydrogels. The neutral (uncharged) sugar-based gels swelled as much as 100-fold in deionized water at a cross-linker concentration of 0.5 mol %. The incorporation of ionic groups (i.e., acrylic acid moieties) into the network resulted in a dramatic increase in the swelling ratio; however, the swelling fell to the level of the corresponding neutral gel in electrolytic solutions (NaCl or $CuCl_2$ solution with concentration $>10^{-3}$ M). Conversely, no salt effect was observed for the neutral gels. This poly(α-methyl galactoside 6-acrylate) hydrogel has potential applications as a water-absorbent and as a biocompatible material.

Introduction

The present investigation has been undertaken to develop α-methyl galactoside 6-acrylate-based hydrogels, novel cross-linked sugar acrylate polymers, which have potential applications as water absorbents and biocompatible materials. Sugar-containing polymers have been prepared chemically.[1-3] For example, Pastoriza and Bertorello reported the chemical synthesis of sucrose methacrylate esters followed by free radical polymerization of the sucrose methacrylate. The resulting poly(sucrose methacrylate) hydrogel contained a mixture of sucroester moieties with acylation on various hydroxyl groups of the sugar.[1] Elias and co-workers were able to regioselectively synthesize styrene monomers with sugar pendant groups (3-(4-vinylphenyl)-propyl β-D-glucopyranoside).[2,3] This synthesis involved a number of blocking and deblocking steps, however, making the process tedious and subject to byproduct formation.

Recently, we have developed a chemoenzymatic method to synthesize highly regioselective sugar-containing polymers and hydrogels.[4-8] This method involves the acryloylation of sugars employing a regioselective enzymatic synthesis, followed by free-radical polymerization of the resulting sugar acrylate monoester.[4] If the polymerization is performed in the presence of cross-linkers, hydrogels are formed; however, in the initial studies the properties of the hydrogels were not described.[5-8] In the present study, the mechanical strength and swelling properties of poly(α-methyl galactoside 6-acrylate) hydrogels were extensively characterized in comparison to polyacrylamide-based hydrogels. The stability of these hydrogels as a function of pH and temperature was also studied.

Results and Discussion

Synthesis of α-Methyl Galactoside 6-Acrylate and Hydrogels. Our chemoenzymatic synthetic strategy was to enzymatically prepare the α-methyl galactoside 6-acrylate monomer followed by chemical polymerization of the monomer in the presence of a common water-soluble cross-linker, *N,N'*-methylenebis(acrylamide) (BIS), to give a cross-linked poly(sugar acrylate), as shown in Scheme 1. As reported previously,[4,8] linear poly(sugar acrylates) are completely water soluble in all proportions tested (up to at least 75% w/v). As a result, the cross-linked networks prepared in this study are highly hydrophilic and form hydrogels when placed in water.

The monomer, α-methyl galactoside 6-acrylate, was synthesized by reacting α-methyl galactoside with vinyl acrylate in anhydrous pyridine catalyzed by lipase P (from *Pseudomonas cepacia*). Typical reaction kinetics for this reaction are given elsewhere.[7] TLC and GC showed that a single monoester isomer was formed. ^{13}C NMR analysis indicated acylation at the C-6 position, consistent with previous results.[5,7,9-13] Importantly, no higher order acrylic acid esters (i.e., di-, tri-, etc.) were produced. This ensured that gel formation would require the presence of an external cross-linking agent. Moreover, only the 6-monoester was produced. Thus, no mixed isomers are present and a highly regular polymer structure was expected.

Polymerization was performed in water to take advantage of the high aqueous solubility of the sugar monomer and the high swellability of the resulting hydrogel. Along with BIS a water-soluble initiator (2,2'-azobis[2-(2-imidazolidinyl)propane] dihydrochloride (VA-044)) was used. This initiator decomposes at a relatively low temperature ($t_{1/2}$ = 10 h at 46 °C), limiting potential hydrolysis of the sugar monomer. Gelation at 55 °C occurred within 10 min under the given conditions. To ensure complete reaction, the mixture was maintained at 55 °C for an additional 2 h. Polymerization in the presence of 15% (w/v) monomer and 2% (mol % relative to total monomer) BIS resulted in a gel that absorbed ca. 45-fold its dry weight in water.

Hydrolysis of the sugar–acrylic acid ester bond during polymerization was a potential concern. However, titration of the free acid groups in a similarly prepared linear poly(α-methyl galactoside 6-acrylate) with 0.01 M NaOH revealed ca. 0.15 mol % free acrylic acid was incorporated into the polymer.[8] Hence, the hydrogel prepared by chemoenzymatic means is essentially nonionic and hydrolysis of the sugar ester moieties during polymerization and gelation was minimal.

Gel Swelling and Elastic Moduli. Gel formation was only observed for initial monomer concentrations

* To whom correspondence should be addressed.
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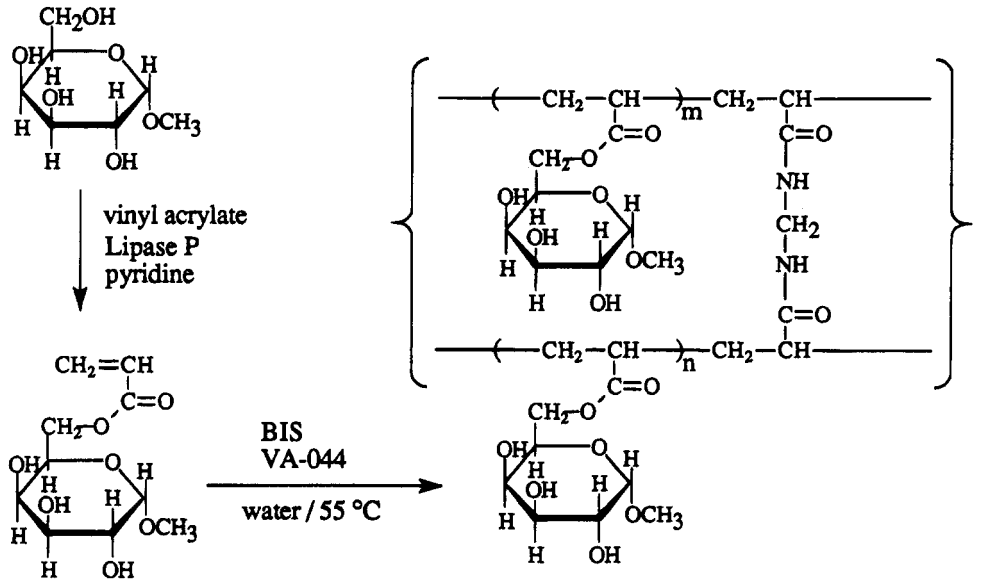
Scheme 1. Synthesis of Poly(α-methyl galactoside 6-acrylate) Hydrogel with BIS as Cross-Linker



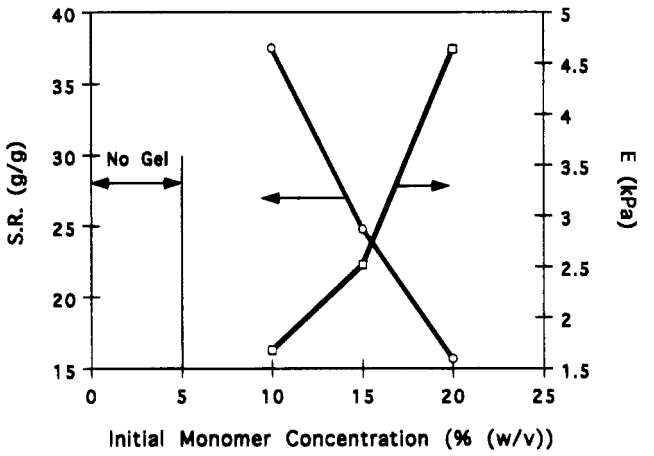


Figure 1. Effect of monomer concentration on the swelling ratio and elastic moduli of poly(α-methyl galactoside 6-acrylate) hydrogels (swollen in H_2O) (with 2 mol % BIS). Note: at 5% (w/v) of monomer concentration, gelation occurred; however, the formed gel was too soft to handle.

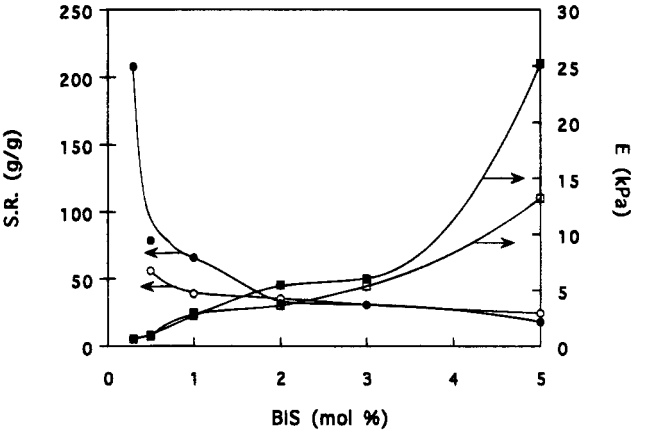


Figure 2. Swelling ratio and elastic moduli of α-methyl galactoside acrylate (α-gal) and acrylamide (AAm) based hydrogels (15% (w/v) monomer for α-gal, 5% (w/v) monomer for AAm): (○) swelling ratio for AAm, (●) swelling ratio for α-gal, (□) elastic modulus for AAm, and (■) elastic modulus for α-gal.

≥5% (w/v). This is not unusual, and critical monomer concentrations for gel formation have been observed for other hydrogels, such as polyacrylamide.[14-17] The effect of initial monomer concentration on the swelling ratio and elastic modulus of poly(α-methyl galactoside 6-acrylate) (PMG) gels with 2 mol % BIS are shown in Figure 1 and summarized in Table 1. For 5% (w/v) initial monomer concentration the gel was too weak to be used for an elastic modulus measurement. An inverse relationship between the equilibrium swelling ratio and the elastic modulus was observed for initial monomer concentrations ≥10% (w/v). Doubling the monomer concentration to 20% (w/v) resulted in a nearly 3-fold increase in elastic modulus at the sacrifice of a 60% decrease in swelling.

A similar relationship between swelling ratio and monomer concentration has been observed for polyacrylamide hydrogels.[16,17] Erman and Mark[16] explained this behavior through the formation of chain entanglements which increased the network elasticity. At higher initial concentrations of monomer, chain entanglements will increase, suppressing junction fluctuations by acting as additional cross-links and, thus, increasing the elasticity.

The concentration of BIS cross-linker had a more dramatic impact on gel swelling and strength. For these studies the initial monomer concentration was fixed at 15% (w/v) while cross-linker concentration was varied from 0.3 to 5 mol %. The swelling ratio and elastic modulus of the sugar-based hydrogels were a strong function of the concentration of cross-linker (Figure 2 and Table 1). At the lowest BIS concentration, the sugar-based gel absorbed more than 200 times its weight in water; however, the elastic modulus was only 0.5 kPa. The swelling ratio decreased as the BIS concentration increased such that at 1 mol % BIS the swelling ratio was 70. The elastic modulus increased ca. 6-fold to 3 kPa. Above 1 mol % and up to 5 mol % BIS the swelling ratio dropped further to ca. 20 along with a dramatic increase in the elastic modulus of the gel to ca. 25 kPa.

The behavior of the sugar-based hydrogel as a function of cross-linker concentration is similar to that observed with poly(acrylamide) (PAAm)-based hydrogels (Figure 2). PAAm gel formation required 0.5 mol % BIS in the presence of 5% (w/v) acrylamide monomer. The sugar-based hydrogels appear to have swelling and strength properties comparable to those for the more established PAAm gels except that at very low cross-

Table 1. Mechanical and Swelling Properties of Hydrogel Samples

w_0[a] (% w/v)	BIS[b] (mol %)	elastic modulus (kPa) ball[c]	elastic modulus (kPa) DMA[d]	SR[e]	ϕ[f]	ν_t[g] (mol/m^3)	ν_e[h] (mol/m^3)	$10^{-6}M_c$[i]	χ[j]
Poly(α-methyl galactose)									
15	0.33	0.45		210	0.0037	0.13	0.36	3.7	0.43
15	0.50	0.91		7	0.0098	0.52	0.52	2.5	0.48
15	1	2.7		65	0.012	1.3	1.5	0.90	0.46
10	2	1.7		37	0.021	4.4	0.76	1.7	0.48
15	2	4.0	17	31	0.027	5.9	1.6	0.81	0.50
20	2	4.7		16	0.052	11	1.5	0.86	0.51
15	3	6.0		29	0.027	8.6	2.5	0.55	0.49
15	5	25		18	0.045	24	8.6	0.15	0.49
Poly(acrylamide)									
5	0.50	0.80		54	0.014	2.6	0.40	3.3	0.50
5	1	2.8		39	0.020	7.3	1.3	1.0	0.49
5	2	3.4		34	0.023	17	1.5	0.90	0.49
5	3	5.4	5.4	29	0.027	30	2.2	0.59	0.49
5	5	13		24	0.033	62	4.9	0.27	0.49

[a] w_0 is the initial concentration of monomer in the reaction mixture. [b] BIS is the moles of BIS cross-linker per mole of sugar–ester repeat units. [c] These values of the elastic modulus were determined using the indentation method. Sample to sample variability is ca. ±50%. [d] These values of the elastic modulus were determined using DMA. [e] SR = swelling ratio which is the weight of the swollen gel over that of the dry gel. [f] Volume fraction of polymer in the swollen gel. Sample to sample variability is ca. ±15%. [g] Theoretical cross-link density is calculated assuming each BIS molecule in the gel acts as a cross-link. [h] The actual cross-link density is calculated from the elastic modulus and swelling using the ideal rubber model. [i] The molecular weight of the polymer chain between cross-links. [j] The polymer–solvent interaction parameter.

linking ratios, the sugar-based hydrogels swell tremendously, albeit with low mechanical strength. This suggests that the sugar-based gels may be used either as homopolymers or as copolymers with acrylic acids to form the basis of superabsorbents much like PAAm. Unlike PAAm,[18] the sugar-based gels are expected to be bioerodable, biocompatible, and nontoxic.

We calculated the cross-link density, ν, molecular weight between cross-links, M_c, and polymer–solvent interaction parameter, χ, of the gels (Table 1) using the affine ideal rubber model.[19–21] The effective cross-linking density (ν_e) was calculated from the stress–strain data using the relationship[19]

$$\tau = RT\nu_e\phi_2^{1/3}(\lambda - \lambda^{-2}) \quad (1)$$

where τ is the applied stress and λ is the ratio of the deformed length to undeformed length. The molecular weight between cross-links was calculated as $M_c = \varrho/\nu_e$ where ϱ is the polymer density. Values of M_c are ca. 10^6, indicating the gels are macroporous (Table 1). The theoretical cross-linking density (ν_t) was calculated by assuming every molecule of BIS forms two cross-links. The elastic modulus values (E) were determined from the stress–strain data.

The cross-link densities compare well with those reported in the literature for hydrogels with similar swelling ratios. In general, as expected, the observed cross-link density increased with increasing BIS or initial monomer concentration. However, the theoretical cross-link density increased much faster than the observed. For example, as the BIS concentration was increased from 0.33% to 5%, the observed cross-link density increased 24-fold while the theoretical density increased 180-fold. Indeed, at low BIS concentrations ν_t is less than ν_e while at BIS concentrations greater than 0.5% ν_t is greater than ν_e. As noted above, this results largely from cross-linking due to chain entanglements which will have a more pronounced impact at low cross-linker concentrations. At higher concentrations ν_e increases more slowly than ν_t, in part because some of the BIS will produce terminal chains or non-load-bearing chains which will not act as an observed cross-link. In addition, there may be blocks of BIS forming at the higher concentrations although we have no direct evidence for block formation.

Table 2. Complex Modulus and tan δ as a Function of Frequency for Poly(α-methyl galactoside) and Polyacrylamide Hydrogels at 25 °C

	frequency (hz)	complex modulus (kPa)	tan δ[a]
poly(α-methyl galactoside) (2% BIS, 15% initial conc)	0.2	2.55	0.20
	1	3.07	0.38
	10	5.30	1.11
	50	7.60	2.25
polyacrylamide (3% BIS, 5% initial conc)	0.2	8.05	0.11
	1	9.6	0.32
	10	13.4	0.55
	20	17	0.55
	50	66.5	1.85

[a] tan δ is the ratio of the loss modulus to the storage modulus.

Values of the polymer-solvent interaction parameter, χ, were calculated using the Flory relationship.[20]

$$\ln(1 - \phi_2) + \phi_2 + \chi\phi_2^2 + \nu_e V_1(\phi_2^{1/3} - 2\phi_2 f^{-1}) = 0 \quad (2)$$

where ν_e is the effective cross-linking density, V_1 is the molar volume of water, and f is the functionality of the cross-linking agent. The polymer–solvent interaction parameter was essentially constant at ca. 0.50 for both PMG and PAAm (Table 1).

Dynamic mechanical analysis (DMA) was used to determine the complex modulus and tan δ (the ratio of the loss modulus to the storage modulus) vs frequency for the sugar-based hydrogel and PAAm (Table 2). The value of the complex modulus increased with increasing frequency and was comparable in magnitude to the elastic modulus. The value of tan δ, which indicates the fraction of recoverable energy, also increased with increasing frequency. Similar results have been reported for natural hydrogels such as fibrin networks.[21] The elastic and complex moduli for these materials were essentially constant from 5 to 80 °C, indicating that their mechanical properties are stable over a broad temperature range including physiological temperatures.

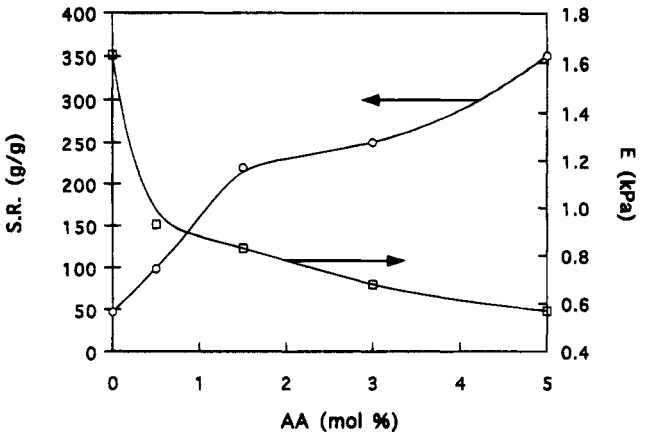


Figure 3. Swelling ratio and elastic moduli of poly(α-methyl galactoside 6-acrylate) hydrogels (swollen in H_2O) vs acrylic acid (AA) content in the hydrogels (10% (w/v) monomer/2 mol % BIS).

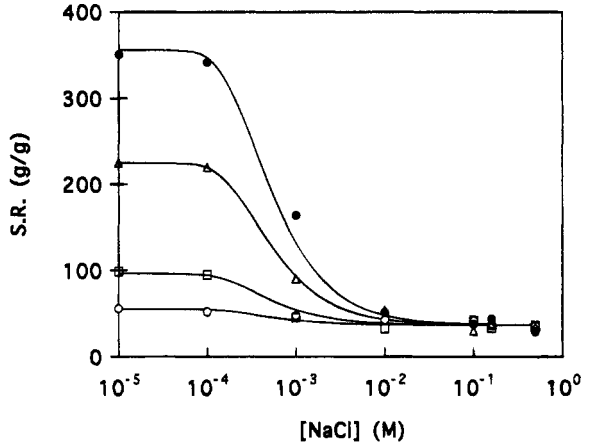


Figure 4. Effect of NaCl concentration on the swelling ratio of poly(α-methyl galactoside 6-acrylate) hydrogels with different acrylic acid (AA) contents: (○) 0 mol % AA, (□) 0.5 mol % AA, (△) 1.5 mol % AA, and (●) 5 mol % AA.

Hydrogels Based on Copolymers with Acrylic Acid. The swelling of PAAm is dramatically increased in deionized water in the presence of acrylic acid moieties incorporated into the polymer network.[22] It was of direct interest, therefore, to examine whether a similar phenomenon is observed with PMG. As shown in Figure 3, the incorporation of acrylic acid dramatically increased the swelling ratio of the PMG hydrogel in water. The swelling ratio increased with increasing acrylic acid content in the range studied (0–5 mol % acrylic acid) and from 45 for pure sugar-based hydrogel to ca. 350 for the hydrogel containing 5 mol % acrylic acid. As expected, an inverse relationship was observed between the swelling ratio and the elastic modulus.

Salt Effects. The sugar-based hydrogel containing no acrylic acid was essentially insensitive to the concentration of NaCl (from 10^{-5} to 0.5 M) with a swelling ratio of ca. 50 over that range of salt concentration (Figure 4). Increasing the acrylic acid content resulted in a higher swelling ratio at NaCl concentrations below 10^{-3} M, yet at NaCl concentrations >0.01 M, the swelling for all of these gels was essentially the same and independent of the acrylic acid content. Electrolytes are known to reduce the solvation around charges, thereby lowering the number of water molecules that interact with the polymer matrix of the hydrogel.[23,24] Higher salt concentrations result in fewer (or perhaps weaker) polymer–water interactions and reduced swelling. The dramatic drop in swelling for all charged gels in the presence of ca. 1 mM NaCl is consistent with the concentration of acrylic acid moieties in the hydrogels. Similar results were obtained with $CuCl_2$ as the salt. Once again, no salt dependence was observed with the uncharged sugar-based hydrogel. The charged gels lost their high swelling in the presence of ca. 10^{-4} M $CuCl_2$. The lower concentration of $CuCl_2$ required for swelling reduction as compared to NaCl is consistent with the higher ionic strength, larger ionic radius, and weaker water binding of the divalent Cu as compared to the monovalent Na.

Thermal and pH Stability. The swelling ratio of both charged and uncharged sugar-based hydrogels was essentially independent of solution pH from 1.5 to 8.5. Above pH 9, base-catalyzed hydrolysis of the sugar–acrylate ester bonds resulting in the limited generation of free acrylic acid. For example, incubation for 24 h at pH 10 hydrolyzed ca. 1% of the ester bonds. The sugar-based gels were thermally stable and the swelling was essentially independent of temperature up to about 37 °C. Above this temperature thermal hydrolysis occurred slowly. For example, treatment for 4 days at 37 °C (pH 7) hydrolyzed about 0.5% of the ester groups while treatment for 24 h at 55 °C (pH 7) hydrolyzed ca. 1.5% of the ester bonds.

Conclusions

A new generation of sugar-based hydrogels was synthesized from an enzymatically prepared monomer, α-methyl galactoside 6-acrylate, using BIS as the cross-linker. The swelling ratio and elastic modulus of this hydrogel were comparable to those of polyacrylamide hydrogels. The gels are macroporous suggesting their potential use for controlled release of macromolecular therapeutic agents such as viruses and proteins. The incorporation of ionic groups (i.e., acrylic acid moieties) into the network resulted in a dramatic increase in the swelling ratio; however, the swelling fell to the level of the corresponding neutral gel in electrolytic solutions (NaCl or $CuCl_2$ solution with concentration $>10^{-3}$ M). Conversely, no salt effect was observed for the neutral gels. The swelling of the gels was essentially independent of pH from pH 1.5 to 8 and independent of temperature up to 37 °C. The sugar-based hydrogels are stable, with slow hydrolysis of the sugar ester group only for pH >9 or T > 37 °C. This poly(α-methyl galactoside 6-acrylate) hydrogel has potential applications as a water absorbent and as a biocompatible material.

Experimental Section

General Procedures. Lipase P (from *P. cepacia*) was obtained from Amano Enzyme Co. (Troy, VA). HPLC grade pyridine was used after drying with 3 Å molecular sieves obtained from EM Science (Gibbstown, NJ). The water content of the dried pyridine was found to be less than 0.01% (v/v), as measured by the sensitivity limit of the Karl–Fischer method. Acrylamide (AAm) and acrylic acid were obtained from Aldrich Chemical Co. (Milwaukee, WI) and were used without further purification. α-Methyl galactoside was purchased from Sigma Chemical (St. Louis, MO), and vinyl acrylate from Tokyo Kasei (Portland, OR). The radical initiator 2,2′-azobis[2-(2-imidazolidinyl) propane] dihydrochloride (VA-044) was obtained from Wako Chemicals USA, Inc. (Richmond, VA) as a gift. Silica gel (flash column chromatography grade) was purchased from J. T. Baker (Philipsburg, NJ). All other chemicals and solvents were of the highest purity commercially available.

Buffer solutions of different pH values were prepared as follows (with salt concentration of 5 mM): HCl/NaCl (pH 1.5), potassium acid phthalate ($KHC_8H_4O_4$)/HCl for pH 2.75 and 4,

Na_2HPO_4/NaH_2PO_4 for pH 7.4 and 8.5, K_2CO_3/HCl for pH 9.25, and K_2CO_3/NaOH for pH 10.27. pH studies in the absence of salt were performed by adjusting the pH of deionized water with either dilute HCl or NaOH. NaCl and $CuCl_2$ solutions were prepared by dissolving the salt in deionized water with the pH maintained at ca. pH 7 by addition of dilute HCl and NaOH.

Optical rotations were measured at 589 nm (Na line) at 25 °C by a JASCO DIP-360 optical polarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker WM 360-MHz instrument with D_2O as the solvent and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as the internal reference. Qualitative analysis of α-methyl galactoside 6-acrylate was performed by TLC on a silica gel plate with 72:5:4 ethyl acetate/methanol/water as the eluent solvent. H_2SO_4 (10% v/v) in ethanol was used as the charring reagent for TLC. Quantitative analysis of α-methyl galactoside was performed by gas chromatography as detailed elsewhere.[5,7]

Enzymatic Synthesis of α-Methyl Galactoside 6-Acrylate. α-Methyl galactoside (19.4 g, 0.1 mol) and 40 g of lipase P were added to 300 mL of anhydrous pyridine. The reaction was initiated by the addition of 24 g (0.24 mol) of vinyl acrylate. The mixture was stirred (200 rpm) at room temperature for 2 days. By TLC analysis, the conversion of the monomer was found to be ca. 60%. A monoester was the sole product. The enzyme was removed from the reaction mixture by centrifugation. The solvent was removed by rotary evaporation under reduced pressure at 35 °C. The oily residue was applied to a flash column for silica gel chromatography with an elution system consisting of ethyl acetate:methanol:water (74:5:4). The solvent fraction corresponding to the monoester was removed under reduced pressure, dissolved in water, and freeze dried for 24 h, giving 9.8 g (39.5%) of α-methyl galactoside 6-acrylate product. Mp: 164–166 °C (polymerized after melting), $[\alpha]^{20}_D$ +137.5 ° (*c* 1, H_2O).

^1H NMR (D_2O): δ 3.41 (3H, s, O–CH_3), 3.85 (2H, t, unresolved, H-2 and H-3), 4.06 (1H, s, H-4), 4.16 (1H, dd, J = 7.5, 3.6 Hz, H-5), 4.35 (1H, dd, J = 11.7, 8.0 Hz, H-6), 4.41 (1H, dd, J = 11.7, 4.1, H-6), 4.85 (1H, d, J = 2.5 Hz, H-1), 6.02 (1H, dd, J = 10.5, 1.1 Hz, H-3′), 6.23 (1H, dd, J = 10.5, 6.23 Hz, H-2′), 6.47 ppm (1H, dd, J = 17.3, 1.0 Hz, H-3′).

^{13}C NMR (D_2O): δ 57.91 (CH_3O–), 67.05 (C-6), 70.9 (C-2), 71.16 (C-4), 72.04 (C-3), 72.15 (C-5), 102.34 (C-1), 130.13 (HC=, C-2′), 135.77 (H_2C=, C-3′), 171.08 ppm (C=O, C-1′).

Anal. Calcd for $C_{10}H_{16}O_7$: C, 48.39; H, 6.50; O, 45.11. Found: C, 48.55; H, 6.33; O, 45.31.

Synthesis of Poly(α-Methyl Galactoside 6-acrylate) Hydrogels. At room temperature (22 °C), the solubility of α-methyl galactoside 6-acrylate in water is about 10% (w/v). By heating to about 35 °C, its solubility is increased to greater than 20 wt %. Generally, the hydrogels were synthesized in a 15% (w/v) monomer solution; however, a series of hydrogels were prepared from monomer solutions with various concentrations (from 5 to 20% (w/v)) to study the effect of monomer concentration on the properties of the resulting hydrogels.

The general method for hydrogel synthesis is as follows: 1 mL of a mixed solution containing 15% (w/v) of α-methyl galactoside 6-acrylate and the desired amount of BIS (0.3–5 mol % relative to monomer) was degassed in an 8 mL vial for 10 min at reduced pressure by connecting to a water aspirator. To this solution, 15 μL of 10% VA-044 solution (0.15% (w/v) relative to solution mixture) was added under N_2. The vial was capped, and the polymerization reaction was allowed to proceed at 55 °C for 2 h. The gelation time was generally about 10 min. The resulting gel (sample size: diameter 1.5 cm, thickness 0.5 cm) was removed from the vial and immersed in water at room temperature for 96 h. During this period, fresh water was exchanged daily. The purpose of this treatment was to wash out any impurities or unreacted species and to allow the sample to reach swelling equilibrium. The swelling was followed during this time to ensure that equilibrium was attained.

Swelling of Hydrogels and Swelling Ratio Measurement. The hydrogel samples swollen in water were cut into 80 mg samples (approximately 0.4 × 0.4 × 0.5 cm^3 in size) and were immersed in buffer solutions at different pH values or in NaCl and $CuCl_2$ salt solutions at various concentrations ranging from 10^{-6} to 0.5 M. The equilibrium swelling was reached within 24 h for these samples. The swelling ratios (SR) of the hydrogel samples were measured using a TA Instruments Model 2950 thermogravimetric analyzer (TGA). The sample (ca. 20 mg) was purged with dry N_2 at 65 °C until constant weight was attained (45–60 min). The SR was calculated by dividing the wet weight by the dry weight of the sample. The standard deviation of SR was less than 5%.

Elastic Modulus Measurements. Elastic moduli were measured by two techniques. The first was an indentation test. In this method a stainless steel ball bearing was placed on the sample, and the indentation depth of the ball, h, was measured.[25] The elastic modulus of the hydrogel sample, E, was calculated as

$$E = \frac{3(1 - v^2)F}{40000h^{3/2}r^{1/2}} \tag{3}$$

where r is the radius of the ball (cm), v, is the Poisson coefficient (assumed to be 0.5), h is the depth of indentation (cm), E is Young's modulus (kPa), and $F = ma$, where m is the weight of the ball (g) and a = 980 cm^2/s^2. The relative standard deviation of the resulting modulus was ca. 25%.

The modulus was also determined for several samples using a Perkin-Elmer DMA-7 dynamic mechanical analyzer. For these measurements a 15 mm stainless steel cup and plate accessory was used. The cup was filled with water during measurements so that the sample would have no water loss. To avoid sample movement, a piece of 600 grit wet/dry sandpaper was glued to the bottom of the cup using spray-mount adhesive.[26] Both static (elastic modulus) and dynamic (complex modulus) measurements were made with standard errors of ca. 15%. Loads of <1 kPa were used to avoid permanent sample deformation. The best reproducibility was obtained for sample thicknesses less than 3 mm. The elastic modulus and swelling ratio data were further analyzed as described above (eqs 1 and 2) to determine the cross-link density, the molecular weight between cross-links, and the polymer–solvent interaction parameter χ.

Both DMA and the indentation method were used to calculate the elastic modulus for several samples (see Table 1). The values determined by the two methods are similar; however, the error for the DMA method is about 10% while that for the indentation method is 25%. While the DMA method is more precise, the indentation method still provides qualitatively useful information, particularly since the batch-to-batch variability of the hydrogels was ca. 50%.

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