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Highly swelling hydrogels from ordered galactose-based polyacrylates

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Abstract

High swelling galactose-based hydrogels have been prepared using a chemoenzymatic procedure. Regioselective acylation of β-*O*-methyl-galactopyranoside in nearly anhydrous pyridine with lipase from *Pseudomonas cepacia* yields the 6-acryloyl derivative (Compound **I**). Further lipase-catalysed acylation of the monoacrylate derivative in nearly anhydrous acetone yielded 2,6-diacryloyl-β-*O*-methyl galactopyranoside (Compound **II**) that can act as a cross-linker with a structure similar to that of the sugar-based monomer. The high selectivity of enzyme catalysis yielded apparently highly regular hydrogel networks with swelling ratios at equilibrium ranging from 170 to 1100, elastic moduli ranging from 0.005 to 0.088 MPa and calculated mesh sizes ranging from 1160 to 6600 Å. These values are far higher than conventional uncharged or lightly charged hydrogels at similar elastic moduli. Gel swelling was fast, with 75% of the equilibrium swelling value reached in a fractional time of 0.17. Non-selective chemical acryloylation of β-*O*-methyl galactopyranoside followed by polymerization yielded a far lower-swelling hydrogel than that obtained using selective enzyme catalysis. These results indicate that the highly regular polymer structure achieved by regioselective enzyme-catalysed acylation yields relatively strong and highly swellable materials. Sugar-based hydrogels, such as those described herein, may find particular use as biomaterials because of their high water content, homogeneity, stability and expected non-toxicity. A wide range of pore sizes can be attained, suggesting that they may also be especially useful as matrices for enzyme immobilization and controlled delivery of biological macromolecules.

Keywords: Sugar-based hydrogels; Sugar poly(acrylate)s; Sugar-based cross-linker; High swelling networks

1. Introduction

Hydrogels are lightly cross-linked polymer networks that swell in water. At equilibrium their swollen water content is high, ranging from about 30 to more than 99 wt.%. Hydrogels are unique because of this large amount of 'resident' water, which acts as a solute transport medium and as a plasticizer, permitting these materials to behave viscoelastically. High swelling gels are widely used as superabsorbents because of their high degree of hydrophilicity, low density and low cost. They are also the material of choice for contact lenses because of their low elastic modulus and, in some cases, biocompatibility. Hydrogels are finding an increased use in medicine and biomedical engineering because of their capacity for rapid solute transport and physical resemblance to living tissue [1], and their influence on solute permeability [2, 3]. Thus, hydrogels have made excellent matrices for drug-delivery systems and enzyme immobilization [4, 5].

Although the constituent polymer of some hydrogels is a cross-linked polysaccharide, the majority of hydrogels originate from synthetic monomers which yield polymeric architectures with both backbone and pendant groups. Usually the former is aliphatic and relatively hydrophobic, whereas the pendant groups can be hydrophilic and polar such as hydroxyl, hydroxylalkyl, amide or pyrollidone, and may include electrically charged groups such as carboxyl, *N*-alkylamide or sulphonate [6, 7]. Cross-linking agents such as di(meth)-acrylates and epichlorohydrin form covalent chain junctions [8].

Conventional hydrogels suffer from a number of limitations. A very high water content is often desirable but it is generally not possible to attain greater than 95 wt%

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unless the polymer contains a significant fraction of ionizable repeat units [9, 10] which are strongly affected by solution pH and ionic strength. The polymer networks tend towards structural heterogeneity, complicating gel characterization and modelling. Finally, common monomers are often toxic (e.g. acrylamide, acrylonitrile) [6], difficult to purify (2-hydroxyethyl methacrylate) [6], or difficult to copolymerize (*N*-vinyl pyrollidone) [11].

Hydrogels based on mono- or disaccharide acrylates do not experience these drawbacks. Among the various ways to prepare sugar-based acrylates [12], we have found that enzymatic acryloylation provides the high degree of regioselectivity necessary for resulting polymer regularity, and the technique is simple to perform [13–15]. In the present work, we describe the synthesis and physical characterization of a hydrogel based solely on two derivatives of galactose; 6-acryloyl-β-methyl galactopyranoside and the novel cross-linker 2,6-diacryloyl-β-methyl galactopyranoside, both prepared using lipase-catalysed transesterification in nearly anhydrous organic solvents. The resulting hydrogels provide equilibrium water contents above 95%. Their unique chemistries also result in unusual swelling behaviour in which the elastic modulus at a given swelling ratio is higher than that of conventional hydrogels. This is important in the generation of new, highly swellable and strong gels.

2. Experimental

2.1. Materials

Reagent-grade dimethylformamide (DMF) was purchased from Aldrich, Milwaukee, WI, USA, and reagent-grade ethyl acetate and methanol were purchased from Fisher, Pittsburgh, PA, USA. All solvents were >99.95% pure. All water used was filtered, distilled and deionized. Lipase P, from *Pseudomonas cepacia*, was purchased from Amano (Troy, VA, USA). The free radical initiators, 2-2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (AIPD) and 2-2′-azobisisobutyronitrile (AIBN), were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Polysciences (Warington, PA, USA), respectively. Monomer identification was performed using a carbohydrate column (Waters Inc., Milford, MA, USA). ^{13}C NMR spectra were recorded on a Bruker AMX 600-MHz instrument with trimethylsilane (TMS) as the internal reference.

2.2. Methods

2.2.1. Monomer synthesis

Compound **I** (6-acryloyl-β-methyl galactopyranoside) was synthesized as described earlier [14]. An isolated yield of 75% was obtained. Characterization of **I** was performed using optical rotation, ^{1}H and ^{13}C NMR, and elemental analysis: $[\alpha]_D^{25}$ − 10.1 (c1, DMF); ^{1}H NMR (DMSO-d_6) δ 3.53 (dd, J = 9.91, 7.90 Hz, H-2), 3.56 (3 H, s, CH_3O–), 3.67 (1 H, dd, J = 9.91, 7.90 Hz, H-3), 3.97 (1 H, m, H-5), 3.99 (1 H, d, J = 3.52 Hz, H-4), 4.34 (1 H, d, J = 7.90 Hz, H-1), 4.38 (1 H, dd, J = 11.7, 4.8 Hz, H-6), 4.40 (1 H, dd, J = 11.7, 7.7 Hz, H-6), 6.03 (1 H, dd, J = 10.58, 0.92 Hz, H-3′), 6.24 (1 H, dd, J = 10.58, 17.37 Hz, H-2′), 6.47 (1 H, dd, J = 17.37, 0.92 Hz, H-3′); ^{13}C NMR (DMSO-d_6) δ 59.97 (CH_3O–), 66.49 (C-6), 71.41 (C-4), 73.34 (C-2), 75.23 (C-5), 75.39 (C-3), 106.6 (C-1), 129.9 (C-2′), 136.2 (C-3′), 170.9 (C=O); Anal. Calcd. for $C_{10}H_{16}O_7$: C, 48.39; H, 6.69; O, 45.16. Found: C, 48.38; H, 6.45; O, 45.29.

Compound **II** (2,6-diacryloyl-β-methyl galactopyranoside) was synthesized using a portion of the finished enzymatic reaction mixture in which Compound **I** was formed. Specifically, 1 g of **I** was added to 100 ml of acetone (pre-dried over 4 Å molecular sieves) and 7 ml of vinyl acrylate was then added slowly, followed by 10 g of Lipase P. The reaction was allowed to proceed for 16 h at 25°C, and was stopped by removing the enzyme by filtration. Total enzymatic conversion of **I** to **II** was 36%, as determined by HPLC (83:17 (v/v), CH_3CN:H_2O). The acetone was then removed by rotary evaporation at 25°C in the presence of 30 g of chromatographic silica and **II** was purified by flash chromatography (72:5:4, ethyl acetate:MeOH:H_2O). The isolated yield of **II** was 30%. $[a]_D^{25}$ − 10.1 (c1, DMF); ^{13}C NMR (DMSO-d_6) δ 58.97 (CH_3O–), 66.49 (C-6), 71.41 (C-4), 68.72 (C-2), 75.23 (C-5), 77.30 (C-3), 105.0 (C-1), 130.6 (C-2″), 137.1 (C-3″), 170.9 (C=O′), 129.9 (C-2′), 136.2 (C-3′), 170.9 (C=O″); Anal. Calcd. for $C_{13}H_{18}O_8$: C, 51.68; H, 5.96; O, 42.36. Found: C, 51.65; H, 5.92; O, 42.43.

2.2.2. Polymer synthesis

The network poly-*co*-(6-acryloyl-β-methylgalactopyranoside/2,6-diacryloyl-β-methylgalactopyranoside) (hydrogel Network **I**), was synthesized from Compound **I** and the cross-linker Compound **II** in the manner described below. Different amounts of cross-linker were used in individual syntheses, creating a series of four polymers (Networks **Ia–d**). As an example, Network **Ib** was synthesized as follows: Compound **I** (2.0 g) was dissolved in 11.3 ml of DI water, creating a 15 wt.% monomer solution Compound **II** (10.9 mg) was then added, resulting in a solution with 0.5 mol% cross-linker relative to Compound **I**. The AIPD initiator (10 mg) was then added resulting in a solution of 0.5 wt.% AIPD, also relative to Compound **I**. The solution was placed in a polymerization tube and aspirated for 15 min to remove dissolved oxygen. After removal from the aspirator, the tube was immediately sealed and placed in a constant temperature bath at 55°C. Nitrogen was then passed through the system at a rate of 3 ml min^{-1} (at STP) and

the polymerization was allowed to proceed for 25 min, forming Network **Ib**. The reaction was terminated by removing the gel-like material from the polymerization tube. The residual initiator and unreacted monomer were removed by washing with copious amounts of DI water followed by immersion of the gel in a stirred DI water bath for 24 h. The isolated yield of Network **Ib** from Compound **I** was 85%. Networks **Ia**, **Ic**, and **Id** were synthesized in a manner identical to that for **Ib**, except 0.1, 1.0 and 5.0 mol% Compound **II** were used, respectively. The average isolated yield of Networks **Ia–d** from Compound **I** was 86%. Polymer charge was measured by base titration. To that end, 3.65 g swollen hydrogel were placed in 10 ml of distilled water under stirring. The charges were titrated with 0.015 M NaOH.

2.2.3. Gel water content and elastic modulus determination

The relative amount of water in hydrogels can be defined as the swelling ratio at equilibrium (SRE), which is the weight of the swollen gel divided by the weight of dried polymer network. SREs were measured using a Dupont Model 2950 thermogravimetric analyser (TGA), and all water used in gel SRE measurements was filtered, distilled and deionized. As described above, a typical SRE measurement consisted of a 10 mg sample of dry network placed in a beaker containing 100 ml of DI water. The network was allowed to swell for 12 h to reach equilibrium. A 25–50 mg fragment of the gel was cut away with a spatula, placed into the TGA sample pan, and its precise initial weight was recorded, as determined by the TGA microbalance. The sample was then automatically loaded into the TGA furnace heated to 65°C and was permitted to dry (de-swell) until no further weight loss was observed (ca. about 1 h). The final weight of the dry polymer network, as determined by the TGA microbalance, was recorded and the gel SRE was calculated. For any given hydrogel type this procedure was performed in triplicate, and the final reported SRE was the average of the three TGA measurements with a standard error of 1%.

Hydrogel elastic moduli were measured using a ball indentation method developed by Hertz [16]. The elastic modulus is directly related to Poisson's ratio v, ball weight F. ball radius r, and depth of the induced indentation h:

$$E = \frac{3(1 - v^2)F}{4h^{1.5}r^{0.5}}. \quad (1)$$

For E measurements, hydrogels were formed with at least one perfectly flat surface. To that end, immediately prior to gel synthesis, a disc-shaped mould 6 mm in diameter and 3 mm in height was introduced into the polymerization tube. After gel formation the mould was removed and the resulting disc-shaped gel was placed into DI water and permitted to swell in the usual manner. The swollen gel retained its original shape. A steel ball-bearing of known weight was carefully balanced on the gel surface and the resulting surface indentation was measured. The elastic modulus was then calculated from Eq. (1), based on an average of three measurements with a standard error of 7%. A Poisson ratio of 0.5 was chosen as being representative of elastic materials with no polymer crystallinity or voids [16] and is consistent with highly swellable materials.

2.2.4. Measurement of network swelling kinetics

In a typical transient swelling ratio measurement, a 4 mg rectangular sample of dry network was placed in a beaker containing 100 ml of DI water. The network was allowed to swell for a specified time and was then removed from the beaker. Entrained surface water was removed by blotting and the sample was quickly weighed and returned to the beaker for continuation of swelling.

3. Results and discussion

The syntheses of Networks **Ia–d** are shown in Fig. 1. The lipase from *Pseudomonas cepacia* catalyses the regioselective acryloylation at the 6-hydroxyl moiety of *β-O*-methyl galactopyranoside in anhydrous pyridine to give the monoacrylate (Compound **I**) in 75% isolated yield. Conversion of **I** into the 2,6-diacrylate cross-linker (Compound **II**) was performed in anhydrous acetone, also by *P. cepacia* lipase, resulting in an isolated yield of 36%. The authenticity of Compound **II** was verified by ^{13}C NMR which revealed O-acylation at the C-6 and C-2 hydroxyls. Acylation of the C-2 hydroxyl group results in a downfield shift of the peaks corresponding to C-2 and C-1 and an upfield shift (1–3 ppm) of the peaks corresponding to C-3 [17]. Shifts of this nature can be seen upon comparison of the spectrum of **II** with that of **I**, indicating secondary-OH acylation at C-2. This is consistent with the previously reported acylation patterns of the related *P. fluorescens* lipase towards galactosides in non-aqueous media [18]. Elemental analysis of **II** shows that the proportions of C, H and O correspond to that calculated for **II**.

Polymer synthesis in the presence of 0.5% (mol/mol) of **II** yielded the hydrogel Network **Ib**. Base titration of Network **Ib** indicated that 0.74 ± 0.10% of all polymer repeat units were hydrolysed to acrylic acid residues, thus providing a very small number of presumably dispersed network charges. Although a small number of charges were evident from the polymerization process, Network **Ib** did not experience a detectable loss of sugar pendant groups over a period of several weeks at 40°C. Thus, the hydrogel formed is stable. Similar charge densities were observed for Networks **Ia, c, d**.

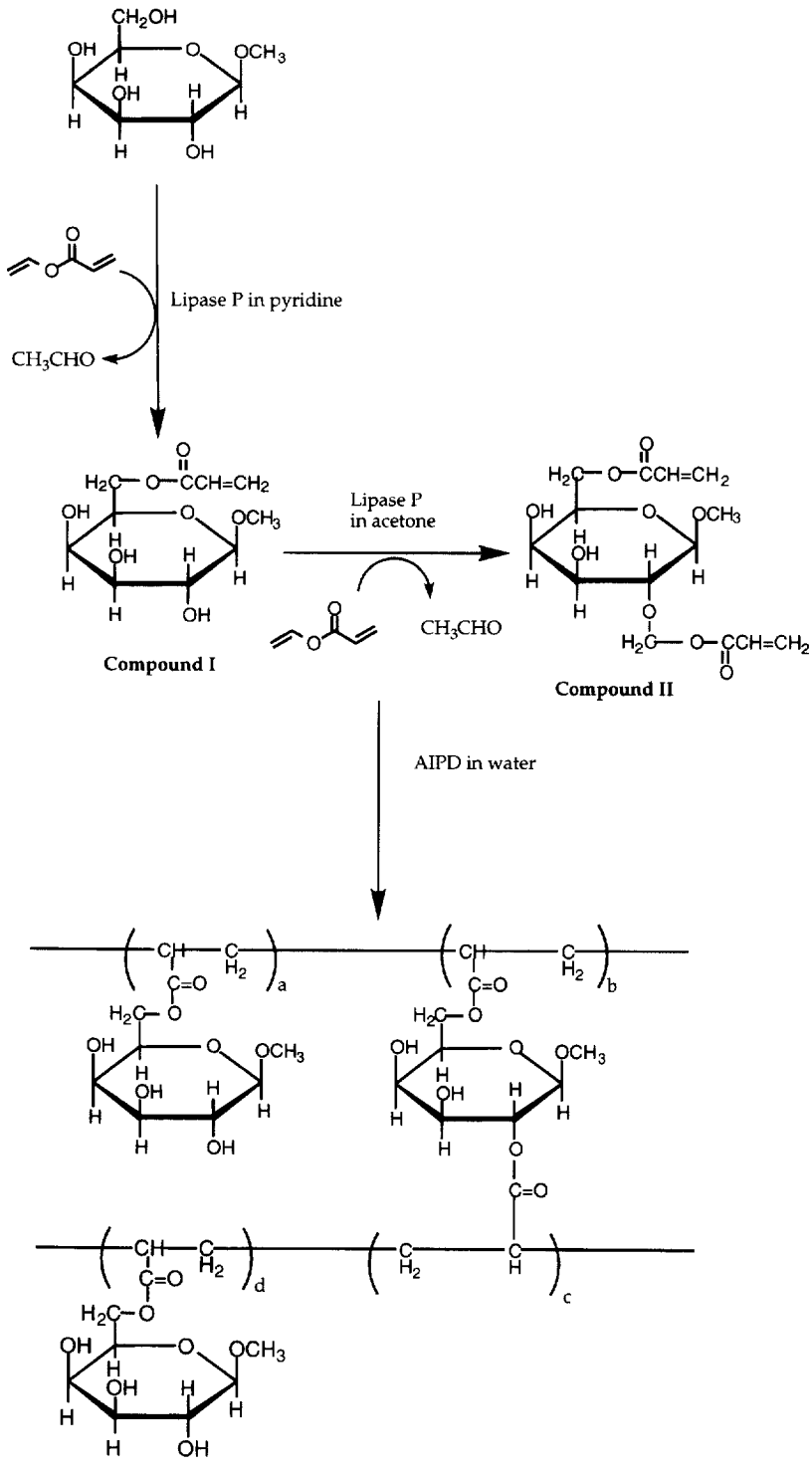

Fig. 1. Preparation of Network **I** using chemoenzymatic synthesis (poly-6-acryloyl-β-*O*-methylgalactopyranoside-*co*-2,6-diacryloyl-β-*O*-methylgalactopyranoside). See text for definitions of Networks **Ia–d**. The polymer chain lengths *a* and *d* are expected to be large. Chain lengths *b* and *c* are expected to be small, and ideally unity in a lightly cross-linked network.

3.1. Swelling characteristics of the galactose-based hydrogels—comparison to conventional hydrogels

The SREs of conventional hydrogels range from about 1.5 to over 1000 [6, 19]. SRE is strongly controlled by the chemical properties of the monomer employed and the type and concentration of cross-linker present. Hydrogels containing strong hydrogen-bonding networks (e.g. poly(acrylamide) and hyaluronic acid) swell to a greater extent than hydrogels containing moderate to low hydrogen bonding functionalities (e.g. pHEMA, poly(vinyl alcohol) and poly(*N*-vinyl pyrrolidone)) [11, 20–22]. Incorporation of charge (e.g. poly(acrylic acid)-based polymers) has a significant effect on hydrogel swelling. For example, incorporation of only 5% (w/w) acrylic acid into modified starch results in an SRE >300 [23]. Incorporation of 2.4% (w/w) acrylic acid into poly(acrylamide) results in an increase in SRE from about 40 to 276 [9]. Similarly, incorporation of 3% (w/w) methacrylamidopropyl triethylammonium chloride (MAPTAC, a positively charged co-monomer) into poly(acrylamide) results in an increase in SRE to 420 [7]. In general, high SREs above 200 cannot be achieved without some charge present in the polymeric network [9].

Macroscopically, the most significant property of our galactose-based hydrogels (Networks **Ia–d**) are their pronounced hydrophilicity. Hydrogels based on Networks **Ia–d** have high equilibrium water contents (Table 1) which are not generally attainable by conventional hydrogels in the absence of significant (i.e. several percentage) charge incorporation. For example, the SRE of Network **Ic** (containing 1% cross-link ratio) is higher than the SRE of poly(acrylamide) containing 3% (w/w) MAPTAC (containing 0.2% cross-link ratio) [8], even though the latter has a lower cross-link ratio and a higher charge density. Thus, the slightly ionized nature of Networks **Ia–d** (<1%, w/w) coupled with the high degree of monomer hydrophilicity is sufficient to promote a higher degree of swelling than other hydrophilic gels, and demonstrates the unique chemistry afforded by sugar-based polymers.

Table 1
Network properties of poly(6-acryloyl-β-*O*-methylgalactopyranoside)-based hydrogels[a]

Network	SRE	E (MPa)	ϕ_p ($\times 10^4$)	$\bar{X}_C$	ξ (Å)
Ia	1103	0.005	9.54	20325	6640
Ib	619	0.010	17.0	12320	4260
Ic	346	0.021	30.4	7120	2670
Id	171	0.088	61.4	2150	1160
II	27.4	0.251	415	1230	500

[a] Errors based on three independent measurements for E and SRE were ±1% for SRE, ±7% for E and ±10% for calculated values of $\bar{X}_C$.

The dependence of SRE on mole% Compound **II** (cross-linker) is also summarized in Table 1. For example, Networks **Ia–d** underwent a profound drop in SRE as the cross-link ratio was increased, with an SRE nearly 6.5-fold lower at 5% Compound **II** as cross-linker compared with 0.1% Compound **II**. Similar behaviour is observed for other strongly hydrogen-bonding polymeric gels (i.e. poly(acrylamide) and poly(acrylic acid)) [8, 10]. Increasing the cross-link ratio to 20% (Compound **II**) did not result in a further significant drop in SRE (data not shown), thereby suggesting that the hydrophilic nature of the cross-linker maintains the highly hydrogen-bonding structure of Networks **Ia–d** and, therefore, the high swelling characteristics of the hydrogel.

3.2. Network properties of galactose-based hydrogels

The swelling behaviour of common hydrogels is related to the elastic modulus through Eq. (2) [24, 25];

$$E = \frac{RT\phi_0^{2/3}\phi_p^{1/3}}{\bar{X}_C v_1} \tag{2}$$

where ϕ_0 represents the volume fraction of the polymer at network formation (=0.1275), ϕ_p is the volume fraction of the polymer in the swollen state and is obtained directly from values of SRE according to Eq. (3), v_1 is the molar volume of solvent, and $\bar{X}_C$ is the number of repeat units

$$\text{SRE} = \frac{(\phi_w\rho_w + \phi_p\rho_p)}{(\phi_p\rho_p)} \propto 1 + \frac{\phi_w\rho_w}{\phi_p\rho_p} \propto \frac{1}{\phi_p} \tag{3}$$

between cross-links (calculated using Eq. (2)). For gels that obey the affine ideal rubber elastic model [22, 26], the exponents of ϕ_0 and ϕ_p are 2/3 and 1/3, respectively. For gels with low cross-linker concentration, $\bar{X}_C$ is large.

Conventional Flory theory for polymer–solvent interactions is often used at this point to provide information about the degree of similarity between the polymer network and the solvent through calculated χ values [26]. However, while such relationships are valid for many polymer systems in non-polar solvents [27], the more polar the solvent and polymer, the more likely that directional associative forces (e.g. hydrogen bonding, ionic interactions, etc.) will cause significant deviations from the enthalpic model of Flory [28]. Thus, χ values were not evaluated in this work. Instead, there is a distinct property among the galactose-based hydrogel networks that indicates higher E values for a given SRE as compared to conventional hydrogels. For example, Network **Id** has a modulus over twice as high as poly(acrylamide) containing 0.2 mol% BIS (*N*,*N*′-methylenebisacrylamide) cross-linker (0.088 vs. 0.035) [8], yet has an SRE over four times higher (171 vs. 40). Only 60% ionized (at gel synthesis) poly(acrylic acid)-based gels can give a similar combination of high modulus with high SRE. For

example, Yin et al. [10] showed that 60% ionized poly (acrylic acid) has an SRE of 475 with a modulus of 0.019.

Such relative strength imparted to the galactose-based hydrogels may be due to the very high polarity associated with the side-chain sugar moieties on the polymeric backbone which may induce cohesive interactions between polymer chains that strengthen the hydrogel network. Ordinarily, such chain–chain interactions would reduce SRE; however, the highly hygroscopic nature of the sugar moieties is able to endow the hydrogel network with a high degree of water binding. This is a significant feature of the sugar-based hydrogels and demonstrates the potential to achieve highly swellable yet mechanically strong gels.

A critical parameter of hydrogels is their average mesh size, ξ, which is important for assessing the transport properties of solutes, particularly large biomolecules within the hydrogel network. ξ can be calculated through the use of Eqs. (4) and (5) [21, 24]:

$$\bar{r}_0^2 = C_n \bar{X}_c b^2 \tag{4}$$

$$\xi = \phi_p^{-1/3} (\bar{r}_0^2)^{1/2} \tag{5}$$

where $\bar{r}_0^2$ represents the average end-to-end subchain length (in Å) when the gel is unswollen and C_n is the polymer rigidity factor (assumed to be 8.9 by analogy to polar poly(vinyl alcohol) [29]. The symbol b represents the characteristic bond length of the polymer backbone (= 1.54 Å) [21]. The value of $\bar{X}_C$ is easily calculated as a function of cross-link ratios for the different hydrogels in Networks **Ia–d** (Table 1). The number-average molecular weights between cross-links (M_c, calculated directly from $\bar{X}_C$) are large, reflective of the high degree of swelling in the hydrogel. The polymeric chains are, therefore, long and flexible. This further enhances their ability to become solvated in the polymeric network, and may reduce the likelihood of chain entanglements. This latter property also aids in the high degree of swelling observed in the hydrogels.

Eqs. (3)–(5) allow calculation of ξ when E is determined experimentally. For dilute (e.g. $\phi_p < 0.05$) electrically neutral gels in a 'good' solvent, physical scaling laws and several light scattering measurements indicate that the pore size varies with polymer volume fraction with a power-law exponent of -0.75 (Eq. (6)) [24]:

$$\xi = \phi_p^{-0.75} \tag{6}$$

This expression describes networks with a non-Gaussian (i.e. 'real') chain distribution. When fit to such a power-law relationship, a plot of ξ vs ϕ_p for Networks **Ia–d** yields an exponent of -0.70 ($r^2 = 0.964$) (Fig. 2). The close agreement with the theoretical value provided in Eq. (6) suggests that the gel networks behave as real (vs. ideal, Gaussian) entities. The fit to Equation (6) also

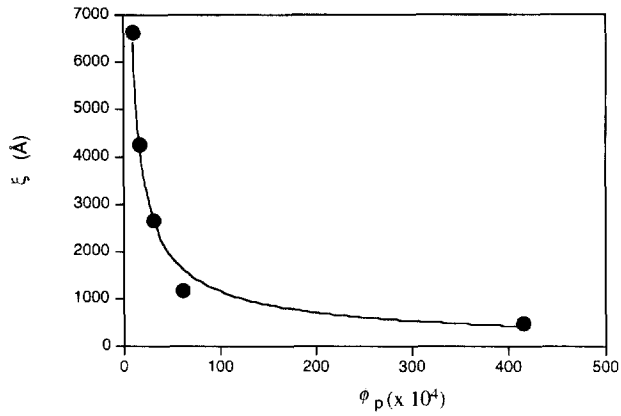

Fig. 2. Effect of polymer volume fraction on calculated average mesh size of hydrogels of Networks **Ia–d**. Individual gels are identified in Table 1.

suggests that the small amount of charged residues present in Networks **Ia–d** do not influence significantly their relatively high E values.

In previous work, we prepared the highly related hydrogel from α-methylgalactoside using methylene bisacrylamide as cross-linker [15]. Both the E and SRE values of this previously prepared hydrogel network were far lower than those obtained in the present study. This indicates that small changes in the polymer network can have a significant impact on the swelling properties of the hydrogel. In particular, BIS is substantially more hydrophobic than the β-*O*-methyl-2,6-diacryloylgalactoside cross-linker (Compound **II**) used in the present study. Moreover, BIS is quite dissimilar structurally to the sugar-based monomer. This may affect the interaction of the sugar monomer and cross-linker in polymerization reactions such that BIS may have a higher reactivity towards itself rather than with the sugar monomer. The sugar diacrylate cross-linker (Compound **II**), however, would be expected to react similarly to the sugar monomer in free radical polymerization, hence resulting in more uniformly distributed cross-links and relatively higher gel strength at low cross-link densities than BIS-based gels.

It was of interest to determine whether the chemoenzymatic methodology for sugar-based hydrogel formation has clear advantages over conventional chemical synthesis. To that end another hydrogel material (Network **II**) was prepared using non-selective chemical synthesis of the galactose-based acrylate monomer. In this case, chemical acryloylation (via acryloyl chloride) followed by silica gel fractionation yielded a mixture of galactose-based mono-acrylate monomers containing acrylate groups at two distinct positions on the pyranose ring structure (most probably the 6-OH and 3-OH positions). In the presence of 5% (w/w) cross-linker **II**, Network **II** swelled about 6.25-fold less than Network **Ic** (Table 1), indicative of a less effectively solvated hydrogel network.

This may be a result of the formation of an irregular polymer chain structure due to the different orientations of sugar pendants bound to the poly(acrylate) backbone. Such irregularity appears to restrict the polymer's rotational degrees of freedom which are necessary for effective solvation, thereby resulting in a lower SRE. Therefore, the cohesive chain interactions observed with Networks **Ia–d** may be at least partly due to the high degree of polymer regularity induced by the regioselective linkage of the sugar moiety to the poly(acrylate) backbone. This demonstrates that the high regioselectivity afforded by enzyme-catalysed transesterification is advantageous for the preparation of highly swellable sugar-based hydrogels.

3.3.1. *Kinetics of galactose-based hydrogel swelling*

The swelling of gels from an initially dry (xerogel) state to a fully swollen state is typically characterized by non-Fickian diffusion of the swelling solvent through the polymer chains. The deviation from Fickian behaviour is caused by several factors, including chain rearrangements during solvation and convective solvent transport, and is most pronounced in systems with strong hydrogen bonding [30]. These processes are dependent on the past history of the gel sample, especially on the method used to dry the polymer initially. Because of these complexities, relatively few studies of hydrogel swelling kinetics have been undertaken [31].

It is possible to characterize gel swelling kinetics through use of an empirical diffusion coefficient (D_p) [31]. The swelling of rubbery polymer networks, i.e. those above their glass transition temperature, in generally planar geometry, is described by the following form of Fick's Law [32]:

$$\frac{SR_t}{SRE} = 1 - \sum_{n=1}^{\infty}\left[\frac{8}{(2n+1)^2\pi^2}\right]\exp\left[-(2n+1)^2\pi^2\left(\frac{D_p t}{L^2}\right)\right] \quad (7)$$

where SR_t is the transient swelling ratio existing at time t, and L is the sample thickness in cm. For non-Fickian kinetics, D_p is simply an empirical constant indicating the rate at which a simple diffusional process would have to proceed to most closely match the observed swelling behaviour. For homogeneous hydrogels, values of D_p calculated from Eq. (7) vary from 1×10^{-8} to 8×10^{-7} cm^2 sec^{-1} [31]. For example, the D_p for polyacrylamide was found to be 3.2×10^{-7} cm^2 sec^{-1} [33].

The time-dependent swelling of Network **Ic** is depicted in Fig. 3. The SRE (346-fold) is reached in about 300 min, and the gel has attained 75% of this value in 50 min, or when t/t_{final} equals 0.167. The data were fit to Eq. (7) with truncation to one term ($n = 0$) yielding Eq. (8). This truncated expression leads to a difference in calculated D_p of less than 1% as compared to the result obtained using Eq. (7).

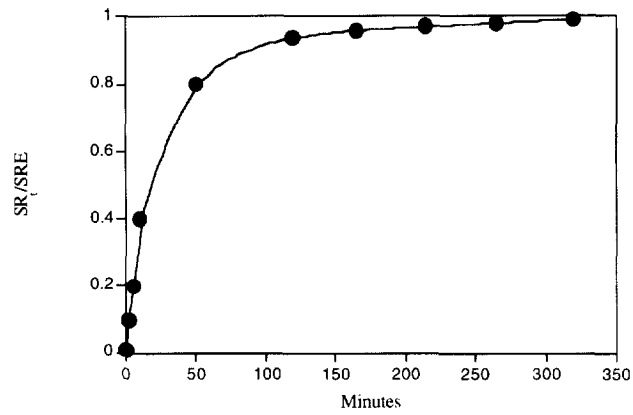

Fig. 3. Swelling kinetics of Network **Ic** in deionized water. See Experimental section for details.

$$\frac{SR_t}{SRE} = 1 - 0.909\exp(-0.0262t) \quad (8)$$

In Eq. (7), $L = 0.10$ cm and the resulting value of D_p is 3.13×10^{-6} cm^2 sec^{-1} for Hydrogel **Ic** (at $SR_t/SRE = 0.5$) with a correlation coefficient, r^2, of 0.991.

Values of D_p for swelling in water are expected to be high for hydrophilic polymers [31] and that of **Ib** is comparable to poly(acrylamide) and poly(vinyl methyl ether) [31, 33]. Direct comparisons are difficult because the physical state of the initially dry polymers may be different. It is, however, clear that **Ic** has a high rate of water uptake.

Hydrogel Network **Ic** was able to retain its high degree of swelling after several cycles of swelling and vacuum drying. No change in the values of SRE were evident following five swelling/drying cycles. This demonstrates that the galactose-based hydrogel does not experience hysteresis and that new polymer chain structures that may form during drying re-equilibrate during subsequent swelling.

4. Conclusions

Hydrogels **Ia–d** are very high swelling with equilibrium swelling ratios ranging from 170 to 1100, and corresponding average mesh sizes ranging from 1160 to 6640 Å. The chain distribution in the polymer networks appears to be non-Gaussian up to cross-link ratios of 5% (w/w). The gel swelling occurs rapidly, reaching 75% of the SRE at a unitless fractional time of 0.17. The extreme hydrophilicity of the gels clearly must stem from the polar nature of the sugars. Moreover, the enzymatic, regioselective monomer formation may result in a highly

ordered spatial distribution of the chain repeat units. This may be an additional reason for the high SREs and rapid swelling rates. Sugar-based hydrogels, such as those described herein, may find particular use as biomaterials because of their high water content, homogeneity, stability and expected non-toxicity. A wide range of pore sizes can be attained, suggesting that they may also be especially useful as matrices for enzyme immobilization and controlled delivery of biological macromolecules.

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