ELSEVIER

0144-8617(95)00082-8

Carbohydrate Polymers **28** (1995) 15–21
Elsevier Science Limited.
Printed in Great Britain.
0144-8617/95/$09.50+.00

Enzymatic and chemoenzymatic approaches to synthesis of sugar-based polymer and hydrogels

X. Chen[a], B.D. Martin[a], T.K. Neubauer[a], R.J. Linhardt[b], J.S. Dordick[a] & D.G. Rethwisch[a]*

[a]*Department of Chemical and Biochemical Engineering; and*
[b]*College of Pharmacy, University of Iowa, Iowa City, IA 52242, USA*

We have prepared a variety of sugar-based polymers using enzymatic and chemoenzymatic synthetic methods. In enzymatic synthesis, the regioselectivity of enzymes was used to copolymerize a sugar and a diester to make poly(sucrose adipate). Sugars including sucrose and glucose derivatives such as α-methyl glucoside have been used. In chemoenzymatic synthesis, the regioselectivity of enzymes was used to make the sugar-based monomers, while standard free radical or condensation methods were used for polymerization. The sugars could be in the backbone (poly(sucrose adipamide)) or as a pendant group (poly(sugar acetylene)s, poly(sugar acrylate)s, and poly(sugar methacrylate)s). We also prepared poly(sugar acrylate) and poly(sugar methacrylate) hydrogels and their copolymers with acrylic acid. The degree of swelling of these gels was studied as a function of pH and ionic strength. Potential applications of these materials include water absorbents, food additives and drug delivery systems.

INTRODUCTION

As illustrated by the wide diversity of natural polysaccharides, enzymes are well suited to synthesize hydrophilic materials based on sugars. Many of the current generation of hydrophilic polymers are prepared by chemical techniques (e.g., polyvinyl alcohol, polyacrylates, etc.). Typical chemical catalysts provide the chemoselectivity required for the preparation of high molecular weight poly(esters, amides, ols, acrylates), etc., while enhancing reaction rates. There is an increasing need, however, to impart additional selectivity (e.g., regio- or stereo-) to the synthesis of polymers (Black *et al.*, 1963; Chen & Tsai, 1990; Wulff & Dhal, 1990). This is particularly important in the synthesis of optically active polymers or the synthesis of linear polymers from monomers with functionality greater than two. For example, sucrose contains eight hydroxyl groups all with reactivity toward chemical acylation. Non-enzymatic linear polycondensation using synthetic catalysts requires the blocking of six groups followed by polymerization and deblocking. Such a task is tedious and results in a mixture of isomers and, presumably, a highly irregular polymer structure.

Recent advances in enzymatic catalysis in non-aqueous media have shown that enzymes are useful catalysts for the synthesis of polyesters and phenolic resins (Wallace & Morrow, 1989; Margolin *et al.*, 1987; Dordick *et al.*, 1987; Ryu *et al.*, 1989). Unlike conventional chemical catalysts, enzymes can synthesize polymers with exquisite selectivity. For example, in previous work we have shown that an alkaline protease from a *Bacillus* sp. catalyses the polycondensation of sucrose and an adipic acid derivative which results in an alternating linear polyester containing sucrose in the backbone (Patil *et al.*, 1991*b*). The high degree of regioselectivity provided by the enzyme enabled sucrose (with eight free hydroxyl groups) to react as if it were a diol. In this situation, no crosslinking was observed.

Our work has focused on the development of enzymatic or combined enzymatic/chemical (e.g., chemoenzymatic) synthetic strategies to prepare sugar-based polymers (Patil *et al.*, 1991*a*, 1991*b*). For complete enzymatic polymer synthesis the enzyme directly catalyses the copolymerization (diacylation) of a sugar with a diacid ester. The result is an alternating copolymer of the sugar and the diacid. Our results with these materials are described in the first section of this paper. In the second section we will discuss chemoenzymatic synthesis in which the polymers are synthesized in two steps. Initially, an enzyme catalyses the regioselective acylation of a sugar. The resulting derivatized sugar (monomer) is then chemically polymerized to give a long-chain, linear polymer. A variety of sugars including glucose, galactose, mannose, and sucrose have been used in these reactions. The

*Author to whom correspondence should be addressed.

intent of this paper is to provide an overview of the variety of sugar-based materials that can be made using enzymatic and chemoenzymatic syntheses.

MATERIALS AND METHODS

Materials

The enzyme Alcalase 2T was obtained from Novo Nordisk A/S, the enzyme Proleather from Amano, sucrose from Sigma, the azo initiator VA-044 (2,2′-azobis(2-(2′-imidazolin-2-yl) propane) dihydrochloride) from Wako, di(2,2,2-trifluoroethyl)adipate and 2,2,2-trifluoroethylmethacrylate (TFEM) from Aldrich, and the cross-linker BIS GenAR (*N*,*N*′-methylenebisacrylamide) from Mallinckrodt. Pyridine was stored over Type 3A molecular sieves, both from EM Science. DMF ChromAR HPLC was purchased from Mallinckrodt. All other chemicals and solvents used in this work were obtained commercially and were of the highest purity available. All water used in preparation and in swelling experiments was Millipore purified (resistivity 18 Mohm cm).

Methods

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker WM360 MHz instrument with D_2O as the solvent and 3-(trimethylsilyl)propionic acid-d_4, sodium salt (TSP) as the internal reference. The swelling ratio of a hydrogel equilibrated in water was determined using a Thermogravimetric Analyzer (TGA) from TA Instruments (model 2950). A small (< 50 mg) piece of gel was cut using a razor blade, blotted dry, and a sample placed on the weighing pan. The initial weight was recorded and the isothermal oven set at 65°C. Measurement was continued until the weight stabilized (between 30 and 60 min). This weight was recorded as the final dry weight. The swelling ratio was calculated as grams of swollen gel/grams of dry gel. Sucrose methacrylates were purified by flash chromatography using a 57 mm diameter column packed with 6 inches of activated silica gel from J.T. Baker Inc. and 1/4 inch of white quartz sand (50–70 mesh) from Aldrich. The eluant was a 17:4:1 ratio of ethyl acetate, methanol and water. Purified monoester was pooled, the eluant removed by rotary evaporation, and the product freeze dried.

Synthesis of monomer

The enzyme was added to pyridine to make a 5% (w/v based on sucrose) solution. Hydroquinone (Fisher) was added in a 1:20 weight ratio of hydroquinone to sucrose as a polymerization inhibitor. The acylating agent trifluoroethyl (meth)acrylate was added in a 3:1 mole ratio to sucrose. The reaction mixture was stirred at room temperature for 48 h after which the reaction was stopped by removing the enzyme by centrifugation. The pyridine was removed by rotary evaporation at 35°C.

Synthesis of hydrogels

Hydrogels were prepared by polymerizing the desired amounts of sucrose methacrylate, crosslinker (BIS), initiator (VA-044), and water in a 2 dram vial with a total liquid volume of 1 ml. The reaction mixture was degassed under vacuum for 5 min and sparged with nitrogen gas for 5 min before placing in a 55°C oil bath. Gelation usually occurred within approximately 20 min; the reaction was allowed to proceed for 2 h. The gels were then removed from the vials and immersed in water for 2–5 days at room temperature, changing the water at least once a day. This extracted uncrosslinked polymer or reactants were still present in the gel and allowed the gels to swell to their equilibrium values. Detailed syntheses of poly(sugar adipate), poly(sucrose acrylamide), poly(sucrose acrylate), and polyacetylenic polymers are given elsewhere (Patil *et al.*, 1991*a*, 1991*b*; Blinkovsky & Dordick, 1993).

RESULTS AND DISCUSSION

Enzymatic incorporation of sugars into linear polymers

Linear polyesters were formed enzymatically by polycondensation of equimolar amounts of a sugar and a diester. These reactions were carried out using pyridine as a solvent. The use of a non-aqueous solvent such as pyridine enables hydrolytic enzymes such as lipases and proteases to catalyse polyester synthesis, often with high degrees of enantioselectivity (Margolin *et al.*, 1987; Wallace & Morrow, 1989). In water, these enzymes would favor the hydrolysis of ester bonds, leading to depolymerization.

We have focused on the incorporation of sugars into polyesters (Patil *et al.*, 1991*a*, 1991*b*; Dordick, 1992). The challenge in such an approach is 2-fold. Firstly, a sugar must function as a diol in this polymerization, though more than two chemically reactive hydroxyls are present. Secondly, an appropriate enzyme must be identified to catalyse this reaction in non-aqueous media. Following a screen of over 60 commercially available lipases and proteases, we identified an enzyme (Proleather from Amano, a *Bacillus* protease) which was capable of polymerizing sucrose with adipic acid derivatives in anhydrous pyridine (Patil *et al.*, 1991*b*). Our selection of solvents was limited. We needed a polar solvent to dissolve reasonable quantities of sugar; however, polar solvents can also inactivate enzymes. For example, while sugars are generally more soluble in

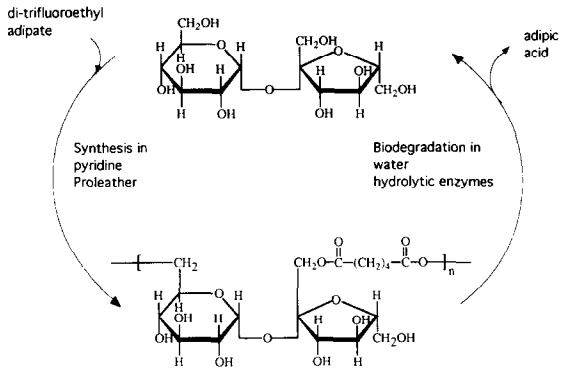

Fig. 1. Enzymatic synthesis and degradation of poly(sucrose adipate).

DMF, DMF inactivated most of the enzymes we tested. Pyridine satisfied both of the required criteria.

Figure 1 depicts the synthesis of poly(sucrose adipate) by the transesterification of sucrose with di(2,2,2-treifluoroethyl)adipate. The trifluoroethylester was used because trifluoroethanol is a weak nucleophile, and this provides a more favorable reaction equilibrium leading to more complete conversion. In addition, enzymes are kinetically activated by esters with good leaving groups. Only the 6 and 1′ positions of sucrose were acylated ensuring that the material was not crosslinked. Water-soluble polymers were prepared which contained over 100 sucrose units. In addition to sucrose, polymers of raffinose, lactose, and fructose have been prepared using this method.

Since these sugar-containing polyesters were prepared by enzymes, it was logical to assume that they would be decomposed by enzymes. In fact, the same enzyme that catalyses the polymerization of sucrose in anhydrous pyridine degrades the polymer in water (see Fig. 1). A variety of lipases catalysed the depolymerization of poly(sucrose adipate) in water, nearly completely breaking it down to sucrose and adipic acid. The polymer is, therefore, prepared and broken down by biological means.

Chemoenzymatic polymer synthesis

The polymers produced by enzymatic polymerization displayed interesting properties. They were water-soluble in essentially all proportions, and our results indicate that they were completely biodegradable. However, the molecular weights were generally low. For example, for poly(sucrose adipate) the molecular weight was typically *c.* 2000. Better results were obtained for poly(raffinose adipate) with a molecular weight of *c.* 20000. The molecular weight limitation is thought to have arisen from several factors:

1. To achieve a high molecular weight in a polycondensation reaction, the condensation products (in this case, trifluoroethanol) must be removed or the molecular weight will be limited by equilibrium conversion. While trifluoroethanol was selected to give a favorable equilibrium, equilibrium will still limit conversion. In addition, any traces of water in the system will greatly limit the final molecular weight by leading to hydrolysis of the ester linkages.
2. Exactly stoichiometric amounts of the diol (sugar) and diester are required to achieve high molecular weights. In addition, none of the reactants should be consumed in any side reactions.
3. High molecular weight polymer may be a poor substrate for the enzyme. Therefore, as molecular weight increases the reaction slows.

A further drawback with enzymatic polymer synthesis was the slow rate of catalysis – the sucrose polyester synthesis was complete only after 3 weeks (Patil *et al.*, 1991*b*). For comparison, typical acid- or base-catalysed polycondensation reactions are typically completed in a matter of hours or minutes. However, the enzyme is only needed for the highly selective step(s) in polymer synthesis (such as monomer preparation). Conventional chemical catalysis could then be used for the bulk polymer synthesis from the enzymatically prepared monomers. These combined chemical/enzymatic (or chemoenzymatic) processes would have advantages inherent to each type of catalysis – namely, high selectivity imparted by the biocatalyst and high reactivities (and sometimes novel chemistries) associated with chemical catalysts. Below, we describe the chemoenzymatic synthesis of several classes of sugar-based polymers. In all cases, selective enzymatic modification of a sugar is performed initially, followed by a non-selective chemical polymerization.

Poly(sucrose adipate) gel

As indicated above, the sucrose polyesters were of fairly low molecular weight; however, they contained a large number of reactive hydroxyl groups which were available for subsequent reaction. In a sense we can consider the poly(sucrose adipate) to be a pre-polymer that could be used in a subsequent reaction to make a higher molecular weight polymer. This was carried out using methylene diisocyanate to crosslink the lower molecular weight polymer fragments. The diisocyanate reacts with hydroxyl groups to form urethane linkages. Reaction of a diisocyanate with hydroxyl groups in two different poly(sucrose adipate) molecules leads to a crosslink and the formation of a poly(sucrose adipate) gel. However, this is a non-selective reaction and any of the hydroxyl groups could react randomly. To achieve gelation, high diisocyanate/free hydroxyl ratios were required. Typical reaction ratios were 2–4 moles diisocyanate per mole of hydroxyl group. As a result the gels were not particularly hydrophilic and absorbed only their own weight in water (swelling ratio of 2).

Poly(sucrose adipamide)

The enzymatic preparation of poly(sucrose adipate) was slowed in part by the low solubility of sucrose in pyridine. As noted above, polymerization required *c.* 1:1 stoichiometry of the diester and diol which means the concentration of the comonomer, di(2,2,2-trifluoroethyl)adipate, was also low. The presence of excess di(2,2,2-trifluoroethyl)adipate speeds up the reaction and leads to the formation of sucrose 6,1′-di(trifluoroethyladipate), a bifunctional monomer which can be used in a subsequent polymerization step. Poly(sucrose adipamide) was synthesized by copolymerizing sucrose 6,1′-di(trifluoroethyladipate) and ethylenediamine in *N*-methylpyrrolidone (NMP) as shown in Fig. 2 (Patil *et al.*, 1991*a*). Moderate-sized linear polymers with $M_n = 4800$ and $M_w = 8100$ were produced with sucrose in the polymer backbone. A substantial byproduct (*c.* 50%) was found to be sucrose monoadipate (the ester linkage either at the C-6 or C-1′ position), presumably formed by the reaction of ethylenediamine with the internal ester linkage between the sucrose and the adipate derivative.

Poly(sugar acetylene)s

Regioselective chemoenzymatic synthesis has been extended to the preparation of poly(sugar acetylene)s (Blinkovsky & Dordick, 1993). The strategy in this case was to use a selective, enzymatic reaction to attach an acetylene group to the sugar, and to form the poly(sugar acetylene) in a subsequent step (see Fig. 3). Propargyl alcohol was used as a glycosyl acceptor in the transglycosylation reactions of glycosidases with various disaccharides including lactose, maltose and cellobiose. For example, reaction of propargyl alcohol with lactose catalysed by β-galactosidase in aqueous buffer containing 25% (v/v) propargyl alcohol resulted in the stereospecific formation of propargyl-β-D-galactopyranoside in 42% yield. The β-galactosidase reaction is absolutely specific for the formation of β-galactosides. Polymerization of propargyl-β-D-galactopyranoside with

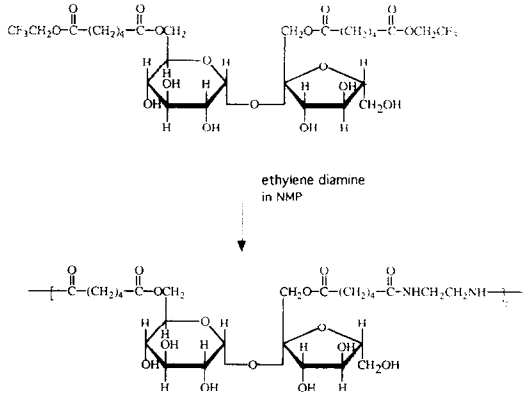

Fig. 2. Chemoenzymatic synthesis of a poly(sucrose adipamide).

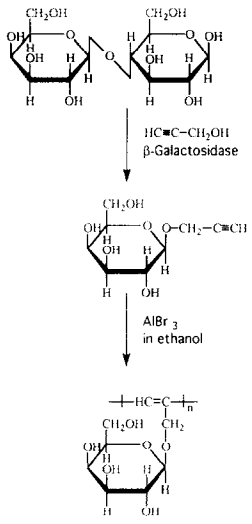

Fig. 3. Chemoenzymatic synthesis of a polyacetylene – Poly(Propargyl-β-D-galactopyranoside). NMP is the solvent *N*-methylpyrrolidone.

$AlBr_3$ in ethanol resulted in the formation of oligomeric poly(acetylenic) species ($M_w + 1300$). Similar polymers have been prepared from propargyl-β-glucoside and propargyl α-glucoside (from cellobiose and maltose, respectively). These materials are water-soluble and may be useful as hydrophilic acetylenic compounds for development into electrically conducting hydrophilic films or resins. In addition to poly(acetylenic) materials, poly(ethylenic) compounds have also been prepared chemoenzymatically (Blinkovsky & Dordick, 1993). Transgalactosylation of lactose with allyl alcohol results in the formation of a galactoside with a double bond aglycon. Polymerization by free radical catalysts results in polymers with $M_W > 30\,000$.

Poly(sugar acrylate)s and poly(sugar methacrylate)s

Perhaps the largest group of polymers we have prepared by chemoenzymatic means are polyacrylates and polymethacrylates that contain sugars as pendant groups. For simplicity, in this discussion (meth)acrylate will be used to indicate that either acrylate or methacrylate species can be used. Once again, the large number of reactive hydroxyl groups on sugars presents a challenge for selective acylation. However, in this case monoacylation is required to form linear polymers. Diacylated (or higher acylated) (meth)acrylate derivatives would result in crosslinking. In the presence of vinyl (meth)acrylate, a number of bacterial proteases catalysed the monoacylation of sucrose in the 1′ position using a bacterial subtilisin (an alkaline protease) in pyridine. Yields of sucrose 1′-acrylate in excess of 80% were obtained in 24 h (Chen *et al.*, 1994).

Polymerization of sucrose 1′-(meth)acrylate was performed in water using an ammonium persulfate (AP)/$FeSO_4$ or an azo initiator VA-044 (2,2′-azobis(2-(2′-imidazolin-2-yl) propane) dihydrochloride) (Chen *et*

al., 1994; Neubauer, 1994). Similar reactions were carried out with a variety of other sugars and sugar derivatives including α- and β-methylgalactoside, α- and β-methylglucoside, and α-phenylgalactoside (Martin *et al.*, 1992).

While the exact structure of these polymers in solution has not been determined, we have carried out molecular modeling using the SYBYL molecular modeling package of Tripos, Ass. Figure 4 shows that a molecular model of the poly(*O*-methylglucoside acrylate), prepared by energy minimization of a 40-mer polymer in water, appears to give a helical network of *O*-Me glucoside residues surrounding the non-polar polyacrylate backbone. Thus, the hydrophilic sugar groups shield the hydrophobic polyacrylate backbone. This is consistent with the structures of many natural polysaccharides such as agarose which exist in double or triple helical form.

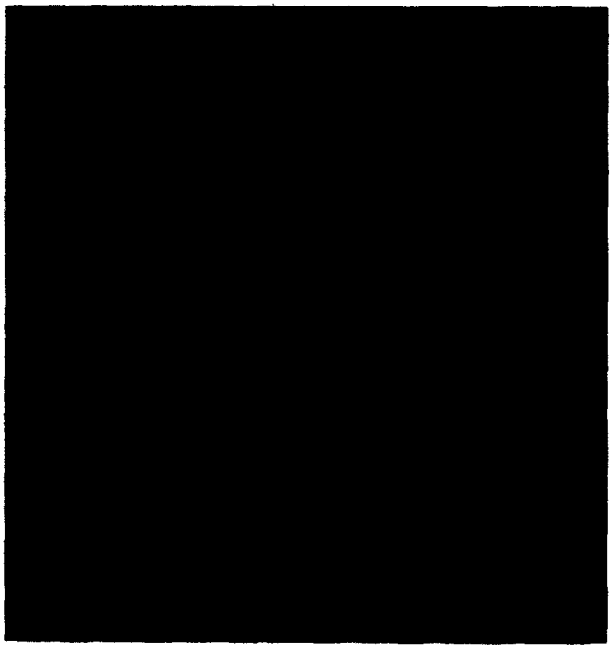

Fig. 4. Computer-generated molecular model of poly(*O*-Me-galactoside 6-acrylate).

Because of the hydrophilicity of the sugar groups, the poly(sugar acrylate)s are highly water soluble and a number of applications for these linear polymers can be anticipated. For example, we are preparing densified samples by casting these poly(sugar acrylate)s. These will be used to determine the physical strength and processability of these materials. If the correct properties are achieved, one could envision a water-soluble plastic for use as a packaging material. However, because of the multiple hydroxyls remaining on the sugar groups there are many different ways to customize these materials. This leaves open the possibility for additional applications. For example, attachment of ionic groups to free hydroxyl moieties on the polymer will result in mutual repulsion causing increased spreading of the polymer chains in an aqueous solution. These ionic polymers are expected to be functional as viscosity enhancing agents and as flocculants in the purification of municipal water supplies (Glass, 1989).

Poly (sugar acrylate) and poly (sugar methacrylate) hydrogels

We have extended the methodology of preparing sugar-containing polymers to the synthesis of hydrogels from sugar-based starting materials (Martin *et al.*, 1992). Potential applications of such hydrogels include drug delivery matrices, bioimplantables, contact lens materials, and functional components of permselective membranes (Kim *et al.*, 1992; Murphy *et al.*, 1988). Once again, the initial step is the enzyme-catalysed acylation of sugars in pyridine with vinyl acrylate to the corresponding monoacrylate derivatives (Fig. 5). Sucrose and a number of monosaccharide derivatives

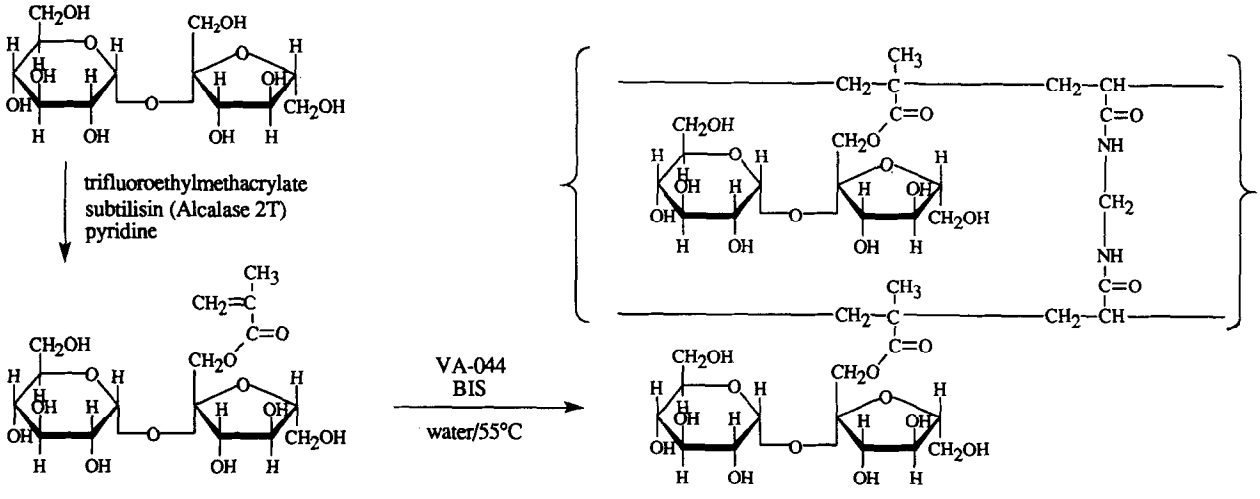

Fig. 5. Chemoenzymatic synthesis of poly(sucrose methacrylate) hydrogel. VA-044 is the initiator, 2,2′-azobis(2-(2′-imidazolin-2-yl) propane) dihydrochloride and BIS is the crosslinking agent, *N*,*N*′-methylenebisacrylamide.

have been employed. The monosaccharides are particularly appealing because sugars with different glycons and aglycons can be used to impart different properties to the resulting hydrogel matrix.

The sucrose methacrylate was polymerized in water with 0.15 mol% VA-044 as the initiator and *c.* 1–2% (w/w) of β-methylglucoside 2,6-diacrylate or *N,N'*-methylenebisacrylamide (BIS) as the crosslinker. Increasing the density of crosslinks usually leads to decreased swelling, but increased strength. So a trade-off must be made to achieve the desired balance of properties. The diacrylate crosslinker employed in our studies is tailored to function for our sugar-based materials. It has several important attributes. Firstly, it is a hydrophilic material, so when it is used as a crosslinker, it will not dramatically diminish the hydrophilicity of the sugar monomers. Secondly, the crosslinker is a sugar-based acrylate and should polymerize at nearly the same rate as the sugar acrylate monomers. This should guarantee uniform crosslinking throughout the hydrogel matrix and block copolymer formation is avoided.

As is generally the case with hydrogels, the water absorbency of the material is a strong function of the synthetic conditions. Important variables include initial monomer concentration, crosslink density, reaction temperature, and the absence or presence of ionic groups in the polymer. As expected, increasing the crosslinker content results in lower water absorbencies; however, even at 20% (w/w) diacrylate crosslinker, a poly(α-methylgalactoside acrylate) hydrogel still swells to 72 times its weight in water. Thus, the sugar-based crosslinker does not dramatically affect the hydrating capacity of the hydrogels. Figure 6 shows the effect of changing the initial monomer concentration in the polymerization solution on the swelling of the resulting poly(sucrose 1'-methacrylate) hydrogels. As the initial concentration of monomer in the preparation solution increases from 5 to 25 wt percent, the swelling ratio in deionized water decreases from 23 to 4. Note that these samples all had a fixed crosslink concentration of 2 mol% BIS.

The poly(sugar (meth)acrylate) hydrogels contain no charged species; therefore, their swelling is essentially independent of ionic strength and is independent of pH from pH 1.5 to 9. Incorporation of ionic groups in a hydrogel leads to increased swelling due to charge–charge repulsion and an increase in the osmotic pressure of water in the gel. Therefore, a series of samples was prepared by copolymerizing sucrose 1'-methacrylate and acrylic acid. Acrylic acid was added because it is ionized in the pH range investigated (near pH 7). The effect of the ionic strength of the swelling solution on these hydrogels prepared is shown in Fig. 7. The swelling of the hydrogel with no acrylic acid was essentially independent of ionic strength. As expected, the swelling of the hydrogels is increased with increasing incorporation of acrylic acid. The swelling of these ionic hydrogels is a strong function of ionic strength. For example, the sample containing 5 mol % AA absorbs *c.* 110 times its weight in pure water, but the swelling ratio rapidly decreases to *c.* 10 at an ionic strength of 0.01 M $CuCl_2$. The swelling changes little for further increases in ionic strength.

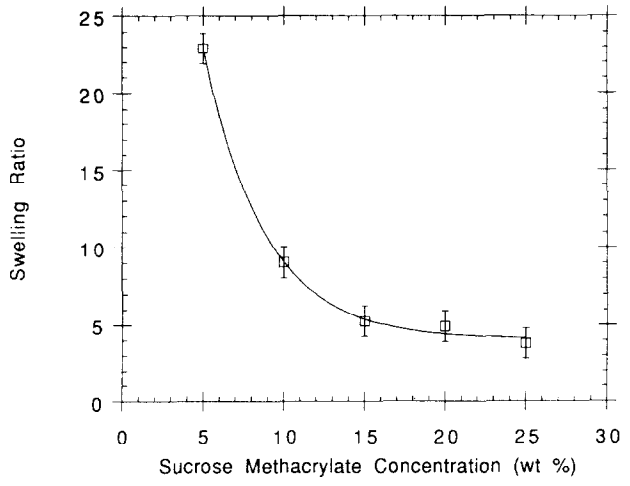

Fig. 6. Swelling ratio as a function of sucrose 1'-methacrylate concentration in the polymerization solution for poly(sucrose 1'-methacrylate) hydrogel. These hydrogels contain 2 mol% BIS crosslinker and the initiator concentration was 0.15 mol% VA-044.

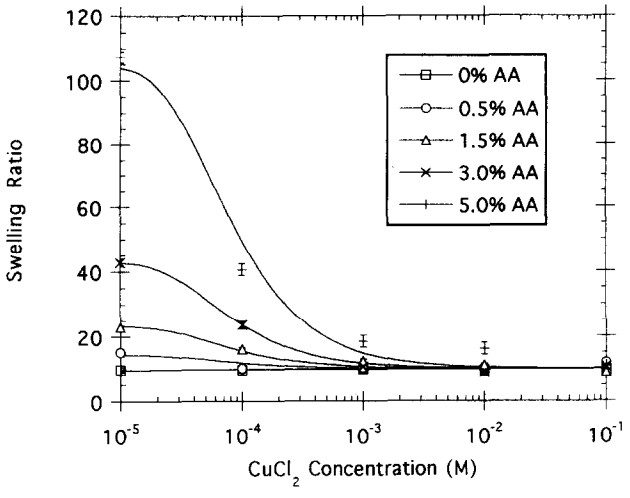

Fig. 7. Swelling ratio as a function of ionic strength and acrylic acid content for poly(co(sucrose 1'-methacrylate/acrylic acid)) hydrogels. These hydrogels contain 2 mol% BIS crosslinker and the initiator concentration was 0.15 mol%

Samples with swelling ratios from 20 to 1000 have been prepared by adjusting the crosslink ratio and the ionic content though at a swelling ratio of 1000 there was little mechanical strength. In general, the mechanical properties of sugar-based hydrogels are strongly influenced by the crosslink content. Below 10% (w/w) diacrylate crosslinker the gels are easily deformed (with elastic moduli of 0.02–0.04 MPa), though they return to

their original conformation when the stress is released. The gels become substantially stronger at 20% (w/w) diacrylate crosslinker and have similar mechanical strength to more conventional hydrogels such as poly(hema) and poly(acrylamide) (Baker *et al.*, 1988). Thus, the high degree of mechanical strength along with high water absorbency appear to be unique attributes of this material.

Based on the results of the linear polymers, the hydrogels are also expected to be biodegradable. Thus, only *c.* 16% (w/w) of the non-swelled polymer (the acrylate backbone) represents an environmentally stable residue. Sugar-based hydrogels, therefore, provide an attractive commercial alternative as water-absorbent materials.

In summary, the chemoenzymatic synthesis of sugar-based hydrogels offers a unique approach to develop non-toxic, highly water absorbent materials for use in applications such as general water absorbents, water treatment additives, and eventually in biomedical devices (Langer & Vacanti, 1993; Werblin *et al.*, 1992). The high specificity of enzymes coupled with the efficiency of chemical polymerization enables an economical approach to hydrogel synthesis as well as for the preparation of a biodegradable matrix. Such materials may have significant commercial potential as replacements for existing water absorbents.

ACKNOWLEDGEMENTS

The authors would like to extend their thanks to the following students and postdocs for their efforts in performing the research described in this work: Joseph O. Rich for the molecular modeling work, and Drs Damodar Patil and Stephen Ampofo for their pioneering efforts in the polymer syntheses. Parts of this work have been funded by grants from the US Army, National Science Foundation, The Sugar Association, USDA, and the Iowa Corn Promotion Board.

REFERENCES

Baker, D.A., Corhill, P.H., Ng, C.O., Skelly, P.S. & Tighe, B.J. (1988). *Polymer*, **29**, 691–700.

Black, W.A.P., Dewar, D.T. & Rutherford, D. (1963). *J. Chem. Soc.*, 4433004439.

Blinkovsky, A.M. & Dordick, J.S. (1993). *Tetrahedron: Asymmetry*, **4**, 1221.

Chen, X., Johnson, A., Dordick, J.S. & Rethwisch, D.G. (1995). *Makromol. Chem. Phys.*, 3567–3579.

Chen, S.H. & Tsai, M.L. (1990). *Macromolecules*, **23**, 5055–5058.

Dordick, J.S. (1992). *Ann. N.Y. Acad. Sci.*, **672**, 352.

Dordick, J.S., Marletta, M.A. & Klibanov, A.M. (1987). *Biotechnol. Bioengng*, **30**, 31–36.

Glass, J.E. Ed. (1989). *Polymers in Aqueous Media, Adv. Chem. Ser.*, Vol. 223, American Chemical Society, Washington, D.C.

Kazanskii, K.S. & Dubrovskii, S.A. (1992). *Adv. Polym. Sci.*, **104**, 97–134.

Kim, S.W., Bae, Y.H. & Okano, T. (1992). *Pharm. Res.*, **9**, 283–290.

Kulicke, W.M. & Nottelmann, H. (1989). In *Polymers in Aqueous Media, Adv. Chem. Ser.*, Vol. 223, ed. J.E. Glass. American Chemical Society, Washington, D.C., pp. 15–44.

Langer, R. & Vacanti, J.P. (1993). *Science*, **260**, 920–926.

Margolin, A.L., Crenne, J.-Y. & Klibanov, A.M. (1987). *Tetrahedron Lett.*, **28**, 1607–1610.

Martin, B.D., Ampofo, S., Linhardt, R.J. & Dordick, J.S. (1992). *Macromolecules*, **25**, 7081–7085.

Murphy, S.M., Hamilton, C.J. & Tighe, B.J. (1988). *Polymer*, **29**, 1887–1893.

Neubauer, T.K. (1994). Chemoenzymatic synthesis and characterization of sucrose 1′-methacrylate hydrogels. M.S. Dissertation, University of Iowa.

Patil, D.R., Dordick, J.S. & Rethwisch, D.G. (1991*a*). *Macromolecules*, **24**, 3462–3463.

Patil, D.R., Rethwisch, D.G. & Dordick, J.S. (1991*b*). *Biotechnol. Bioengng*, **37**, 639–646.

Ryu, K., Stafford, D.R. & Dordick, J.S. (1989). *ACS Symposium Ser.*, **389**, 141–157.

Wallace, J.S. & Morrow, C.J. (1989). *J. Polym. Sci. Part A: Polym. Chem.*, **27**, 2553–2567.

Werblin, T.P., Patel, A.S. & Barraquer, J.I. (1992). *Refractive and Corneal Surgery*, **8**, 23–26.

Wulff, G. & Dhal, P.K. (1990). *Macromolecules*, **23**, 4525–4527.