ELSEVIER

Carbohydrate Polymers 40 (1999) 183–190

Carbohydrate Polymers

Synthesis and characterization of dextran-based hydrogel prepared by photocrosslinking

S.H. Kim, C.Y. Won, C.C. Chu*

Fiber and Polymer Science Program, Department of Textiles and Apparel, Biomedical Engineering Program, Cornell University, Ithaca, NY 14853-4401, USA

Received 9 December 1997; received in revised form 1 April 1999; accepted 2 April 1999

Abstract

A polysaccharide-based hydrogel was prepared by photocrosslinking of modified dextran. Dextran was first bromoacetylated by bromoacetyl bromide and was subsequently reacted with sodium acrylate for incorporating a vinyl group. The acrylated dextran was then irradiated by a long-wave UV lamp for photocrosslinking. Reaction products (bromoacetyl dextran, acrylated dextran, and hydrogel) were characterized by elemental analysis, FT-IR, ^{1}H-NMR, and ^{13}C-NMR. The prepared dextran hydrogels showed a wide range of swelling behavior in different pH media, depending on the degree of substitution in bromoacetyl dextrans.

Keywords: Hydrogel; Dextran; Bromoacetyl bromide; Swelling

1. Introduction

Hydrogel is a material, when placed in excess water, able to swell rapidly and retains large volumes of water in its swollen three-dimensional structure without dissolution (Park, Shalaby & Park, 1993). Recently, intelligent hydrogels, which can respond to an external stimuli, such as pH, temperature, and magnetic field, were developed and generated a lot of interest in this area (Dagani, 1997; Li, Hu & Chen, 1997; Nagasaki & Kataoka, 1997; Park & Orozco-Avila, 1992; Park & Orozco-Avila, 1993; Park & Hoffman, 1991; Park & Hoffman, 1994). However, most of the advanced hydrogel systems mentioned above were developed from synthetic non-biodegradable polymers. There is a need to develop non-toxic biodegradable hydrogels for biomedical applications. As a result of their abundant sources and variety, natural polysaccharides would be good candidates as starting materials in preparing intelligent hydrogels, especially for use as drug delivery, artificial muscles, and wound healing.

In this paper, a synthetic method to use dextran as the starting material to make hydrogels is described. Dextran is a naturally occurring non-toxic biodegradable polymer and widely used in many biomedical areas (Mora & Pato, 1990). Dextran contains primarily (1 → 6) linked α-D-glucopyranosyl residues and carries, on average, three hydroxyl groups per glucose residue in the structure (Sidebotham, 1974). In our study, these hydroxyl groups were first converted to bromoacetyl groups, and the resulting pendant bromoacetyl groups in dextran were used for the subsequent attachment of vinyl groups to make a hydrogel precursor. The degree of substitution was controlled at the bromoacetylation level. Photocrosslinking was employed to create a network of the chemically modified dextran because of the convenience of photocrosslinking over other conventional crosslinking method (Andreopoulos et al., 1996; Elisseeff, Anseth, Langer & Hrkach, 1997; Sawhney, Pathak & Hubbell, 1993; Vyavahare & Kohn, 1994). The photocrosslinked hydrogels were subjected to various pH buffer solutions, and their swelling characteristics were studied.

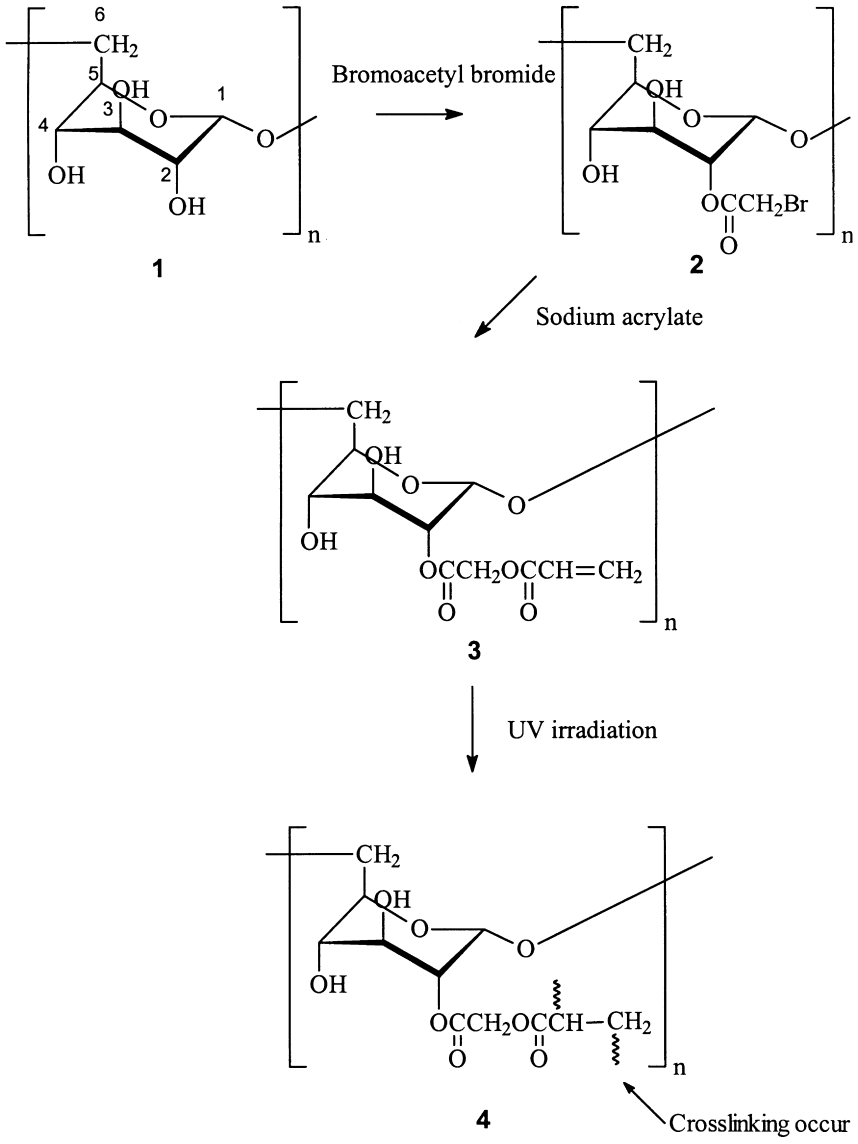
2. Materials and methods

2.1. Materials

Dextran (MW = 70 000 with 5% branching) was obtained from Sigma Chemical Co. (St. Louis, MO, USA) and dried at 60°C in a vacuum oven. Acrylic acid, bromoacetyl bromide, 2,2′-dimethoxy-2-phenyl acetophenone, dimethyl formamide, pyridine, and sodium hydroxide were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Lithium chloride was obtained from Aldrich Chemical Co. and dried at 70°C in a vacuum oven. Standard buffer solutions were obtained from VMR Scientific (West

* Corresponding author. Present address: Drug delivery division, Shearwater Polymers Ins., 2305 Spring Branch Rd., Huntsville, AL 35816, USA.

0144-8617/99/$ - see front matter
PII: S0144-8617(99)00053-3



Scheme 1. Preparation of dextran-based hydrogel.

Chester, PA, USA). A pH 3 buffer solution contained 0.25 M of sodium carbonate and bicarbonate. A pH 7 buffer solution contained 0.05 M of sodium and potassium phosphate. A pH 10 solution contained 0.05 M potassium hydrogen phthalate.

2.2. Preparation of bromoacetyl dextran (1)

Dextran was dissolved in a LiCl/DMF (10 wt.%) solvent system at 80°C under nitrogen. After the dextran was clearly dissolved and cooled down to room temperature, pyridine was added to the dextran solution. The amount of pyridine added was in equimolar ratio to bromoacetyl bromide. The solution was stirred for 10 min Then, bromoacetyl bromide was added at a very slow rate to the dextran solution. The amount of bromoacetyl bromide added ranged from 0.0056 to 0.0168 molar for achieving different degrees of substitution. The reaction was conducted at room temperature for 8 h under nitrogen. The reaction product was precipitated with cold ethyl alcohol, filtered, washed several times with ethanol, and then dried at 50°C in a vacuum oven.

2.3. Preparation of sodium salt of acrylic acid (sodium acrylate) (2)

Acrylic acid was mixed with 10 volumes of absolute ethyl alcohol. Then, sodium hydroxide in ethyl alcohol (10 wt.%) was added to the acrylic acid ethanol solution with a dropping funnel. The amount of sodium hydroxide added was in equimolar ratio to acrylic acid. After the reaction, sodium acrylate was filtered off and dried at room temperature in a vacuum oven.

Table 1
Reactant concentrations and degrees of substitution (DS) for the reaction of dextran with bromoacetyl bromide

#	Reactant concentration		Bromine content (%)	Degree of substitution
	(Glucose)[a]	(Bromoacetyl bromide)[b]		
1	0.0056	0.0056	8	0.19
2	0.0056	0.0084	18	0.49
3	0.0056	0.0112	34	1.42
4	0.0056	0.0168	46	2.99

[a] Moles of glucosyl residues as dextran.
[b] Moles bromoacetyl bromide.

2.4. Preparation of hydrogel precursor, acrylated dextran (3)

Bromoacetyl dextran was dissolved in LiCl/DMF (10 wt.%) at 70°C under nitrogen. An excess amount of sodium acrylate was uniformly dispersed in the bromoacetyl dextran solution. The acrylation reaction was conducted at 40°C for 24 h. Unreacted sodium acrylate was removed by filtration and the product was precipitated from the filtrate with cold ethyl alcohol (10 volume). The acrylated dextran was filtered off, washed several times with ethyl alcohol, and then dried at room temperature in a vacuum oven. Bromoacetyl dextrans having 18 and 46% bromine contents were used as the model compounds to illustrate this acrylation reaction, the subsequent hydrogel formation, and their swelling behavior.

2.5. Hydrogel formation with UV polymerization (4)

Acrylated dextran was dissolved to 40 % (w/v) in a buffer solution (pH 7). Photoinitiator (2,2-dimethoxy-2-phenylacetophenone dissolved in *n*-methyl pyrrolidone) was added to the acrylated dextran buffer solution. The amount of photoinitiator was 3 wt.% of the acrylated dextran. The solution was poured onto the glass plate and irradiated with a long wave UV lamp (360 nm) for 1 h. The resulting hydrogel was washed with deionized water and ethyl alcohol, and then dried in a vacuum oven.

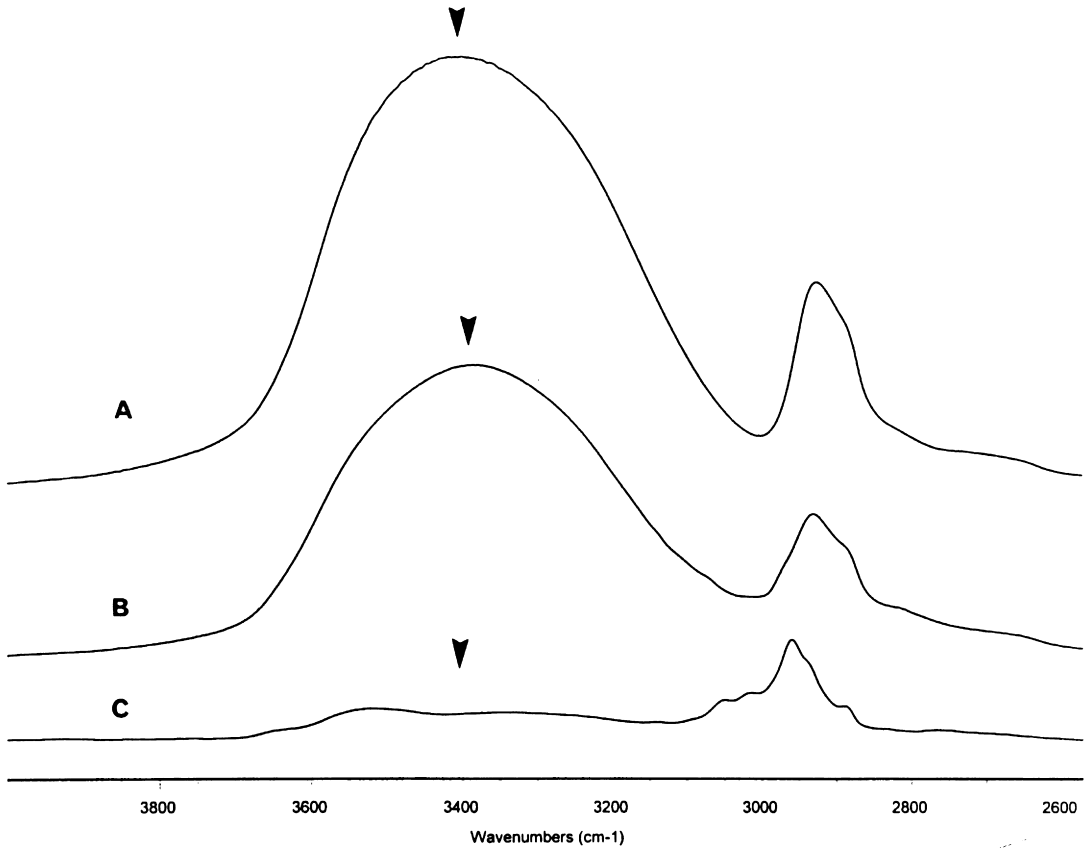


Fig. 1. FT-IR spectra of bromoacetyl dextrans: (a) DS = 0; (b) DS = 1.42; (c) DS = 2.99. Arrow (▼) indicates hydroxyl stretch.

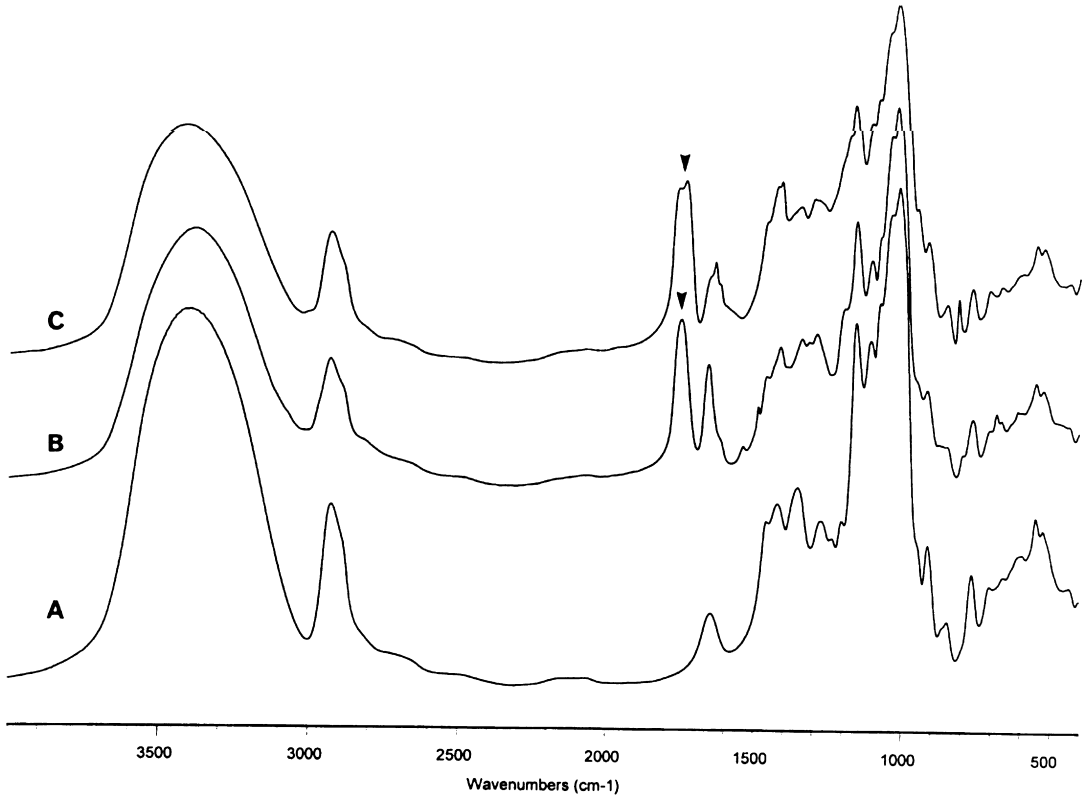


Fig. 2. FT-IR spectra of dextran and its derivatives: (a) Dextran; (b) Bromoacetyl dextran with DS = 0.49; (c) Acrylated dextran with DS = 0.23. Arrow (▼) indicates carbonyl stretch.

2.6. Characterization

Elemental analysis of bromine was conducted for calculating the bromine content of bromoacetyl dextran by use of a Carlo Erba 1106 EA instrument. The degree of substitution was obtained from the following formula (Won, Chu & Yu, 1997):

$$DS = \frac{\text{MW of dextran repeating unit} \times \%Br}{(\text{Atomic wt.of bromine} \times 100) - (\text{MW of ester substituent} - 1) \times (\%Br)}. \quad (1)$$

KBr pellets of reaction products (10 wt.% of KBr powder) were prepared for FT-IR measurement in a Perkin–Elmer Magna-IR560 Spectrometer. ^{1}H-NMR spectra and ^{13}C-NMR spectra were recorded with a Varian Unity spectrometer at 300 MHz. DMSO-d_6 was used as a solvent and tetramethylsilane (TMS) was used as the internal reference.

2.7. Swelling test

Dried hydrogels were weighed and soaked in buffer solutions of different pH: 3, 7, and 10. The soaked hydrogel was removed at a predetermined interval, washed, the surface water was then wiped by a paper towel, and weighed until no further weight change was detected. Swelling ratio of hydrogel was calculated by the following equation (Peng, Yao, Yuan & Goosen, 1994):

$$\text{Swelling ratio}, \% = \frac{W_s - W_0}{W_0} \times 100, \quad (2)$$

where W_s is the weight of a swollen hydrogel, W_0 the weight of a dried hydrogel.

3. Results and discussion

3.1. Bromoacetylation of dextran

The procedure of bromoacetylation of dextran is shown in Scheme 1. The carbonyl carbon in bromoacetyl bromide reacted with the hydroxyl groups in dextran to form esters. The degree of substitution (DS) increased with an increase in the ratio of bromoacetyl bromide to dextran as shown in Table 1. For example, the DS increased from 0.19 to 2.99 as the bromoacetyl bromide concentration increased form 0.0056 to 0.0168 mol. The DS data indicate that a wide range of hydroxyl group replacement, from a fraction to a complete bromoacetylation of all three hydroxyl groups per glucosyl residue in dextran, was achieved.

Highly substituted bromoacetyl dextran (DS > 1.42) has

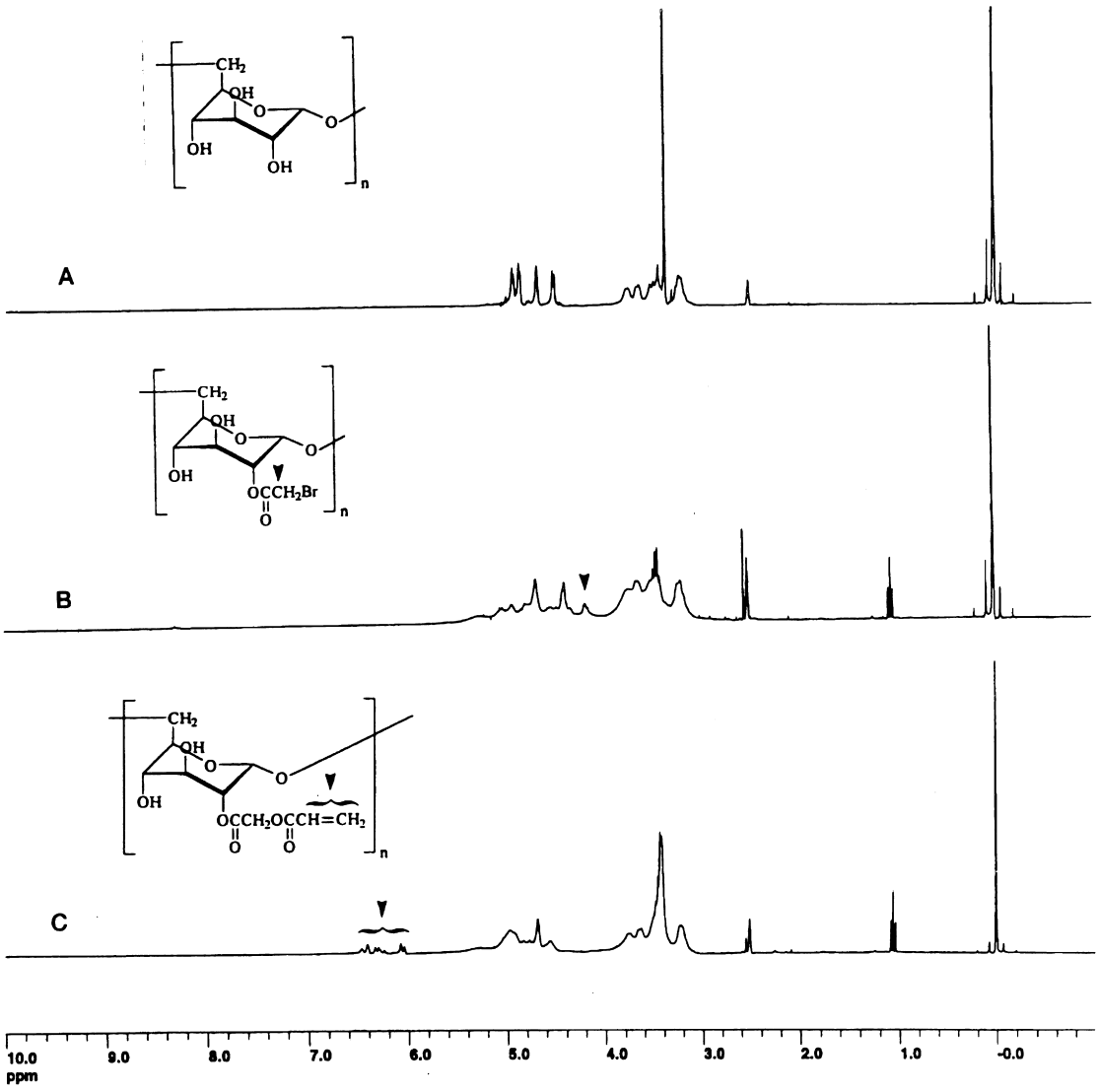


Fig. 3. ^{1}H-NMR spectra of dextran and its derivatives (300 MHz, DMSO-d_6): (a) Dextran; (b) Bromoacetyl dextran with DS = 0.49; (c) Acrylated dextran with DS = 0.23. In spectrum B, arrow (▼) indicates methylene protons in bromoacetyl group. In spectrum C, arrow (▼) indicates protons in acrylic substituents.

low water solubility due to fewer free hydroxyl groups. The collapse of the hydroxyl IR band (3400–3600 cm^{-1}) with an increase in DS (Fig. 1) also supported the observed low water solubility of highly substituted bromoacetyl dextran. The presence of the carboxyl ester group in the bromoacetyl dextran was also confirmed by the appearance of a carbonyl band at 1714 cm^{-1} that was absent in native dextran (Fig. 2).

In ^{1}H-NMR spectrum (Fig. 3), the methylene protons in bromoacetyl groups were shown in δ 4.18 ppm. In ^{13}C-NMR spectrum, the methylene carbon in bromoacetyl groups was shown in δ 40.9 ppm and the ester carbon was shown in δ 167 ppm (Fig. 4).

3.2. Acrylation of bromoacetyl dextran

The incorporation of vinyl groups to bromoacetylated dextran is illustrated in Scheme 1. As shown in the FT-IR spectrum (Fig. 2), it is difficult to identify the vinyl groups (1635–1645 cm^{-1}) of the acrylated dextran due to the existence of the small band (1646 cm^{-1}) of the unmodified dextran. Therefore, NMR would be more suitable for identifying the presence of vinyl groups in the acrylated dextran.

As shown in ^{1}H-NMR spectrum (Fig. 3), the protons in acrylic groups of the acrylated dextran appeared in the δ 6.03–6.46 ppm range. In ^{13}C-NMR spectra (Fig. 4), the appearance of two peaks at δ 128–134 ppm corresponded to the carbons in the vinyl group. In addition, the upward shift of methylene carbon peaks from δ 40.9 to δ 56.6 was observed because of the replacement of the adjacent bromine atom by an ester group.

3.3. Hydrogel formation

The gelation of the acrylated dextran upon 365 nm UV

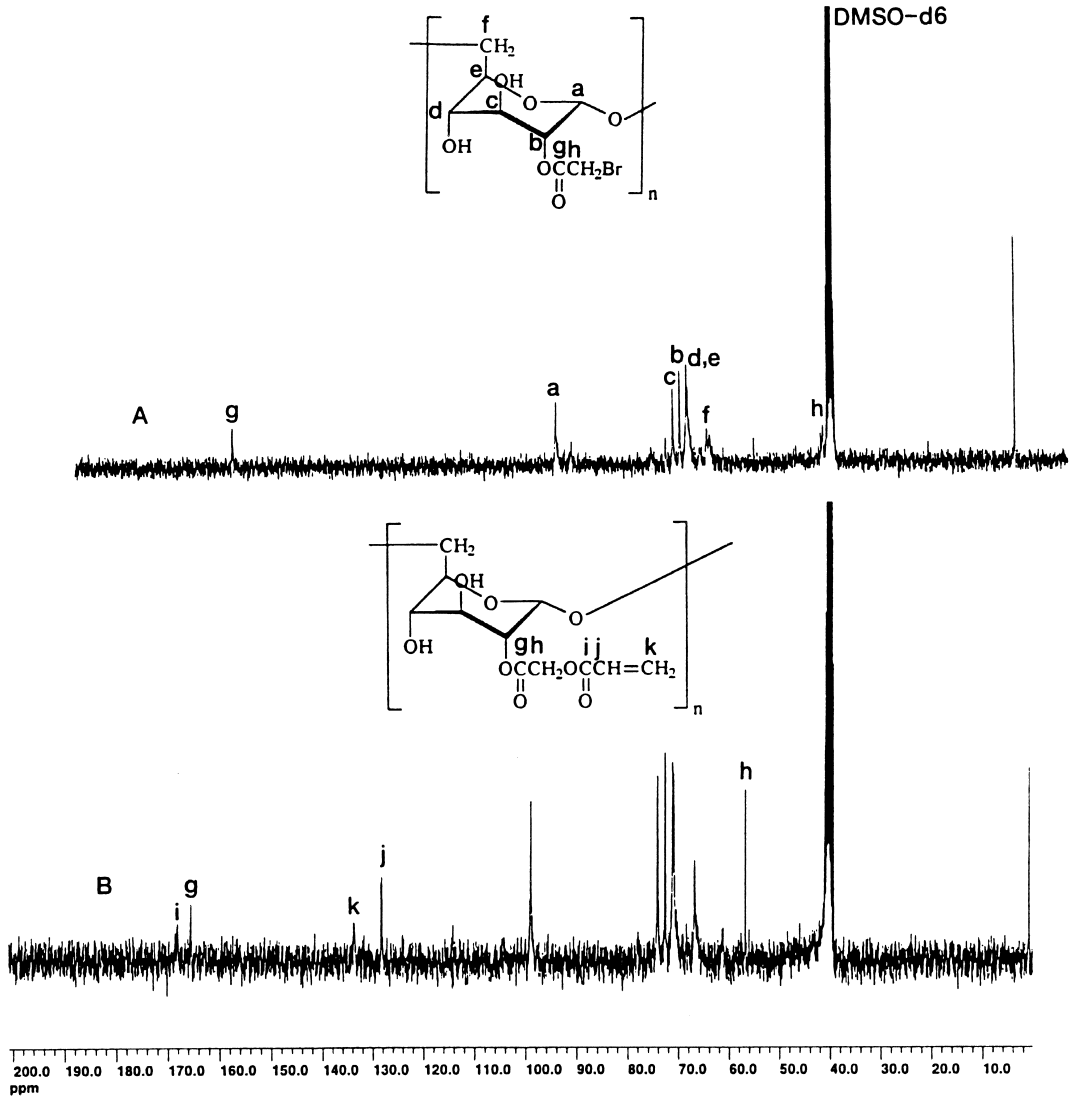


Fig. 4. ^{13}C-NMR spectra of dextran and its derivatives (300 MHz, DMSO-d_6): (a) Bromoacetyl dextran with DS = 0.49; (b) Acrylated dextran with DS = 0.23.

irradiation occurred within 5 min, and irradiation was prolonged for more consumption of the vinyl groups. The reduction of the C=CH_2 band (813 cm^{-1}) was used to indicate the level of consumption of vinyl groups due to crosslinking (Dijk-Wolthuis, Franssen, Talsma, Steenbergen, Bosch & Hennink, 1995). As illustrated in Fig. 5, the FT-IR spectra clearly show the disappearance of the 813 cm^{-1} band through crosslinking.

The choice of UV wavelength for crosslinking during hydrogel formation is critical to the properties of hydrogel. Torikai et al. (1995) reported the effect of UV wavelength on the photoinduced reaction of polymethyl methacrylate. They observed that photosensitized main-chain scission was favored by irradiation at 280 nm, whereas photocrosslinking took place efficiently with exposure to 340 nm. The chain scissions observed by Torikai et al. was mainly C–C backbone scission induced by UV. Although we do not think the long wave UV used in this study would have any adverse effect on dextran, it is not clear at the present stage whether the ether linkages (C–O–C) in dextran would be fragmented by this long wave UV.

3.4. Swelling test

The swelling behaviors of crosslinked dextrans (DS = 0.49 and DS = 2.99) in different pH media are shown in Fig. 6. Generally, the prepared dextran hydrogels showed a wide range of swelling behavior in different pH media, and the level of pH-dependent swelling was related to the degree of substitution.

In the pH 3 buffer solution, the bulk of the overall swelling (>87%) was achieved during the initial 30–40 min, and the swelling ratio increased very slowly thereafter for both hydrogels with a DS = 0.49 and 2.99. The highly substituted dextran hydrogel (DS = 2.99) showed a much lower equilibrium swelling ratio (128%) compared to that of the

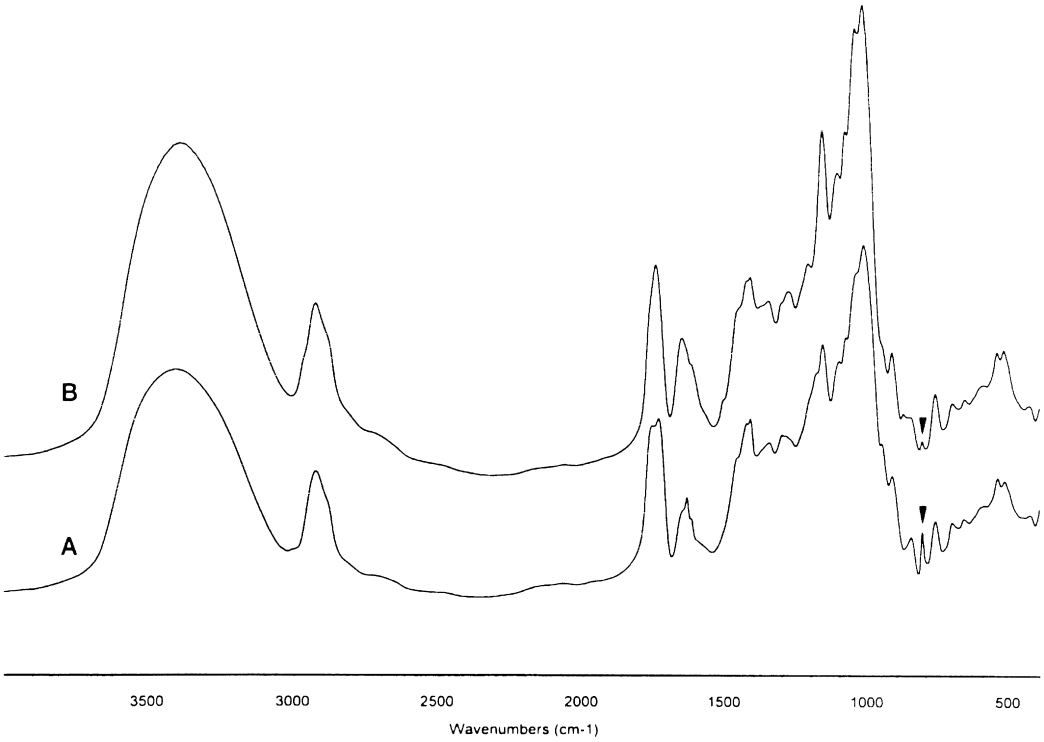


Fig. 5. FT-IR spectra of dextran hydrogels: (a) Acrylated dextran with DS = 0.23; (b) Dextran hydrogel. Arrow (▼) indicates double bond ($C{=}CH_2$) band.

hydrogel with a lower substitution (923%). Dextran hydrogels in pH 3 medium exhibited a good structural integrity when comparing to those in higher pH media. For example, the hydrogel (DS = 0.49) showed a good retention of its structure even after 312 h at low pH.

In the pH 7 buffer solution, the swelling ratios and their rates were very similar for both hydrogels (DS = 0.49 and 2.99). Like those dextran hydrogels in pH 3.0 medium, both hydrogels reached their equilibrium swelling within 1 h of immersion at pH 7.0. Although the highly substituted dextran hydrogel at pH 7 has a swelling ratio similar to that in the lower pH medium, the less-substituted dextran hydrogel (DS = 0.49) at pH 7 has significantly lower swelling ratio (171%) than the same hydrogel at pH 3 (923%).

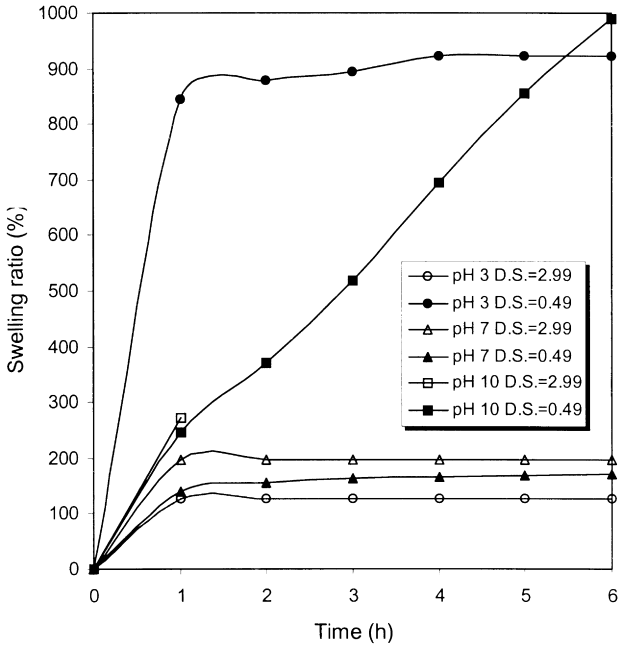


Fig. 6. Swelling ratio of dextran hydrogels with DS = 2.99 (○) and DS = 0.49 (●) in various pH buffer solutions.

The swelling phenomena of these two dextran hydrogels in pH 10, however, were very different from those in the lower pH media. The characteristics of reaching equilibrium swelling at an early stage of immersion found in the pH 7 and 3 media, however, did not appear at pH 10. Instead, both dextran hydrogels, particularly the less substituted one, showed a continuous increase in swelling ratio with time. After reaching to 272% swelling at 1 h, the physical structure of the highly substituted dextran hydrogel began to break down. The less-substituted dextran hydrogel, however, exhibited a nearly linear increase in swelling ratio with time after 1 h, and reached a 990% swelling ratio at 6 h. This less-substituted dextran hydrogel, however, began to dissolve in the buffer medium after 6 h and became completely soluble in the pH 10 buffer medium after 24 h at room temperature.

This faster structure breakdown of dextran hydrogels observed in high alkaline pH was consistent with the reported pH dependent hydrolytic degradation of linear aliphatic polyesters. Chu reported that polyglycolide, polylactides and their copolymers degraded at a much faster rate in a highly alkaline buffer medium than in an acid buffer

medium, and the alkaline-catalyzed hydrolysis of the ester bonds in the crosslinker was also irreversible (Chu, 1981; Chu, Fraunhofer & Greisler, 1997).

The consistently significantly lower swelling ratio of the highly substituted dextran hydrogel over the range of pH 3–10 is believed to be attributed to its higher level of crosslinking, which led to a tighter and more compact structure for limiting water uptake, and limited number of available free hydroxyl groups.

The higher swelling ratio of the less substituted hydrogel (DS = 0.49) at alkaline media might be attributed to the reduction of intermolecular hydrogen bonding capability. Similar observations of the pH-dependent swelling of other types of hydrogels were also reported by others. Bell and Peppas reported that poly(methacrylic acid–*g*-ethylene glycol) hydrogel had a higher swelling ratio as the pH of the medium increased from 7 to 12 (Bell & Peppas, 1994). They suggested the lack of hydrogen bonds between poly-(methacrylic acid) and poly(ethylene glycol) at an alkaline pH medium as the explanation for this behavior.

In conclusion, natural polysaccharides-based hydrogels were synthesized via UV crosslinking of acrylated dextran. The resulting hydrogels showed a wide range of swelling, depending on both the pH of the buffer media and the degree of substitution of acrylic groups.

References

Andreopoulos, F. M., Deible, C. R., Stauffer, M. T., Weber, S. G., Wagner, W. A., Beckman, E. J., & Russell, A. J. (1996). Photoscissable hydrogel synthesis via rapid photopolymerization of novel PEG-based polymers in the absence of photoinitiators. *J. Am. Chem. Soc.*, *118*, 6230–6235.

Bell, C., & Peppas, N. A. (1994). *Biomaterials and drug and cell delivery*, Pittsburgh, PA: Materials Research Society.

Chu, C. C. (1981). The in-vitro degradation of poly(glycolic acid) sutures—effect of pH. *J. Biomed. Mater. Res.*, *15*, 795–804.

Chu, C. C., Fraunhofer, J. A. v., & Greisler, H. P. (1997). *Wound closure biomaterials and devices*, New York: CRC Press.

Dagani, R. (1997). Intelligent gels. *Chem. Eng. News, June*, 26–37.

Dijk-Wolthuis, W. N. E. v., Franssen, O., Talsma, H., Steenbergen, M. J. v., Bosch, J. J. K. -v. d., & Hennink, W. E. (1995). Synthesis, characterization, and polymerization of glycidyl methacrylate derivatized dextran. *Macromolecules*, *28*, 6317–6322.

Elisseeff, J., Anseth, K., Langer, R., & Hrkach, J. (1997). Synthesis and characterization of photocrosslinked polymers based on poly(L-lactic acid–co-L-aspartic acid). *Macromolecules, 30*, 2182–2184.

Li, Y., Hu, Z., & Chen, Y. (1997). Shape memory gels made by modulated gel technology. *J. Appl. Polym. Sci.*, *63*, 1173–1178.

Mora, M., & Pato, J. (1990). Polymeric prodrugs, synthesis and hydrolytic behavior of dextran-bound anticancer agents. *Makromol. Chem.*, *191*, 1051–1056.

Nagasaki, Y., & Kataoka, K. (1997). Poly(silamine)s as intelligent materials. *Chemtech*, *March*, 23–29.

Park, C. H., & Orozco-Avila, I. (1992). Concentrating cellulases from fermented broth using a temperature-sensitive hydrogel. *Biotechnol. Prog.*, *8*, 521–526.

Park, C. H., & Orozco-Avila, I. (1993). Concentrating cellulases using a temperature-sensitive hydrogel: effect of gel particle size and geometry. *Biotechnol. Prog.*, *9*, 640–646.

Park, T. G., & Hoffman, A. S. (1991). Immobilization of arthrobacter simplex in thermally reversible hydrogels: effect of gel hydrophobicity on steroid conversion. *Biotechnol. Prog.*, *7*, 383–390.

Park, T. G., & Hoffman, A. S. (1994). Estimation of temperature dependent pore size in poly(*N*-isopropyl acrylamide) hydrogel beads. *Biotechnol. Prog.*, *10*, 82–86.

Park, K., Shalaby, W. S. W., & Park, H. (1993). *Biodegradable hydrogels for drug delivery*, Lancaster, PA: Technomic Publishing Company.

Peng, T., Yao, K. D., Yuan, C., & Goosen, M. F. A. (1994). Structural changes of pH-sensitive chitosan/polyether hydrogels in different pH solutions. *J. Polym. Sci., Part A: Polym. Chem.*, *32*, 591–596.

Sawhney, A. S., Pathak, C. P., & Hubbell, J. A. (1993). Bioerodible hydrogels based on photopolymerized poly(ethylene glycol)–co-poly(α-hydroxy acid) diacrylate macromers. *Macromolecules*, *26*, 581–587.

Sidebotham, R. L. (1974). *Advances in carbohydrate chemistry and biochemistry*, New York: Academic Press pp. 371–383.

Torikai, A., Hattori, T., & Eguchi, T. (1995). Wavelength effect on the photoinduced reaction of polymethylmethacrylate. *J. Polym. Sci., Part A: Polym. Chem.*, *33*, 1867–1871.

Vyavahare, N., & Kohn, J. (1995). Photocrosslinked hydrogels based on copolymers of poly(ethylene glycol) and lysine. *J. Polym. Sci., Part A: Polym. Chem.*, *32*, 1271–1281.

Won, C. Y., Chu, C. C., & Yu, T. J. (1997). Synthesis of starch-based drug carrier for the control/release of estrone hormone. *Carbohydr. Polym.*, *32*, 239–244.