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# Synthesis and characterization of acrylic acid-containing dextran hydrogels

H.-C. Chiu*, A.-T. Wu, Y.-F. Lin

*Department of Chemical Engineering, National Chung Hsing University, Taichung 402, Taiwan, ROC*

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**Abstract**

pH-Sensitive dextran hydrogels were prepared by free radical polymerization of methacrylate derivatized dextran, acrylic acid and *N*-*t*-butylacrylamide. Incorporation of acrylic acid in hydrogels was confirmed by Fourier transform infrared spectroscopy. The pH-dependent swelling of hydrogels was strongly influenced by the acrylic acid content, conjugation degree of methacrylate moiety with dextran and modified dextran concentration. Intermolecular polymerization that occurred to a greater extent with a lower degree of conjugation of methacrylate and/or higher concentration of modified dextran effectively increased the network density of hydrogels. An increase of acrylic acid reduced the enzymatic degradability of pre-swollen hydrogels by dextranase, although the increased equilibrium swelling was observed.


*Keywords*: Dextran hydrogels; pH-Dependent swelling; Enzymatic degradability

## 1. Introduction

Hydrogels have received considerable interest due to their variety of biomedical and biotechnological applications. Owing to its general good tissue compatibility and possibility for fabrication, hydrogel has been frequently considered as a potential drug delivery system. To achieve site-specific delivery and/or release rate control of therapeutic agents, stimuli-sensitive hydrogels, which underwent changes in gel volume and/or integrity in response to environmental variations under various physiological conditions have been designed and extensively investigated [1–7]. Colon-specific delivery of bioactive compounds that are susceptible to enzymatic degradation in the upper gastrointestinal (GI) tract via hydrogel systems has also attracted increasing attention. Kopecek and his associates prepared pH-sensitive and biodegradable hydrogels by cross-linking of acrylic acid-containing polymeric precursors with an azoaromatic agent as carriers for colonic drug targeting [8,9]. To reduce the extent of intramolecular cyclization, the hydrogel system was also prepared by aminolysis of polymeric precursors containing reactive *p*-nitrophenyl ester in the side chains with those containing amino groups [9]. By exploiting the pH change along the GI tract and due to the presence of azoreductase in colon, therapeutically active proteins and/or peptides can be liberated effectively at the desired site with reduced extent of proteolytic degradation. Temperature/pH-sensitive and biodegradable hydrogels were also prepared from free radical copolymerization of *N*-isopropylacrylamide, *N*-*t*-butylacrylamide (BA), acrylic acid (AAc) and *N*-methacryloylglycylglycine *p*-nitrophenylester, followed by cross-linking of polymeric precursors with cystamine [10]. Similarly, hydrogels became microbially degradable through extensive swelling with rat cecum content of pH 7.4.

Polysaccharide hydrogels have also been exploited as a colon-specific delivery system primarily owing to their enzymatic degradability at the desired sites. Dextran hydrogels can be obtained by several different approaches. Bronsted and Hovgaard prepared hydrogels from cross-linking of dextran with 1,6-hexanediisocyanate in DMSO [11] whereas Edman et al. synthesized dextran hydrogels by chemical incorporation of glycidyl acrylate into dextran in aqueous phase, followed by free radical polymerization of the dextran derivatives in the presence of *N*,*N*′-methylenebisacrylamide as an additional cross-linker [12]. Kim et al. prepared dextran hydrogels from photocross-linking of vinyl group carrying dextrans, which were prepared by sequential reactions of dextrans with bromoacetyl bromide and sodium acrylate [13]. In addition, van Dijk-Wolthuis et al. introduced vinyl group into dextran in a full control

* Corresponding author. Tel.: +886-4285-2636; fax: +886-4285-4734.
*E-mail address:* hcchiu@dragon.nchu.edu.tw (H.-C. Chiu).

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manner of the degree of conjugation by transesterification of glycidyl methacrylate (GMA) with dextran molecules in DMSO at ambient temperature [14,15]. Hydrogels were successfully obtained by free radical polymerization of methacrylate-derivatized dextran (MA-dextran) in aqueous solution using ammonium peroxydisulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) as an initiator system [14,16–21]. Similarly, Vervoort et al. synthesized inulin hydrogels from polymerization of MA-inulin obtained by transesterification of the polysaccharide with GMA [22].

Recently, carboxylate-containing dextran hydrogels were prepared to render the swelling of hydrogels pH-dependent. Regulation of swelling by pH change enables hydrogels to protect protein/peptide drugs from enzymatic hydrolysis in the upper GI tract and enhance drug release at colon via extensive gel swelling and degradation. For example, Chiu et al. prepared pH-sensitive dextran hydrogels by activation of dextran with 4-nitrophenyl chloroformate, followed by conjugation of the activated dextran with 4-aminobutyric acid and cross-linking with 1,10-diaminodecane [23]. Strong influence of pH-dependent swelling on the release of entrapped protein and enzymatic degradation of hydrogels by dextranase was observed. Kim et al. synthesized maleic acid-containing dextran by the reaction of dextran with maleic anhydride in the presence of triethylamine [24]. pH-Responsive hydrogel was prepared from polymerization of maleic acid-carrying dextran by UV irradiation. The dextran hydrogel system exhibited the highest swelling ratio at neutral pH, followed by acidic and then alkaline pH. At pH 10 or higher, disintegration of hydrogels occurred irrespective of the degree of cross-linking [24].

In this study, pH-sensitive dextran hydrogel is synthesized by copolymerization of MA-dextran with AAc and BA. The hydrogel structure is characterized by Fourier transform infrared (FTIR) spectroscopy. In addition, hydrogels are characterized with respect to pH-dependent swelling and swelling reversibility as functions of the degree of MA substitution, content of acrylic acid and concentration of modified dextran. Moreover, the effective network density of hydrogels is evaluated by mechanical measurements and its correlation with the degree of MA conjugation is described as well. Also examined herein is the effect of chemical cross-linking as well as acrylic acid content on the enzymatic degradation of dextran hydrogels by dextranase.

## 2. Experimental

### 2.1. Materials

Dextran (T-70) was obtained from Amersham-Pharmacia, Uppsala, Sweden. Gel permeation chromatography (GPC) (Superose 6, FPLC, Amersham-Pharmacia) showed that the number and weight average molecular weights were 61 000 and 106 000 g $mol^{-1}$, respectively. Glycidyl methacrylate (GMA) was purchased from TCI, Tokyo, Japan and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA), and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) and acrylic acid (AAc) were obtained from Lancaster, Lancashire, UK. Ammonium peroxydisulfate (APS) was purchased from Showa, Tokyo, Japan. AAc was distilled under reduced pressure before use. *N*-*t*-Butylacrylamide (BA) from Acros, NJ, USA, was purified by recrystallization twice from acetone. Dextranase (EC 3.2.1.11, 167 U/mg) from penicillium species was obtained from Sigma, MO and Bio-Rad (Coomassie blue) protein assay reagent purchased from Bio-Rad, CA, USA. Preparation of MA-dextran was carried out as described elsewhere [14,15]. In brief, GMA and DMAP were added to a solution of dextran in DMSO and the solution was stirred at room temperature for 48 h. The reaction was stopped by adding an equal molar amount of HCl to neutralize DMAP. The solution was subsequently subjected to ultrafiltration (MWCO 500, Amicon 8400) for 72 h. After purification, the product was collected by lyophilization. The structure of MA-dextran was characterized by FTIR (Beckman Paragon 500) and $^{1}H$ nuclear magnetic resonance (NMR) (Varian VXR-300 MHz) spectroscopies. For the $^{1}H$ NMR measurements, the sample was run in $D_2O$ (25 mg/ml) at ambient temperature with solvent suppression. The degree of GMA substitution (DS) was expressed as the number of methacrylate groups per 100 anhydroglucoside units [14]. The average molecular weights of dextran derivatives were checked by means of GPC (Superose 6, FPLC, Pharmacia). In this study, MA-dextrans with four different degrees of substitution (theoretical DS of 6, 12, 20 and 30, respectively, as calculated from the molar ratios of GMA to glucopyranose residues in the reaction mixtures) were prepared.

### 2.2. Hydrogel syntheses

pH-Responsive dextran hydrogels were prepared by free radical copolymerization of MA-dextran, AAc and BA in phosphate buffer (pH 8.5) using APS and TMEDA as an initiator system [14]. MA-dextran (45 mg) was dissolved in phosphate buffer (0.3 ml) and AAc (13.9 μmol, 5 mol% based on 100 glucopyranose residues) and BA (41.6 μmol, 15 mol%) in DMSO (20 μl) were added. This solution was extensively vortexed. APS (5 μmol) and TMEDA (18 μmol) in phosphate buffer (0.1 ml, separately) were subsequently added and copolymerization proceeded at room temperature for 72 h. Dextran hydrogel was carefully removed and washed thoroughly in distilled water under stirring for 7 days. Dextran hydrogels were also prepared by a final concentration of ca 150 mg/ml of MA-dextran in phosphate buffer while the added amounts of APS and TMEDA were maintained constant. In addition, AAc with concentrations of 0, 10, 15 and 20 mol% was, respectively, utilized to introduce various amounts of carboxylate groups into hydrogels.

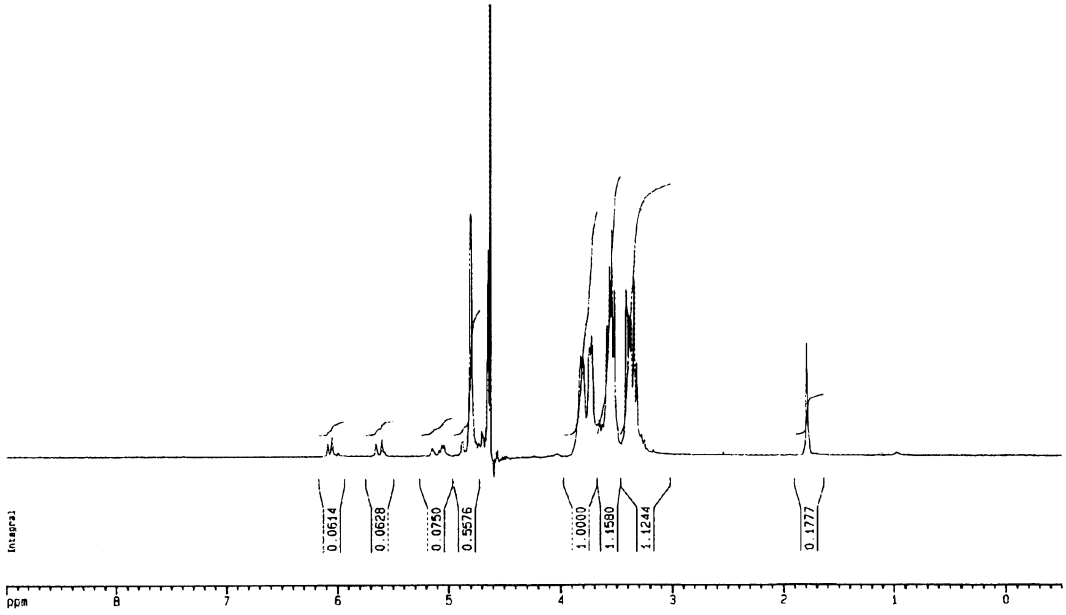


Fig. 1. $^1$H NMR spectrum of MA-dextran with DS 10.6.

### 2.3. Structure analyses of dextran hydrogels

To confirm the incorporation of AAc spectrometrically, dextran hydrogels were synthesized by copolymerization of MA-dextran and AAc in the absence of BA. Hydrogels were washed thoroughly with distilled water for 7 days to remove unreacted monomers and/or AAc oligomers. Hydrogel was dried in vacuo at 50°C for 120 h and subsequently powdered and ground with KBr and FTIR measurements.

### 2.4. Swelling characterization of hydrogels

Hydrogels were dried in vacuo at 50°C for 72 h. The pre-weighed dry gels were placed in various pH (2.0, 5.0, 7.4 and 10.0) buffer solutions. Ionic strength of all buffer solutions was adjusted to 0.01. The experiment was carried out for 72 h to ensure a state of equilibrium swelling. The swelling ratio, herein, is defined as $(W_s - W_d)/W_d$, where $W_s$ and $W_d$ are the weights of swollen and dry gels, respectively [25].

Swelling reversibility of dextran hydrogels was studied by measuring the gel swelling in response to repeated changes in pH between 2.0 and 7.4. After equilibrium swelling at pH 7.4, the gel was withdrawn and placed in pH 2.0 buffer solution. The degrees of swelling were determined at various time intervals. The time evolution of gel hydration at pH 7.4 was measured again and the procedure was repeated.

### 2.5. Estimation of effective network density of hydrogels

Effective network density of pH-responsive dextran hydrogels was evaluated by determining the modulus of elasticity in compression as described elsewhere [26,27]. Hydrogel at equilibrium swelling from pH 7.4 buffer was cut into 1-cm-in-diameter piece and the equilibrium heights of swollen gel under various compressive stresses were determined at 25°C using a bench comparator (Ames 135). The equilibrium modulus of elasticity was obtained from the following equation [27]:

$$F/A = -G(\lambda - \lambda^{-2}) \quad (1)$$

where $F/A$ is the compressive pressure applied, $G$ the modulus of elasticity and $\lambda = h/h_o$ (where $h$ and $h_o$ are the equilibrium heights of deformed and original gels, respectively). The value of $G$ was obtained from the slope of a linear plot of $F/A$ versus $-(\lambda - \lambda^{-2})$ with at least six different weights applied. The degree of effective cross-linking (the number of elastically effective chains in gel) of dextran hydrogels was calculated according to the following equation [27]:

$$v_e = G/(RTv_{2,s}^{1/3}\langle\alpha\rangle_o^2) \quad (2)$$

where $v_e$ is the degree of effective cross-linking in mol/m$^3$, R the gas constant, $T$ the absolute temperature, $v_{2,s}$ the polymer volume fraction at the equilibrium swelling state and $\langle\alpha\rangle_o$ indicates an isotropic dilatation factor of hydrogels in a dry state and is equal to $v_{2,r}^{1/3}$ according to Ref. [28] where $v_{2,r}$ is the polymer volume fraction at the relaxed state. Volume additivity was assumed and 1.61 g cm$^{-3}$ for the density of MA-dextran was employed in calculation [29].

### 2.6. Enzymatic degradation of dextran hydrogels

In a solution of dextranase (2.5 μ/ml, ca 0.42 U/ml) in 10 ml of McIlvain buffer solution, pH 5.6, a swollen gel (pre-weighed from the dry state) at the equilibrium state from the same buffer was immersed. The reaction was performed at 37°C in a constant temperature water bath. At various time intervals, hydrogels were withdrawn and

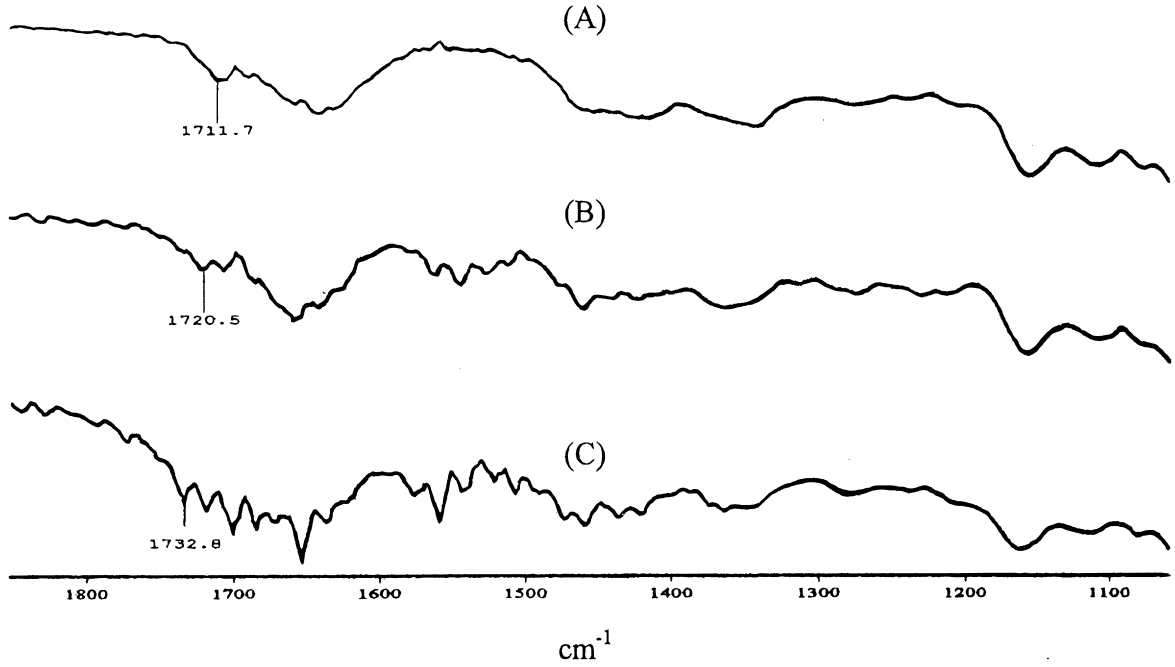


Fig. 2. FTIR spectra of MA-dextran with DS 4.7 (A) and the corresponding dextran hydrogels in the absence of AAc (B) and presence of 15 mol% of AAc (C) after 0.2 h of polymerization.

washed with distilled water and dried in vacuo at 50°C for 48 h. The degraded hydrogel was weighed. The extent of degradation was expressed in the percentage of weight loss of dry gels. In addition, after 15, 30, 60 and 120 min of incubations, Coomassie blue reagent (0.1 ml of 5-time diluted Bio-Rad reagent solution with distilled water) was separately added into the reaction mixtures and the color change in hydrogels was closely examined.

## 3. Results and discussion

Different approaches have been adopted to prepare dextran hydrogels [11–14,23,24,30–33]. Recently, Kim et al. and Chiu et al. separately synthesized pH-responsive dextran hydrogels for potential drug delivery applications [23,24]. In this study, pH-sensitive dextran hydrogels were prepared first by synthesis of vinyl group carrying dextran, followed by free radical copolymerization of AAc, BA and the resultant dextran derivative in aqueous phase using APS and TMEDA as initiator system. A constant molar ratio of BA (15 mol% with respect to glucopyranose residues) in the reaction mixture was employed to enhance the mechanical strength of hydrogel. Introduction of vinyl groups onto dextran molecules was conducted by reaction of GMA with dextran in DMSO at room temperature. Conjugation of GMA with dextran has been studied in detail elsewhere [14,15]. Transesterification of GMA with hydroxyl groups at positions 2 and 3 of glucopyranose residues of dextran in approximately a 1:1 ratio, led to direct attachment of methacryloyl groups to dextran molecules and formation of MA-dextran [15]. Fig. 1 shows an NMR spectrum of MA-dextran (DS = 10.6), which was obtained from a transesterification reaction with a molar ratio of GMA at 0.12 to glucopyranose residues (theoretical DS 12.0). The results are consistent with those documented previously [14,15]. Whereas the integrated peaks at $\delta$ 5.60 and 6.08 ppm and at $\delta$ 1.80 ppm were attributed to protons of $CH_2$=C and methyl groups of MA moieties, respectively, the integrated signals of $\delta$ 4.87 ppm and between $\delta$ 3.25 and 3.82 ppm were assigned to anomeric and remaining protons of dextran molecules [14,15]. Accordingly, the DS of each MA-dextran, calculated as $(z/3y) \times 100$ where $z$ and $y$ are the integrated areas of proton peaks at $\delta$ 1.80 and 4.87 ppm, was 4.7, 10.6, 16.6 and 26.8 in correspondence to theoretical DS of 6, 12, 20 and 30, respectively. An average value of more than 84% of MA conjugation was obtained. Characterization of MA-dextran by FTIR also agreed with its structure. Along with the appearance of absorption peaks attributed to dextran at 764 and 3425 $cm^{-1}$, typical peaks at ca 1711 and 815 $cm^{-1}$ assigned, respectively, to the carbonyl and double bond of methacrylate groups were observed (Fig. 2A) [14]. The GPC spectra also indicated the essentially identical elution profiles between dextran and MA-dextrans, irrespective of the DS of MA-dextran (data not shown).

In the absence of BA during polymerization, the structures of AAc-containing dextran hydrogels were analyzed by FTIR. Fig. 2(B and C) shows the FTIR spectra of hydrogels prepared from MA-dextran with DS 4.7 in the absence and presence of 15 mol% of AAc, respectively. As described previously, a typical peak at ca 1711 $cm^{-1}$

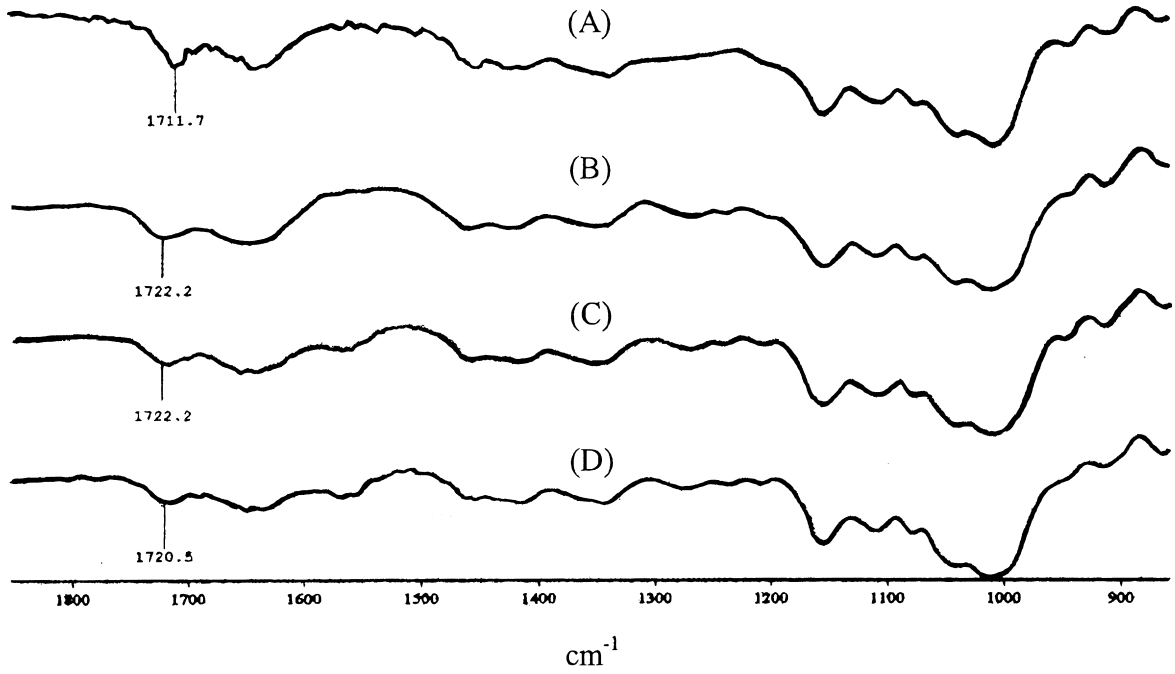


Fig. 3. FTIR spectra of MA-dextran with DS 16.6 (A) and the corresponding dextran hydrogels containing 0 (B), 10 (C) and 20 mol% (D) of AAc after 72 h of polymerization.

(Fig. 2A) was assigned to the carbonyl (C=O) groups of unpolymerized MA-dextran. Polymerization of MA-dextran for 0.2 h led to the appearance of an additional peak at ca 1720.5 $cm^{-1}$ (Fig. 2B). It was attributed to the carbonyl of polymerized methacrylate moiety. The spectrum also indicates the existence of unpolymerized MA moieties in modified dextran molecules after 0.2 h of polymerization. The peak at 1711.7 $cm^{-1}$ completely disappeared after polymerization for 1 h. Incorporation of AAc was confirmed by the formation of an extra peak at ca 1732 $cm^{-1}$ for the carbonyl of AAc units (Fig. 2C). Notably, the copolymerization of MA-dextran with AAc was incomplete after 5 h. Continual FTIR monitoring of the reaction indicated that a polymerization of ca 36 h was required to bring about a full reaction. Longer reaction times resulted primarily from the ionic interaction of AAc with TMEDA and will be shown quantitatively in our following report. The FTIR spectra of dextran hydrogels from MA-dextran DS 16.6 after polymerization of 72 h, however, show a broadened peak at ca 1722.2 $cm^{-1}$, irrespective of the incorporated amounts of AAc (Fig. 3).

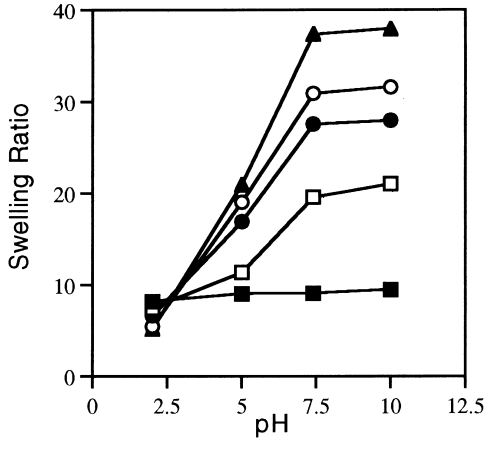


Fig. 4. Equilibrium swelling of dextran hydrogels containing 0 (■), 5 (□), 10 (●), 15 (○) and 20 (▲) of AAc as a function of pH (DS = 4.7 and concentration = 90 mg/ml).

Incorporation AAc during gel formation renders the swelling of dextran hydrogels pH-dependent. Fig. 4 shows the equilibrium swelling of dextran hydrogels (DS = 4.7; concentration = 90 mg/ml) under various pH conditions. The state of equilibrium swelling was reached within 3 h. Dextran hydrogels, in the absence of AAc showed an average swelling ratio of ca 9.0, irrespective of the pH of the medium. This is significantly higher than those observed by Hennink et al. from the same dextran hydrogel system, primarily owing to the differences in the aqueous concentrations of MA-dextran during gel formation [18]. By increasing the pH from 2.0 to 7.4, AAc-containing dextran hydrogels exhibited increased equilibrium swelling. Afterwards, the change in equilibrium swelling became rather insignificant. Ionization of AAc in gels occurring at a pH higher than gel p$K_a$ led to an increase in the hydrophilicity and charge repulsion and, as a consequence, in the water uptake of hydrogels. At pH 7.4, ionization of more than 99% of AAc was attained and further increase in gel swelling by increasing pH was rather restricted.

The equilibrium swelling of hydrogels at pH 7.4 was enhanced in an approximately proportional manner to

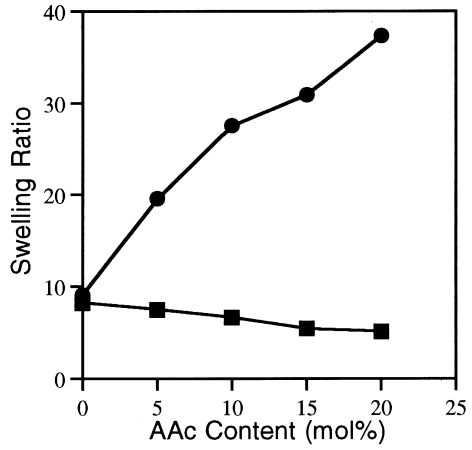


Fig. 5. Equilibrium swelling of dextran hydrogels as a function of AAc content at pH 2.0 (■) and 7.4 (●) (DS = 4.7 and concentration = 90 mg/ml).

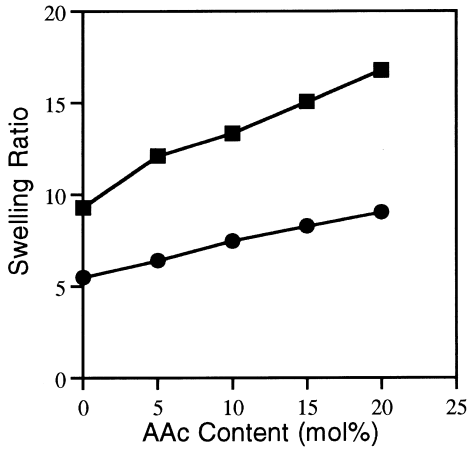


Fig. 7. Equilibrium swelling of dextran hydrogels prepared from MA-dextran with DS 10.6 and concentrations 90 (■) and 150 mg/ml (●) as a function of AAc content at pH 7.4.

AAc content ranging from 0.0 to 20.0 mol% (Fig. 5). It was reported that pH-sensitive hydrogels prepared from copolymerization of more than 12 mol% of (meth)acrylic acid with hydrophobic monomers exhibited the same magnitudes of swelling in response to pH change [5]. However, herein, the effect of AAc content on gel hydration remained even at the high levels of AAc. Furthermore, along with the variation in pH, discontinuous sharp phase transition of dextran hydrogels was not observed, irrespective of their AAc content. Siegel and Firestone prepared a series of ionic gels from copolymers of *n*-alkylmethacrylate with *N*,*N*-dimethylaminoethyl methacrylate (DMA) [34]. In response to pH change, they observed volume transition more clearly from methylmethacrylate-DMA gels than *n*-butyl- (or *n*-hexyl)methacrylat-DMA gels [34]. As widely recognized, a sharp phase transition of hydrogels in response to pH change requires incorporating hydrophobic monomers in gels [34,35]. Compared to ionic gels containing sufficient amounts of hydrophobic comonomers, the pH-dependent

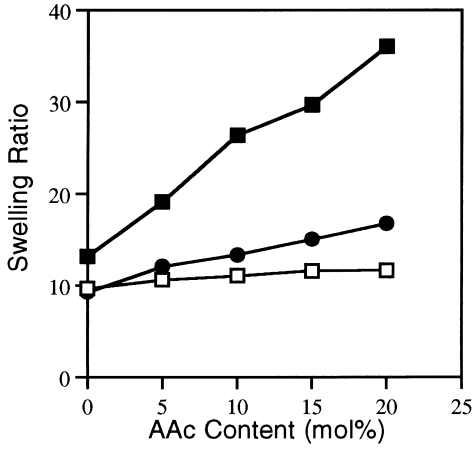


Fig. 6. Equilibrium swelling of dextran hydrogels with DS 4.7 (■), 10.6 (○) and 16.6 (□) as a function of AAc content at pH 7.4 (concentration = 90 mg/ml).

swelling behavior of dextran hydrogels manifests the importance of the hydrophilic nature of dextran molecules. The effect of the osmotic pressure from counterions surrounding the fixed charged moieties in gels on swelling at higher pH is reduced by the favorable mixing of dextran with water molecules. Accordingly, incorporation of increased amounts of AAc in dextran hydrogels is probably required for distinct volume transition to occur. However, incorporation of higher amounts of AAc resulted in rapid swelling at pH 7.4, followed by disintegration and dissolution of dextran hydrogels. Adding a higher amount of BA in order to reduce the favorable mixing of polymer chains with water was also restricted owing to an early phase separation during gel formation. The equilibrium swelling of hydrogels at pH 2.0 decreased with an increase in AAc content although the differences were relatively small (Figs. 4 and 5). At pH 2.0, AAc was mostly un-ionized and the hydrophobicity of polymer matrix had increased somewhat, leading to a decrease in gel hydration. Owing to insignificant difference in the hydrophilic/hydrophobic nature between carboxylic groups of AAc and hydroxyl of dextran, the reduction in the equilibrium swelling was rather limited. Kim et al., however, observed that the swelling of dextran–maleic acid hydrogels increased with an increase in the conjugation of maleic anhydride with dextran even under pH 2.0 conditions [24].

Fig. 6 shows the equilibrium swelling of hydrogels from MA-dextrans with different DS (4.7, 10.6 and 16.6, respectively, and conc. = 90 mg/ml) and various amounts of AAc at pH 7.4. Hydrogels from MA-dextran DS 4.7 exhibited significantly higher equilibrium swelling than those from 10.6 and 16.6. Also, the dependence of swelling on AAc from hydrogels with DS 4.7 was more pronounced. While the swelling of dextran hydrogels from MA-dextran with DS 16.6 increased only ca 21% as the amounts of AAc increased from 0 to 20 mol%, hydrogels with DS 4.7 showed a significant increase in hydration by 173%.

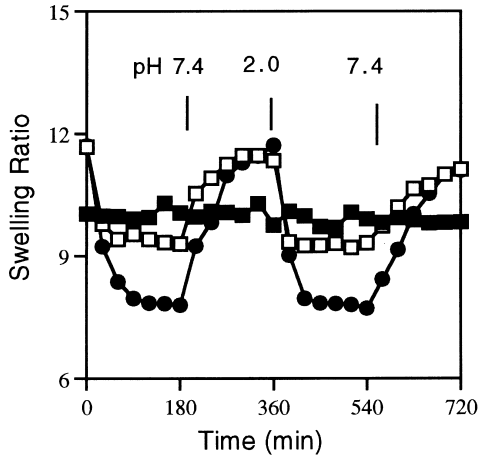


Fig. 8. Swelling reversibility of dextran hydrogels (DS = 16.6 and concentration = 90 mg/ml) with incorporation of 0 (■), 10 (□) and 20 mol% (●) of AAc after repeated changes in pH between 2.0 and 7.4.

Owing to the fact that a high DS leads to a high degree of chemical cross-linking and physical interpenetration of polymer network, the mobility of dextran molecules and gel hydration were limited. Consequently, the influence of AAc from hydrogels with DS 16.6 on swelling was much reduced. In contrast, the differences in equilibrium swelling between hydrogels with DS 10.6 and 16.6 were relatively insignificant. This can be explained by two factors. First, the aqueous mobility of polymer chains was severely limited as a sufficiently high degree of cross-linking occurred. A difference by 2.1 in the equilibrium swelling ratios between hydrogels with DS 16.6 and 26.8 (AAc = 15 mol%) at pH 7.4 further illustrates this. Secondly, the varying degrees of chemical cross-linking between hydrogels with DS 4.7 and 10.6 were more pronounced than those between 10.6 and 16.6 owing to high extents of intermolecular polymerization at low DS (see the description below).

The effect of MA-dextran concentration on the equilibrium swelling of hydrogel was also studied. In Fig. 7, hydrogels were prepared separately from 90 and 150 mg/ml of MA-dextran (DS = 10.6) with various amounts of AAc. The degree of gel hydration increased with decreasing polymer concentration (Fig. 7). Increasing polymer concentration obviously enhanced the physical entanglement of polymer chains and ultimately reduced the swelling capability of hydrogels. In addition, the extent of intermolecular polymerization might also increase by an increase in the concentration of MA-dextran. Similarly, the influence of the increase in polymer concentration on the reduction of pH dependence of swelling was also observed primarily owing to the increased physical entanglement and chemical cross-linking of polymer chains.

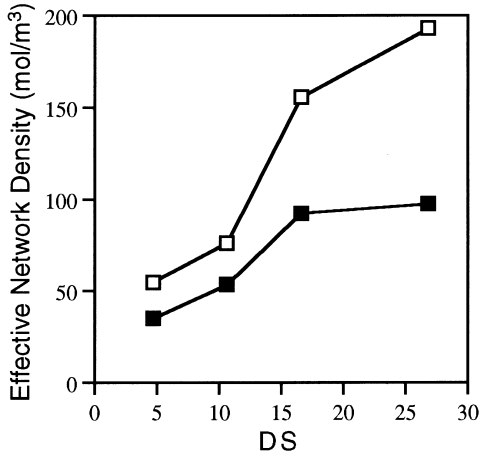


Fig. 9. Effective network density of dextran hydrogels as a function of DS. Hydrogels were prepared from MA-dextran concentrations of 90 (■) and 150 mg/ml (□) in the presence of 10 mol% of AAc.

The hydrophilic nature of dextran hydrogels rendered a slow response in swelling–deswelling behavior to repeated pH changes as compared to general synthetic pH-sensitive hydrogels (Fig. 8). It is generally believed that the incorporation of hydrophobic comonomers is required for a rapid response in phase transition to pH changes [34,35]. Increasing the hydrophilicity of (meth)acrylic acid-containing hydrogels also decreased the response rate to pH variation [25]. Dextran hydrogels without AAc exhibited pH-independent swelling. The amplitude of reversible swelling–deswelling of dextran hydrogels after repeated changes in pH between 2.0 and 7.4 was influenced by the AAc content. During the repeated changes in pH, the hydrogel system exhibited a reversible pattern with a faster response in deswelling than swelling. Upon the exposure of hydrogels from pH 7.4 to 2.0, the favorable interactions on swelling from the mixing of polymer chains with solvent and ion osmotic pressure were reduced. The elastic retractile force of the network became predominant in gel deswelling. On the other hand, the slow increase in gel hydration in response to pH change from 2.0 to 7.4 was primarily due to the balance of the increased mixing and ion osmotic force with the unfavorable network retractile response.

The effective network density of dextran hydrogels was evaluated by mechanical compression measurements [27]. Fig. 9 shows the cross-linking density of hydrogels as a function of DS and concentration of MA-dextran. Increases in both the DS and concentration of MA-dextran enhanced the effective network density of hydrogels. Transesterification of GMA with dextran introduced vinyl groups onto the polysaccharide molecules and rendered the polysaccharide intermolecularly polymerizable. The increase in DS, increases the possibility of cross-linking of dextran molecules, particularly at high MA-dextran concentration. At low DS, polymerization occurred mainly in an intermolecular pattern, irrespective of the concentration of MA-dextran. Intramolecular polymerization occurred to a larger extent at a high DS and low MA-dextran concentration. Consequently, the effective network density of hydrogels only increased slightly. The intramolecular polymerization decreased with increased MA-dextran concentration, leading to a significant increase in the degrees of effective

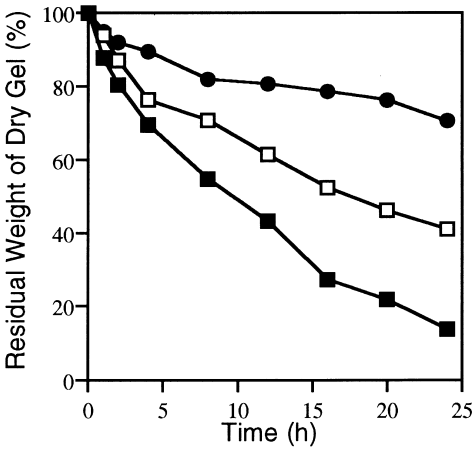


Fig. 10. Time-dependent degradation of dextran hydrogels from MA-dextrans with DS 4.7 (■), 10.6 (□) and 16.6 (●) (concentration = 90 mg/ml) by dextranase in pH 5.6 buffer at 37°C.

cross-linking. However, van Dijk-Wolthuis et al. indicated that increasing MA-dextran concentration lowered conversion of MA moieties owing to the enhanced physical entanglement and severe mobility restriction of polymer chains after reaching the gel point [14].

As well recognized, the degradability of dextran derivatives by dextranase was significantly affected by the type and extent of chemical modification of dextran molecules [36–39]. Our previous study observed that the release rate of entrapped proteins was significantly influenced by the enzymatic degradation of dextran hydrogels [23]. Fig. 10 demonstrates a decrease in the degradation of dextran hydrogels with an increase in the DS of MA-dextran. While dextran hydrogel from MA-dextran DS 4.7 was significantly degraded (more than 83% in the weight loss) within 24 h, a slight decrease (ca. 23%) in the residual weight of dry gel with DS 16.6 was

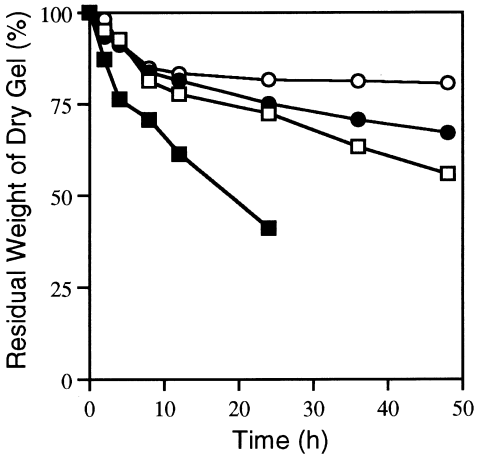


Fig. 11. Time-dependent degradation of dextran hydrogels from MA-dextrans with (DS = 10.6 and concentration = 90 mg/ml) with 0 (■), 5 (□), 10 (●) and 20 mol% (○) of AAc by dextranase in pH 5.6 buffer at 37°C.

observed. The Coomassie blue assay indicates the existence of dextranase inside hydrogels as well as bulk degradation. The intensity of blue color inside hydrogels increased significantly with time for hydrogels with DS 4.7. Hydrogels with DS 10.6 displayed a similar, yet less intense reaction. Reduction in the degradation rate of dextran hydrogels with an increase in the DS can therefore be attributed to the effect of steric hindrance of network structure and breakage of consecutive arrangement of unsubstituted glucopyranose residues. The decrease of the dextranase concentration inside hydrogels with an increase in DS may significantly limit the hydrogel degradation via bulk mechanism. Matheson and McCleary reported that penicillium dextranase contains six subsites for binding six successive glucopyranose rings of the substrates [40]. Although the enzymatic cleavage of MA-dextran was found possibly at a glycosidic bond between a methacrylated glucopyranose residue and an unsubstituted one, Franssen et al. reported that the degradation of intramolecularly polymerized methacrylated dextran and dextran hydrogels occurred at the fastest rate with 18 or more consecutive unsubstituted glucopyranose residues [38]. The unsubstituted chain segments shorter than six residues from polymerized methacrylated dextran become nondegradable by dextranase. In this study, hydrogels from MA-dextran with DS 16.6 were degraded slightly probably owing to the uneven distribution of MA conjugation along the dextran molecules.

Surprisingly, the incorporation of AAc in dextran hydrogels reduced the enzymatic degradability of hydrogels by dextranase (Fig. 11). A significant reduction in gel degradation occurred even at an incorporated AAc level of only 5 mol%. The degradability of dextran hydrogels further decreased with an increase in the incorporated amounts of AAc. The degradation was carried out by immersing the hydrogels at equilibrium swelling from McIlvain buffer, pH 5.6 into the dextranase solution of the same buffer. Higher equilibrium swelling of hydrogels with higher amounts of AAc was clearly observed. With Coomassie blue protein assay, our results indicated that the enlarged mesh size of highly swollen hydrogels did not facilitate the penetration of the enzyme into hydrogels. The influx of dextranase into hydrogels decreased probably owing to the enhanced internal osmotic pressure of hydrogel, which resulted from the high local accumulation of counterions surrounding AAc during pre-swelling process. However, the elevated osmotic pressure in hydrogels might also retard the penetration of Coomassie blue reagent into gels and lead to improper conclusion. Nonspecific association that occurred between the enzyme with AAc might also alter the natural conformation of the enzyme and reduce its activity for degrading the substrates. However, the current data regarding the reduced degradability of AAc-containing dextran hydrogels are not conclusive and further investigation is required.

## 4. Conclusion

In this study, pH-sensitive dextran hydrogels were prepared from free radical copolymerization of MA-dextran with AAc and BA. The DS of MA-dextran was determined by NMR whereas the incorporation AAc into hydrogels was confirmed by FTIR spectroscopy. Equilibrium swelling of dextran hydrogels increased with an increase in pH. The extent of increased swelling was dependent on the AAc content. An increase in either the DS or concentration of MA-dextran, however, reduced the capability of hydrogels undergoing volume change in response to pH alteration. AAc-containing dextran hydrogels also showed reversible swelling–deswelling behavior in response to repeated changes in pH. Owing to the hydrophilic nature of dextran, slow response of hydrogels was observed. Intermolecular polymerization that took place to a larger extent by a low DS and/or high concentration of MA-dextran increased the effective network density of hydrogels. The enzymatic degradability of dextran hydrogels by dextranase was reduced with an increase in the DS of MA-dextran owing to the breakage of consecutive arrangement of glucopyranose residues and steric hindrance of the increased cross-linking network system. Moreover, the degradation of pre-swollen hydrogels was also reduced with an increase in the AAc content probably owing to the increased osmotic pressure by the local accumulation counterions, which surrounded the fixed charged moieties in hydrogels.

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