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FTIR-ATR measurements of the ionization extent of acrylic acid within copolymerized methacrylated dextran/acrylic acid networks and its relation with pH/salt concentration-induced equilibrium swelling

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Abstract

In this study, dextran hydrogels were obtained by free radical copolymerization of methacrylated dextran with acrylic acid (AAc) in aqueous solution. The fractions of dissociation of AAc units within hydrogel in response to changes in pH and ionic strength of external aqueous solution were determined by FTIR-ATR spectroscopy. The influence of small ion concentration within hydrogel on the dissociation constant of AAc follows the Debye–Hückel behavior. Based on the results from the FTIR-ATR measurements, the total ion concentration difference inside and outside the hydrogel was determined according to the Donnan equilibrium theory, taking account of the counterion condensation effect quantitatively. The swelling response of hydrogels to changes in external pH and ionic strength was governed mainly by the ionic osmotic pressure due to the accumulation of diffusible ions within hydrogels. The energy balance between the osmotic and elastic retractile responses indicates the non-Gaussian behavior of elastically effective subchains with increasing swelling.

Keywords: Dextran hydrogels; Donnan equilibrium theory; Counterion condensation

1. Introduction

Dextran hydrogels have received increased attentions due to their variety of applications in biochemical and biomedical fields. Different approaches to preparation of dextran hydrogels have also been exploited extensively [1–7]. For example, hydrogels can be obtained by transesterification of glycidyl methacrylate (GMA) with dextran, followed by radical polymerization of methacrylated dextran (MA-dextran) in aqueous solution at ambient temperature, using ammonium peroxydisulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) as an initiation system [6,7]. Introduction of vinyl group onto dextran molecule can be conducted in a full control manner simply by varying the molar ratio of GMA and dextran in the feed under mild reaction conditions [6]. The physicochemical properties, enzymatic degradability and protein release behavior of polymerized MA-dextran networks have also been thoroughly studied [8–18].

The aforementioned approach to synthesizing MA-dextran also offers a facile route to prepare pH-responsive dextran hydrogels. In our previous reports, pH-sensitive hydrogels were obtained from radical copolymerization of methacrylated polysaccharides with acrylic acid in aqueous phase [19–21]. The pH effects on the polymerization yield and incorporation efficiency of AAc, using APS and TMEDA as the initiation system were examined [20]. It was also observed that the cross-linking density increased with increasing the AAc content due to the enhanced intermolecular covalent connection of MA moieties by the AAc bridging effect [20,21]. Quantitative analysis of swelling thermodynamics based on the Gaussian model also indicated that the increase in the cross-linking density with increasing the AAc content was a key factor in regulating the sensitivity of swelling to changes in pH and ionic strength of external aqueous solutions [22].

In this study, FTIR-ATR spectroscopy was employed to determine the content of AAc units within hydrogels and the degree of dissociation of AAc as functions of pH and ionic strength of external aqueous solution. It is generally recognized that the penetration depth of Evanescent wave

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from FTIR-ATR spectroscopy is limited to only several micrometers from the sample surface. Therefore, the sliced hydrogel specimens for the FTIR-ATR measurements were obtained from different parts of the synthesized hydrogels. In addition, the calibration curve for determination of the concentration of the ionized AAc units within hydrogel was established from the aqueous solution of MA-dextrans with poly(sodium acrylate) in varying molar ratios of anhydroglucoside and AAc residues. The total ion concentration difference is quantitatively evaluated according to the Donnan theory by taking account of counterion condensation effect and its performance is correlated with the swelling response due to changes in external pH and salt concentration.

2. Experimental

2.1. Materials

Dextran (T-70) obtained from Amersham-Pharmacia was dried in vacuo for 72 h before use. Gel permeation chromatography (Superose 6, calibrated with dextran standards (Polysciences); FPLC, Amersham-Pharmacia; RI detector, RI-930 Jasco) indicated that the weight and number average molecular weights were 106,000 and 61,000 g mol^{-1}, respectively. Poly(sodium acrylate) obtained from Aldrich has a weight average molecular weight of 15,000 g mol^{-1}. GMA was obtained from TCI and (*N*,*N*-dimethylamino)pyridine, TMEDA and AAc were from Lancaster. AAc was vacuum-distilled before use. APS was purchased from Showa and used as received. Deionized water used in buffer preparation was produced from Milli-Q Synthesis (Millipore). Preparation of MA-dextran was carried out by transesterification of GMA with dextran, as described elsewhere [6,7]. The degree of substitution (DS) of MA-dextran, defined as the number of methacrylate moieties per 100 anhydroglucoside residues was determined by ^{1}H-NMR [6]. The approach to preparing copolymerized dextran/AAc networks has been described in detail in our previous paper [22]. In brief, free radical copolymerization of MA-dextran (DS 4.9, 45 mg) with AAc (60 and 150 μmol, respectively) was carried in borate buffer (0.5 ml) at room temperature for 72 h, using APS and TMEDA as an initiation system. Prior to the polymerization reaction, the solution was vortexed extensively and purged with nitrogen for 10 min and the final pH was adjusted to 8.5. Hydrogels were thoroughly washed by placing them in deionized water with stirring for at least 7 days. Precaution was taken to prevent hydrogels from damage by stirring. The polymerization yields were in the range from 84 to 88% by weight.

2.2. FTIR-ATR measurements

2.2.1. Measurements of the AAc content

Hydrogels were placed in Tris/HCl buffer solution (pH 8.0, I 0.01) for at least 3 days. Buffer was changed every 12 h until no further change in pH. It is assumed that, at this pH, the AAc units within hydrogels are fully ionized. The residual amount of water at hydrogel surface was removed carefully by soft tissue paper. For each FTIR (Spectrum One, Perkin–Elmer)-ATR (Miracle, Pike) measurement, the hydrogel was sliced into small specimens with smooth surfaces from different parts of hydrogel. The specimen was scanned 20 times in a wavenumber range from 600 to 2000 cm^{-1} with a resolution of 2 cm^{-1}. The FTIR-ATR measurement of each sample was performed at least in triplicate. The baseline correction was carried out using the same Tris/HCl buffer as a reference under identical conditions. From the scanning spectra of hydrogel specimens, the areas of transmittance bands at 1555 and 1017 cm^{-1} from the intense asymmetric stretching of carboxylate ($-COO^-$) groups of AAc units and symmetric stretching of $-C-O-(H)$ of anhydroglucoside residues coupled with the adjacent $-C-C-(O)$ stretching vibration, respectively, were integrated. The AAc content was determined by the area ratio of these two transmittance bands in correlation with the mole ratio of AAc units to anhydroglucoside residues from the established calibration curve.

Calibration data was obtained by the FTIR-ATR determination of poly(sodium acrylate) and MA-dextran in Tris/HCl aqueous buffer. Calibration solutions were prepared by employing MA-dextrans with 3 different DS (4.9, 10.1 and 11.6) and 8 different molar ratios of AAc units of poly(sodium acrylate) to anhydroglucoside residues of each MA-dextran. The measurement was performed as described previously and the integrated area ratio at 1555 and 1017 cm^{-1} evaluated. Following the Beer's law, the proportional constant (K) can be obtained as

$$A_{1555}/A_{1017} = K(N_{\mathrm{AAc}}/N_{\mathrm{glucoside}}) \qquad (1)$$

Here A_{1555}/A_{1017} is the integrated area ratio at these two frequencies and $N_{\mathrm{AAc}}/N_{\mathrm{glucoside}}$ the molar ratio of AAc units to anhydroglucoside residues in aqueous solution.

2.2.2. Measurements of the degree of dissociation of AAc units within hydrogel

Hydrogels were placed separately in HCl aqueous solutions in pH range from 3.3 to 6.4. The ionic strength was adjusted by addition of NaCl to 0.01 and 0.1, respectively. The solution was changed constantly until no further change in pH. Typically, 5 days are required to reach the equilibrium swelling in HCl solution at pH lower than 4.0 and 3 weeks at pH higher than 5.0. Hydrogels were sliced into small specimen samples as previously described. The FTIR-ATR measurements of hydrogels and baseline correction were performed in the wavenumber range from

600 to 2000 cm^{-1} with the resolution of 2 cm^{-1}. The integrated areas of the transmittance bands at 1555 and 1017 cm^{-1} of small specimen samples from different pH and ionic strengths were determined. The measurements were performed in triplicate from different specimen samples. The dissociation degree of AAc units within hydrogel was obtained from the area ratio of the transmittance bands at these two frequencies in comparison with that of hydrogel sample from pH 8.0 in Tris/HCl buffer.

2.3. Measurements of equilibrium swelling

Hydrogels were placed in HCl aqueous solution (pH 3.3 ~ 6.4; I 0.01 and 0.1, respectively), replacing the HCl solutions constantly until no further changes in both pH and swelling. Hydrogel was weighed and the swelling ratio ($q = (W_s - W_d)/W_d$, where W_s and W_d are the weights of swollen and dry gels, respectively) determined. In this study, the equilibrium swelling was also represented in terms of the polymer volume fraction ($v_{2,s}$) of a swollen gel. Volume additivity was assumed and 1.61 g cm^{-3} for the density of MA-dextran [23] was employed in the calculation, irrespective of the DS of MA-dextran.

2.4. Measurements of the elastic moduli of the networks

Uniaxial compression of hydrogels under various degrees of equilibrium swelling from HCl aqueous solutions was carried out using tensile testing instrument (Hounsfield, H1KS) equipped with a maximum load of 50 N. The ambient temperature was controlled at 25 °C. The measurement was conducted at a compression rate of 0.4 mm min^{-1}. The experiment was performed in triplicate. The elastic modulus (G) can be determined by the following equation [24]:

$$F/A = -G(H - H^{-2}) \tag{2}$$

Here F/A is the compressive stress applied, G the elastic modulus (Pa) and $H = h/h_0$ where h and h_0 are the equilibrium heights of deformed and original hydrogels, respectively.

With the assumptions that the behavior of cross-linking junctions follows the affine model and the elastically effective subchains fall into the Gaussian distribution region, the cross-linking density (i.e., the concentration of elastically effective subchains of network in dry state) can be obtained by the following equation [25,26]:

$$\nu_e = G/(RTv_{2,s}^{1/3}v_{2,r}^{2/3}) \tag{3}$$

Here ν_e is the cross-linking density in mol m^{-3}, $v_{2,s}$ the polymer volume fraction of hydrogel at equilibrium swelling and $v_{2,r}$ the polymer volume fraction at the relaxed (gel formation) state.

3. Results and discussion

In this study, pH-sensitive dextran hydrogels were obtained by free radical copolymerization of MA-dextran with AAc in aqueous solution at room temperature, using APS and TMEDA as an initiation system. Incorporation of AAc in dextran hydrogels renders the swelling of hydrogels pH-responsive. It has been observed in our previous studies that the cross-linking density of copolymerized MA-dextran/AAc networks was mainly governed by the extent of copolymerized AAc residues within hydrogels and the methacrylation degree of MA-dextran [19,20]. For the latter, an increase in the DS of MA moieties along with dextran molecules undoubtedly increased the degree of the intermolecular covalent connection among dextran molecules and, therefore, reduced their equilibrium swelling. In addition, by the observation of the changes in the cross-linking density, the intramolecular homopolymerization of MA moieties within the same MA-dextran molecule occurred only for MA-dextran with a sufficiently high degree of methacrylation, particularly, at a reduced concentration of MA-dextran during copolymerization [19]. On the other hand, in this study, the difference in the cross-linking densities (28.5 and 38.3 mol m^{-3}, respectively) between dextran hydrogels (DS 4.9) with AAc 7.7 and 11.9 mol% was observed owing to the covalent bridging effect of copolymerized AAc residues as reported previously [20,22]. As a consequence of the covalent bridging from the AAc residues between MA moieties, the response in swelling of MA-dextran/AAc hydrogels to changes in pH and ionic strength of external buffer solutions increased with increasing the AAc content, especially for MA-dextran with a low DS (for example, 4.9 in this study) [22].

FTIR-ATR spectroscopy was employed to determine the content of AAc units within hydrogels and the degree of dissociation of AAc units as functions of pH and ionic strength of external aqueous solution. However, owing to the limitation of the penetration depth of Evanescent wave from FTIR-ATR spectroscopy, the structural data obtained are usually restricted from the surface to only several micrometers inside the sample. In order to avoid the misinterpretation of the result, hydrogels were sliced into small specimens and the samples from different parts of hydrogels were employed in triplicate for the FTIR-ATR measurements. Prior to the measurements of hydrogel samples, aqueous solutions of MA-dextran and poly(sodium acrylate) were used for calibration. Fig. 1 represents the transmittance spectra of MA-dextran (DS 10.1) and poly(sodium acrylate) in three different molar ratios ($N_{\text{AAc}}/N_{\text{glucoside}}$, 12.1, 46.4 and 78.8 mol%) in aqueous solutions, respectively. The characteristic band for the asymmetric stretching of carboxylate anions ($-\text{COO}^-$) of poly(acrylate) occurs at the position of ca. 1555 cm^{-1}. It was also noticed that the feature signal for the symmetric stretching of carbonyl group of acrylic acid at ca. 1730 cm^{-1} disappeared entirely due to the ionization of

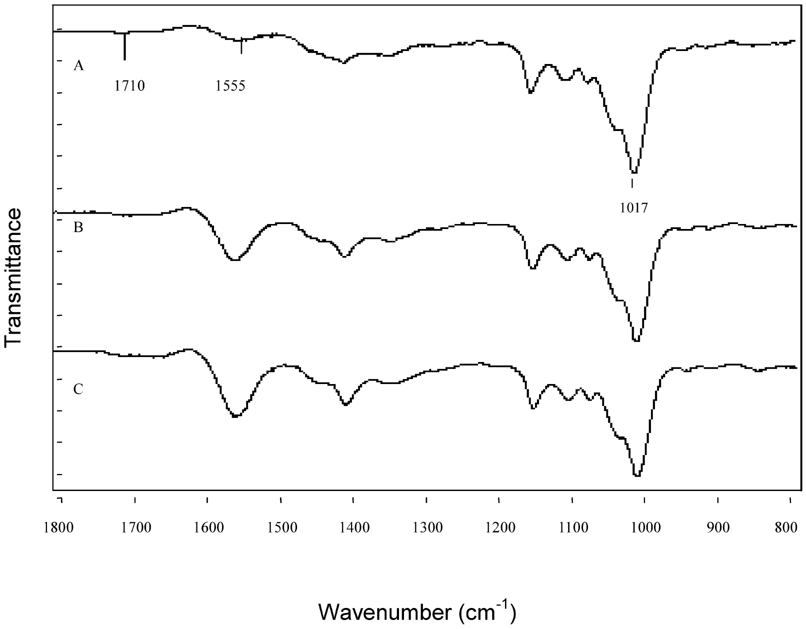

Fig. 1. FTIR-ATR spectra of MA-dextran (DS 10.1) and poly(sodium acrylate) in Tris/HCl buffer solution (pH 8.0, I 0.01). The molar ratios of AAc to anhydroglucoside residues in percentage are 10.8 (A), 43.2 (B) and 75.6 mol% (C), respectively.

AAc whereas the transmittance band at 1710 cm^{-1} was obtained from the carbonyl of MA moiety of MA-dextran. For a clear comparison, the intense band at 1017 cm^{-1} resulting from the stretching of –C–O–(H) coupled with the adjacent –C–C–(O) stretching vibration at 1041 cm^{-1} was selected for representation of MA-dextran concentration. The results show an excellent linear relationship between the integrated area ratio (A_{1555}/A_{1017}) and the molar ratio of AAc to anhydroglucodside residues (Fig. 2). The linear dependence was established by using three different MA-dextrans (DS 4.9, 10.1 and 11.6) and 8 different molar ratios of AAc residues of poly(sodium acrylate) to anhydroglucoside of each MA-dextran. The results also indicate that the relationship is essentially independent of the degree of MA conjugation with dextran molecules. The calibration samples were prepared in the void of chemical cross-linking reaction, but otherwise similar to hydrogels with comparable polymer concentrations in aqueous solutions. Therefore, from the observation of the excellent linear relationship, it is rather evident that the AAc units and anhydroglucoside residues are present homogenously in both the swollen hydrogels and calibration samples. In addition, this also signifies the fidelity of the FTIR-ATR measurements employed in this study, in spite of the limited penetration depth of its Evanescent waves into the specimen structure. According to Eq. (1), the proportional constant (K, 0.0096) with the coefficient of determination (0.99957) was obtained. Further, the AAc content within hydrogel and the degree of dissociation of AAc units in response to different pH and salt concentration of external aqueous solution were accordingly determined. Prior to the measurements of the degree of dissociation of AAc units within hydrogels, hydrogels were placed in HCl aqueous solutions separately with two ionic strengths (0.01 and 0.1) in pH range from 3.3 to 6.4. Instead of buffer solutions, HCl solution was utilized due to its absence of carboxylate group, which might result in an overlapping in FTIR-ATR transmittance band with acrylate anions.

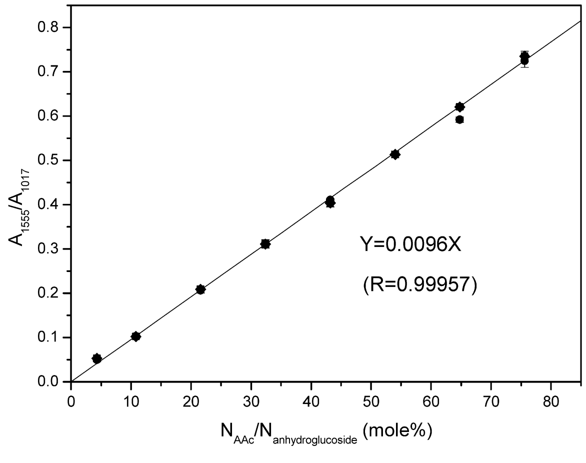

Fig. 2. Relation between A_{1555}/A_{1017} and the molar ratio, $N_{AAc}/N_{glucoside}$, for MA-dextrans with DS equal to 4.9 (■), 10.1 (●) and 11.6 (◆), respectively. The data were obtained from FTIR-ATR measurements in triplicate.

Based upon the FTIR-ATR measurements, the incorporated AAc contents were, respectively, 7.7 and 11.9 mol% with respect to the anhydroglucoside residues within hydrogels from 60 and 150 μmol of AAc monomer added in the copolymerization solutions. After thorough extraction of hydrogels in deionized water with stirring for at least 7 days, the polymerization yields for dextran hydrogels (DS 4.9) with AAc 7.7 and 11.9 mol% were 88 and 84% by weight, respectively. In terms of the incorporation efficiency, they were only 35 and 22% for copolymerization with AAc monomers of 60 and 150 μmol in the reaction solutions, respectively. The increase in the addition amount of AAc monomer in copolymerization reduced the incorporation efficiency of AAc residues within hydrogel. It is reasonable to postulate that the reactivity of AAc monomer in copolymerization reaction is somewhat higher than that of MA moieties attached to dextran molecules mainly owing to the less inherent steric hindrance and higher mobility of AAc. Therefore, the reduction in the incorporation efficiency of AAc with increasing its reaction concentration was resulted from the generation of poly(acrylic acid) homopolymers during copolymerization, which were then entirely or almost entirely removed by extraction.

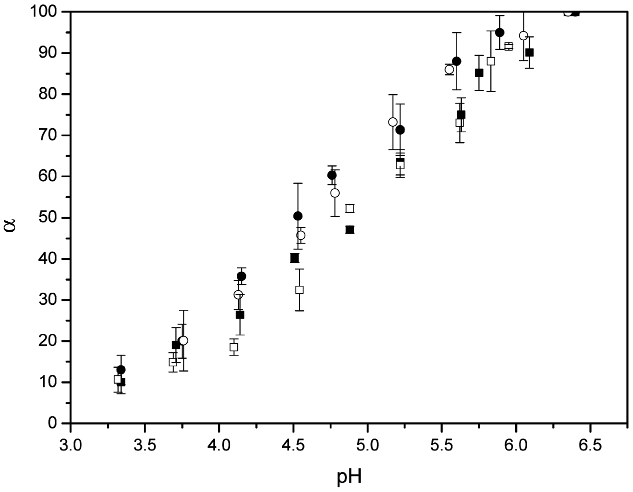

Fig. 4. Degree of dissociation of AAc units for dextran hydrogels (DS 4.9) with AAc contents equal to 7.7 (solid symbols) and 11.9 mol% (open symbols) as a function of external pH in I 0.01 (squares) and 0.1 (circles) of the external aqueous solutions. Error bars represent the standard deviation of triplicate measurements.

Fig. 3 shows the differences in FTIR-ATR spectra of hydrogels (DS 4.9 and AAc 11.9 mol% with respect to the anhydroglucoside moieties) in response to changes in pH (3.7, 5.5 and 6.4, respectively) of external HCl solutions. It clearly demonstrates that the band positions, especially at 1555 and 1017 cm^{-1}, between the spectra of hydrogels and calibration samples (as shown by Figs. 1 and 3) are essentially identical and, from the hydrogel spectra, the integrated area ratio from these two frequencies increases with increasing external pH. Based upon the FTIR-ATR data, the degrees of dissociation of AA units for hydrogels (DS 4.9) with the AAc contents 7.7 and 11.9 mol%, respectively, in response to changes in pH and ionic strength of external aqueous solutions were obtained (Fig. 4). The results indicate that the degree of dissociation of AAc units is higher at $I = 0.1$ than that at $I = 0.01$ of the external solution. For hydrogels with different AAc contents at the same external pH, the degree of dissociation for hydrogel with AAc 11.9 mol% is slightly lower than that for AAc 7.7 mol% due to the lower internal pH (higher proton concentration) within hydrogel with the AAc content equal to 11.9 mol%, as it will be discussed below. Obviously, the fraction of ionization of AAc units was mainly controlled by the pH of aqueous solution within hydrogel. The degree of dissociation is usually expressed as

$$\alpha = \frac{K_{\text{H}^+}}{K_{\text{H}^+} + C'_{\text{H}^+}} \tag{4}$$

where α is the degree of dissociation, K_{H^+} the apparent dissociation constant of AAc units with respect to proton and C'_{H^+} the proton concentration within hydrogel. Owing to the diffusion limitation of fixed acrylate anions from the interior of hydrogel to external aqueous solution, the total ion concentration, especially the counterion concentration,

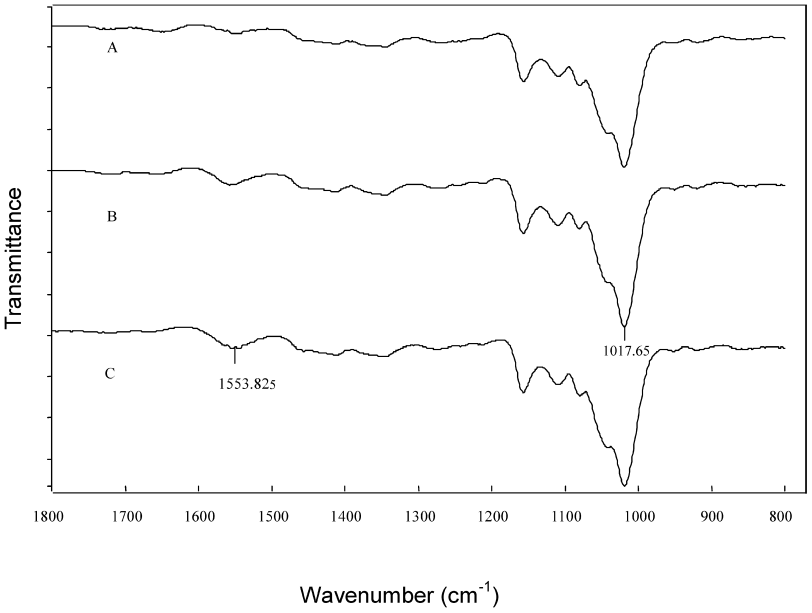

Fig. 3. FTIR-ATR spectra of dextran hydrogels (DS 4.9 AAc 11.9 mol%) in HCl solutions ($I = 0.1$) with pH 3.7 (A), 5.5 (B) and 6.4 (C), respectively.

is usually higher inside the hydrogel than that outside. The concentration of diffusible ion species accumulated within hydrogel can be determined by the Donnan equilibrium theory, which allows quantitative calculation of the distribution of diffusible ions between two separated aqueous solutions with a concentration of un-diffusible ions residing only in one side. Based upon this theory, the concentration of any ion species within swollen hydrogel can be expressed as

$$C_i' = C_i\lambda^{z_i} \tag{5}$$

where C_i' and C_i are the concentrations of ion species i in aqueous solutions inside and outside the hydrogel, respectively, λ is the Donnan ratio and z_i the charge on the species i. Therefore, the proton concentration within hydrogel (C'_{H^+}) in Eq. (4) becomes equal to the product of the Donnan ratio and the external proton concentration ($C_{H^+}\lambda$).

Following the principle of local charge neutrality inside the swollen hydrogel, the Donnan ratio can be obtained from the only real root of the following equation [27,28]:

$$(1 - v_{2,s})\sum z_iC_i\lambda^{z_i} + z_{AAc}\sigma_o v_{2,s}\alpha = 0 \tag{6}$$

Here σ_o and z_{AAc} are the concentration and charge (equal to -1) of AAc units in dry gel, respectively, and $v_{2,s}$ the polymer volume fraction of hydrogel at equilibrium swelling, which was obtained from the swelling measurements as described previously. Since the degrees of dissociation (α) of AAc units in response to different external pH and ionic concentrations were obtained from the FTIR-ATR measurements, the Donnan ratio (λ) can be determined from Eq. (6). Furthermore, based upon the data of α and λ, the dissociation constants (K_{H^+}) of AAc units within hydrogel under different external pH and ionic strengths can be easily determined from Eq. (4) and the results are shown in Fig. 5.

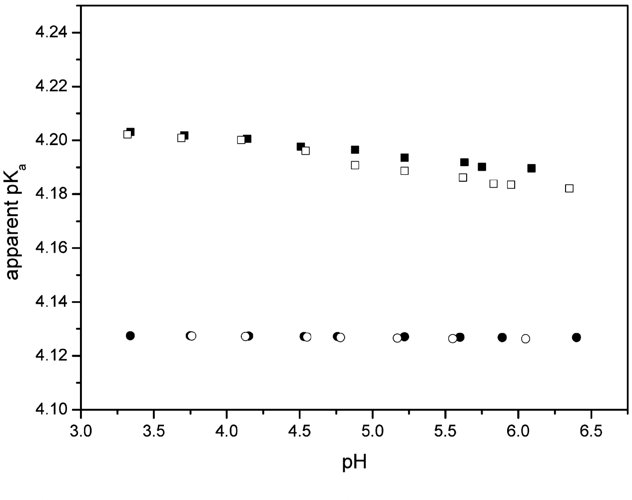

Fig. 5. Apparent dissociation constant of AAc units for dextran hydrogels (DS 4.9) with AAc contents equal to 7.7 (solid symbols) and 11.9 mol% (open symbols) as a function of external pH in I 0.01 (squares) and 0.1 (circles) of the external aqueous solutions, respectively. The data were obtained from Fig. 4.

It is found from Fig. 5 that the apparent dissociation constant of fixed AAc groups within hydrogels in pK_a form decreases with increasing external pH. It is also observed that the K_{H^+} value of AAc units for hydrogel with the AAc content 11.9 mol% is slightly higher than that of 7.7 mol%. In addition, a more pronounced change in K_{H^+} is observed in response to the variation in the ionic strength of external aqueous solutions from 0.01 to 0.1 M. From these observations, it becomes more evident that the dissociation constant of AAc units is strongly related to the total ion concentration within hydrogel, which is regulated by either the AAc content, external pH or external ionic strength, as shown in Fig. 5. With the increase in the total ion concentration inside the hydrogel, the deviations of the concentrations of ionized and unionized AAc groups from their thermodynamic activities and, as a consequence, the apparent dissociation constant from its intrinsic counterpart increase, as it can be described by the Debye–Hückel theory as follows [29]:

$$pK_H = pK_H' - \frac{0.51(2|z_{AAc}| - 1)\sqrt{I'}}{1 + \sqrt{I'}} \tag{7}$$

Here pK_H' is the intrinsic dissociation constant of AAc units in -log form with respect to proton, I' the ionic strength within hydrogel and $|z_{AAc}|$ the absolute value (equal to 1) of the charge of acrylate anion.

However, due to the presence of small ions, especially the accumulation of counterions within hydrogel, the fraction of ionization of AAc units can also be affected by partial condensation of counterion (in this study Na^+ only) due to the presence of fixed acrylate anions [30,31]. Taking account of the effect of counterion condensation, we derive an equation for the true degree of dissociation (α_T) of AAc units within hydrogel as follows:

$$\alpha_T = \frac{K_{H^+}K_{Na^+}}{K_{H^+}K_{Na^+} + \lambda C_{H^+}K_{Na^+} + \lambda C_{Na^+}K_{H^+}} \tag{8}$$

Here K_{Na^+} is the dissociation constant of AAc units with respect to Na^+ and C_{Na^+} the concentrations of Na^+ in the external aqueous solution. Therefore, the true degree of dissociation of AAc units and the Donnan ratio can be obtained by solving Eqs. (5), (6) and (8) together. In calculation of α_T and λ, the value (0.25) of K_{Na^+} was acquired directly from Ref. [31] and the values of K_{H^+} in response to different external pH and ionic strengths were obtained from Fig. 5, respectively. The influence of ionic strength on K_{Na^+} is negligible due to its high degree of dissociation as proved by Eq. (7).

Figs. 6 and 7 show the true degrees of dissociation of AAc units for hydrogels with the AAc contents equal to 7.7 and 11.9 mol%, respectively, as functions of external pH and ionic strength. For hydrogels with AAc 7.7 mol%, the fraction of dissociation of AAc units at low pH became lower at $I = 0.01$ than that at $I = 0.1$ of the external solutions whereas the AAc units were ionized to a higher

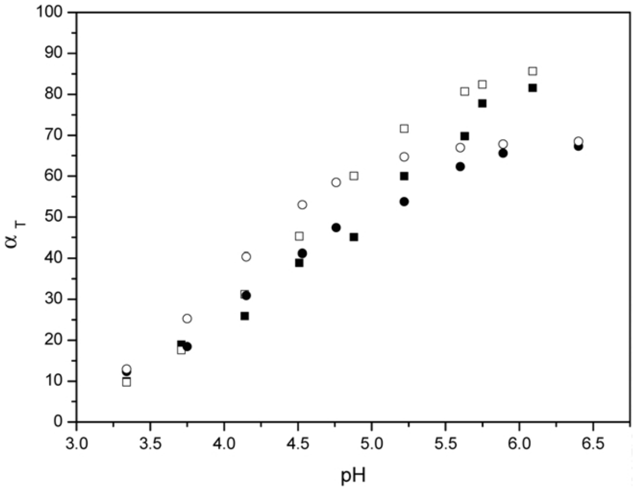

Fig. 6. Relation of the true degree of dissociation of AAc units of dextran hydrogel (DS 4.9 and AAc 7.7 mol%) and pH of the external aqueous solutions with I 0.01 (■) and 0.1 (●), respectively. Theoretical prediction of the dependence of the true degree of dissociation of AAc on external pH is also illustrated in open symbols with I 0.01 (□) and 0.1 (○).

extent at $I = 0.01$ than that at $I = 0.1$ at high pH (Fig. 6). At low pH, the Debye–Hückel effect on the dissociation constant (K_{H^+}) was more significant, leading to a higher fraction of dissociation of AAc units at the higher ionic strength ($I = 0.1$). However, the Debye–Hückel effect becomes negligible at high pH even though the apparent dissociation constant (K_{H^+}) slightly increases. The counterion condensation, on the other hand, reduced the true fraction of dissociation, especially at high pH and ionic strength, where the concentrations of acrylate anion and its counterion within hydrogels were essentially higher. Therefore, α_T of AAc units becomes lower at $I = 0.1$ than that at $I = 0.01$ at high pH. On the other hand, Fig. 7 indicates that, for hydrogels with AAc 11.9 mol% in the external pH range used in this study, the true degree of dissociation of AAc units is higher at $I = 0.01$ than that at $I = 0.1$. From the aforementioned discussion, it can be expected that the result is primarily a consequence of the counterion condensation. To justify this, the degree of dissociation of AAc units was also calculated, using Mathematica (version 4.0) to solve Eqs. (5)–(8) simultaneously, in which the Debye–Hückel effect on the apparent dissociation constant of AAc units and the ionic binding of acrylate anions with counterions have both been taken into account [22]. The intrinsic dissociation constant of AAc units within polymeric network was assumed equal to that of AAc monomer in an ideal aqueous solution and the value (4.25) in its -log form was directly employed in Eq. (7). The results also shown in Figs. 6 and 7 clearly demonstrate their high similarity to the data obtained based upon the FTIR-ATR measurements in the responses of the true degree of dissociation of AAc units to changes in external pH, external ionic strength and the AAc content of hydrogel. The degree of dissociation obtained from the theoretical computation is slightly higher than that obtained from the FTIR-ATR measurements probably owing to the difference in the intrinsic dissociation constant of AAc units in the copolymer network. In the case that if the Debye–Hückel effect and ionic condensation were not considered, the degree of ionization of AAc would be solely controlled by the internal pH of hydrogels as shown by Eq. (4). However, the results in this study indicate that the degree of dissociation of AAc units is influenced by the ionic condensation and Debye–Hückel effect as well.

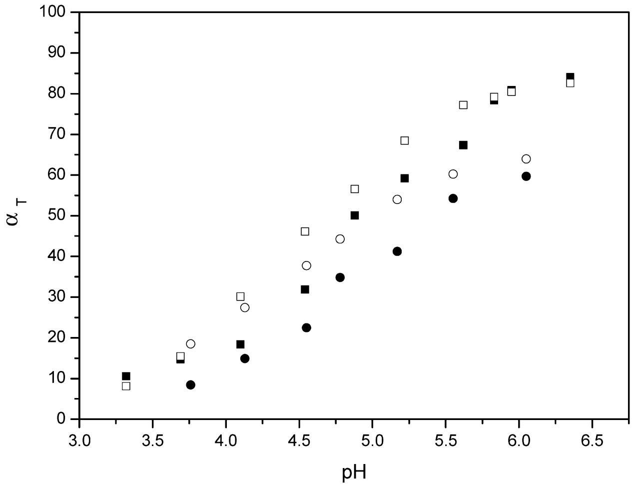

Fig. 7. Relation of the true degree of dissociation of AAc units of dextran hydrogel (DS 4.9 and AAc 11.9 mol%) and pH of the external solutions with I 0.01 (■) and 0.1 (●), respectively. Theoretical prediction of the dependence of the true degree of dissociation of AAc on external pH is also illustrated in open symbols with I 0.01 (□) and 0.1 (○).

Owing to the presence of fixed acrylate anions within hydrogel, the total ion concentration inside the hydrogel is higher than that in the external aqueous solution owing to the local neutrality and equality of chemical potentials of small ions inside and outside the hydrogel as described by the Donnan equilibrium theory. Accumulation of small ions within hydrogel results in a difference in chemical potentials of water molecules outside and inside the hydrogel. Therefore, the ionic osmotic pressure arises in proportion to the total ion concentration difference as described by van't Hoff equation. According to Eq. (5), the total ion concentration difference (ΔC_{total}) can be obtained as follows [27]:

$$\Delta C_{\text{total}} = \sum(C_i' - C_i) = \sum C_i(\lambda^{z_i} - 1) \qquad (9)$$

Fig. 8 shows the variation of the total ion concentration difference for hydrogels with AAc 7.7 and 11.9 mol%, respectively, in response to changes in external pH and ionic strength. The results were obtained based on the data from the FTIR-ATR measurements. It is obvious that ΔC_{total} increases with increasing external pH or AAc content of hydrogel. Undoubtedly, the internal total ion concentration increases with increasing the concentration of ionized AAc units within hydrogel, which is mainly governed by the AAc content of hydrogel and external pH. On the other hand,

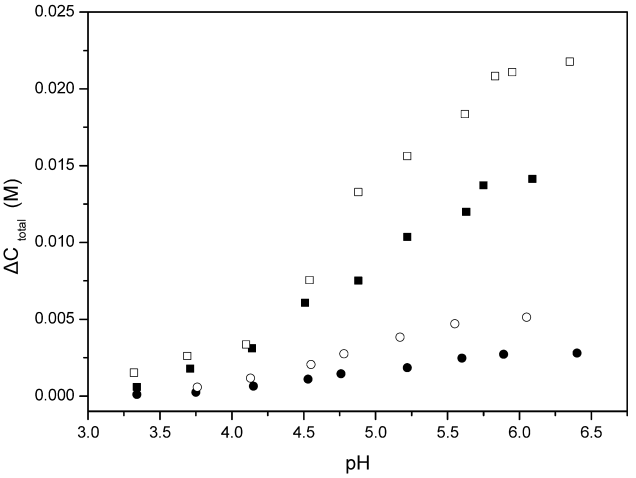

Fig. 8. Effect of the external pH on the total ion concentration difference (ΔC_{total}) for hydrogels (DS 4.9) with AAc contents equal to 7.7 mol% with I 0.01 (■) and 0.1 (●) and 11.9 mol% with I 0.01 (□) and 0.1 (○), respectively.

ΔC_{total} decreases with increasing the external ionic strength due to the ideal Donnan effect [22]. As the salt concentration increases, the Donnan ratio decreases as illustrated in Fig. 9. It can be expected that, in the presence of a sufficiently high concentration of salt, the total ion concentration difference is approximately equal to the concentration of ionized AAc units only as described by the Donnan equilibrium theory [32]. The increase in the Donnan ratio with increasing external pH was also observed owing to the increase in the concentration of fixed acrylate anions within hydrogels. As expected, the total ion concentration difference is predominantly controlled by the Donnan ratio as shown in Figs. 8 and 9 and Eq. (9). The Donnan ratio generated due to the presence of fixed ionized groups within hydrogel is a quantitative index for the partition of free mobile ions between the internal and external of hydrogels. Therefore, the approach adopted herein can also be utilized in similar manner for the evaluation of the ionic osmotic performance in swelling of hydrogels with different ionizable groups.

Fig. 10 shows the swelling response of hydrogels with different AAc contents (7.7 and 11.9 mol%, respectively) to changes in external pH and ionic strength. The swelling of hydrogels increases with increasing external pH owing to the increased ionized AAc units and, as a consequence, the total ion concentration difference. It is observed that the change in swelling follows ΔC_{total} perfectly in response to change in external pH, especially at high pH. Therefore, under such a circumstance, the swelling of hydrogel is governed predominantly by the ionic osmotic performance due to the accumulation of small ions within hydrogel. At low pH, where ΔC_{total} is rather small, the extents of swelling became rather close to those in deionized water (8.7 and 8.4 for hydrogels with AAc 7.7 and 11.9 mol%, respectively). The swelling is driven mainly by the osmotic response from the mixing interaction of polymeric network with water molecules as described by the Flory–Huggins equation [33]. More importantly, at low pH, the higher extent of swelling for hydrogels with AAc 7.7 mol% than that for 11.9 mol% was attributed to the difference in the crosslinking density (the concentration of elastically effective subchains) of hydrogels due to the increased intermolecular covalent connection of MA moieties of MA-dextrans by AAc bridging effect [22].

Following the usual affine thermodynamic model for hydrogel swelling, the swelling of an ionic hydrogel is governed mainly by the balance between the osmotic pressure within hydrogel and elastic retractile force of polymer network [33]. The osmotic pressure arises from the accumulation of small ions within hydrogels and energy changes in the configurational entropy of polymer chains and dilution heat due to the mixing interaction of polymeric chains with water molecules. For the latter, it can be expressed quantitatively by the Flory–Huggins equation

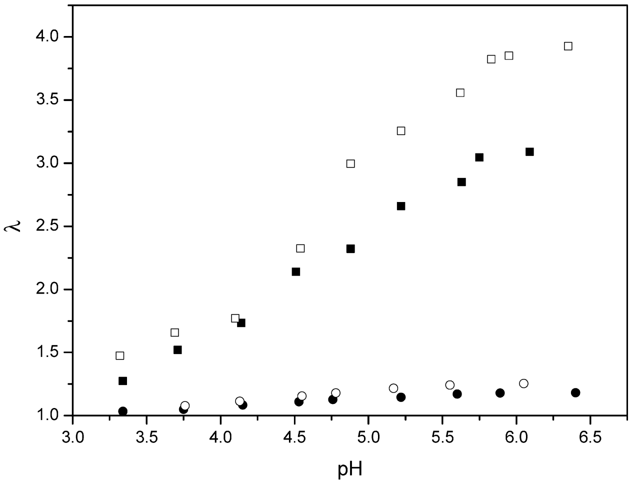

Fig. 9. Effect of the external pH on the Donnan ratio (λ) for hydrogels (DS 4.9) with AAc contents equal to 7.7 mol% with I 0.01 (■) and 0.1 (●) and 11.9 mol% with I 0.01 (□) and 0.1 (○), respectively.

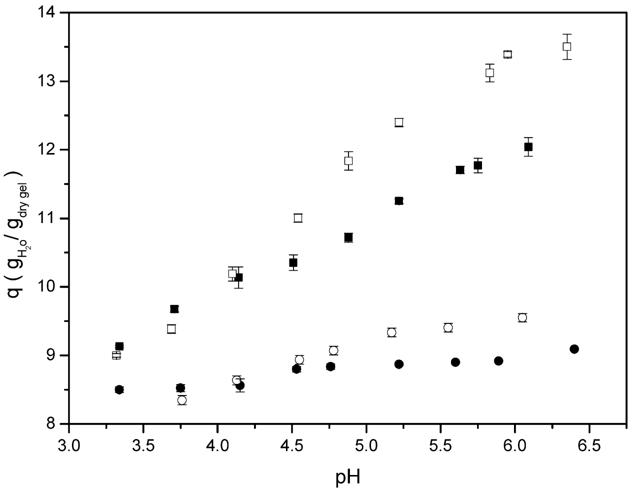

Fig. 10. Relation of the external pH and the equilibrium swelling (q) for dextran hydrogels (DS 4.9) with AAc contents equal to 7.7 mol% with I 0.01 (■) and 0.1 (●) and 11.9 mol% with I 0.01 (□) and 0.1 (○). Error bars represent the standard deviation of triplicate measurements.

[33]. However, at high pH, the swelling is predominantly driven by the ionic osmotic pressure as shown earlier in this study and elsewhere as well [34]. The mixing contribution to swelling becomes negligible as compared to the ionic osmotic performance. The difference in chemical potentials ($\Delta\mu_{\text{ion}}$) between water molecules outside and inside the hydrogel due to the ion accumulation (i.e. ionic osmotic response) can be described as follows:

$$\Delta\mu_{\text{ion}} = RTV_1\Delta C_{\text{total}} \tag{10}$$

where V_1 is the molar volume of water molecules. On the other hand, the counterbalance force to hydrogel swelling is generated from the elastic retractile response of polymer network.

On the other hand, with the well known treatment based on the assumptions of the affine model, Gaussian distribution behavior and tetrafunctionality for cross-linking, the change in chemical potential ($\Delta\mu_{\text{ela}}$) of water molecules induced by the retractile response of polymer network can be described as [33]

$$\Delta\mu_{\text{ela}} = RT\nu_{\text{e}}V_1(v_{2,\text{r}}^{2/3}v_{2,\text{s}}^{1/3} - v_{2,\text{s}}/2) \tag{11}$$

Fig. 11 shows the discrepancy between $\Delta\mu_{\text{ion}}$ and $\Delta\mu_{\text{ela}}$, especially at high external pH, even though the osmotic response from mixing interaction is further neglected. As an only counterbalance contribution, the change in the elastic retractile force in response to hydrogel swelling deviates from its energy balance, strongly indicating that the assumptions, especially that the elastically effective chains follow the Gaussian behavior, are not applicable. The non-Gaussian behavior was induced most probably by the high stiffness and high cross-linking density of polymer network chains. Therefore, based upon the non-Gaussian theory, not only did the dilution effect of swelling on elastic modulus as characterized by the Gaussian behavior (seen in Eq. (3)) disappear, but the retractile response also further increased as a result of a decrease in the theoretical flexible unit of effective subchains due to the high extension of polymer chains at a high degree of swelling [35]. The results of detail analysis in the non-Gaussian behavior of elastic modulus of dextran/AAc copolymerized networks will be presented in our upcoming paper.

4. Conclusion

In this study, copolymerized dextran/AAc networks were obtained from free radical copolymerization of MA-dextran with AAc in aqueous solution, using APS and TMEDA as an initiation system. The AAc content of hydrogel as well as the degree of dissociation of AAc units in response to different external pH and ionic strengths was quantitatively determined by FTIR-ATR spectroscopy. From the FTIR-ATR measurements, it was observed that the change in the apparent dissociation constant of AAc units follows the Debye–Hückel behavior owing to the accumulation of diffusible small ions within hydrogels upon the ionization of AAc units. Taking account of counterion condensation with fixed acrylate anions within hydrogel, the true fraction of dissociation of AAc units and the total ion concentration difference were calculated according to the Donnan equilibrium theory. The results indicate that the swelling response of hydrogels to changes in external pH and ionic strength is predominantly driven by ionic osmotic performance. In addition, from the energy balance analysis for hydrogels at equilibrium swelling, it is observed that the elastic retractile response of AAc/dextran copolymerized network deviated obviously from the Gaussian behavior.

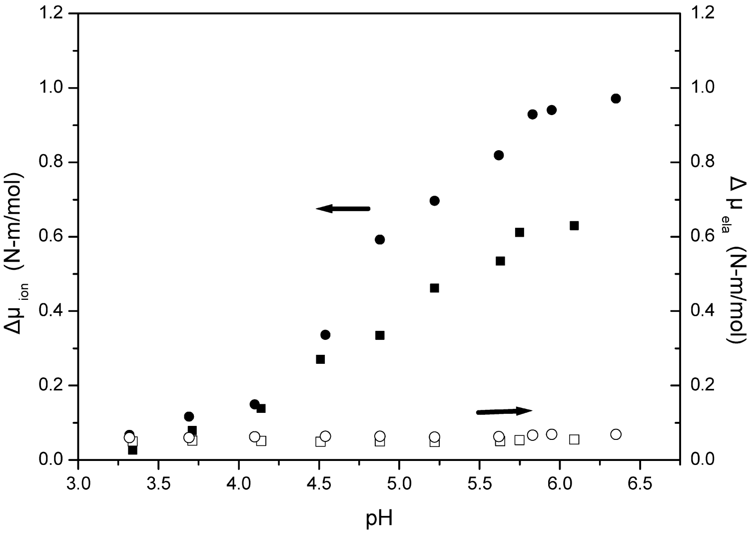

Fig. 11. Effect of external pH on the chemical potential differences due to ionic osmotic ($\Delta\mu_{\text{ion}}$, solid symbols) and elastic retractile ($\Delta\mu_{\text{ela}}$, open symbols) responses of dextran hydrogels (DS 4.9) with AAc contents equal to 7.7 (square symbols) and 11.9 mol% (circle symbols). $I = 0.01$. $\Delta\mu_{\text{ela}}$ was obtained based on the Gaussian model.

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References

[1] Bronsted H, Anderson C, Hovgaard L. Crosslinked dextran—a new capsule material for colon targeting of drug. J Contr Rel 1998;53:7–13.

[2] Kim SH, Chu CC. Synthesis and characterization of dextran–methacrylate hydrogels and structural study by SEM. J Biomed Mater Res 2000;49:517–27.

[3] Kim SH, Won CY, Chu CC. Synthesis and characterization of dextran–maleic acid based hydrogels. J Biomed Mater Res 1999;46:160–70.

[4] Kim SH, Won CY, Chu CC. Synthesis and characterization of dextran-based hydrogels prepared by photocrosslinking. Carbohydr Polym 1999;40:183–90.

[5] Chiu HC, Hsiue GH, Lee YP, Huang LW. Synthesis and characterization of pH-sensitive dextran hydrogels as a potential colon-specific drug delivery system. J Biomater Sci Polym Edn 1999;10:591–608.

[6] Van Dijk-Wolthuis WNE, Franssen O, Talsma H, Van Steenbergen MJ, Kettenes-Van Den Bosch JJ, Hennink WE. Synthesis, characterization and polymerization of glycidyl methacrylate derivatized dextran. Macromolecules 1995;28:6317–22.

[7] Van Dijk-Wolthuis WNE, Kettenes-Van Den Bosch JJ, Van Der Kerk-Van Hoof A, Hennink WE. Reaction of dextran with glycidyl methacrylate: an unexpected transesterification. Macromolecules 1997;30:3411–3.

[8] De Smedt SC, Lauwers A, Demeester J, Van Steenbergen MJ, Hennink WE, Roefs SPFM. Characterization of the network structure of dextran glysidyl methacrylate hydrogels by studying the rheological and swlling behavior. Macromolecules 1995;28:5082–8.

[9] Meyvis TKL, De Smedt SC, Demeester J, Hennink WE. Influence of the degradation mechanism of hydrogels on their elastic and swelling properties during degradation. Macromolecules 2000;33:4717–25.

[10] Meyvis TKL, De Smedt SC, Demeester J, Hennink WE. Rheological monitoring of long-term degrading polymer hydrogels. J Rheol 1999;43:933–50.

[11] Franssen O, Van Ooijen RD, De Boer D, Maes RAA, Herron JN, Hennink WE. Enzymatic degradation of methacrylated dextrans. Macromolecule 1997;30:7408–13.

[12] Franssen O, Van Ooijen RD, De Boer D, Maes RAA, Hennink WE. Enzymatic degradation of cross-linked dextrans. Macromolecules 1999;32:2896–902.

[13] Van Dijk-Wolthuis WNE, Hoogeboom JAM, Van Steenbergen MJ, Tsang SKY, Hennink WE. Degradation and release behavior of dextran-based hydrogels. Macromolecules 1997;30:4639–45.

[14] Franssen O, Vos OP, Hennink WE. Delayed release of a model protein from enzymatically-degrading dextran hydrogels. J Contr Rel 1997;44:237–45.

[15] Hennink WE, Franssen O, Van Dijk-Wolthuis WNE, Talsma H. Dextran hydrogels for the controlled release of proteins. J Contr Rel 1997;48:107–14.

[16] Hennink WE, Talsma H, Borchert JCH, De Smedt SC, Demeester J. Controlled release of proteins from dextran hydrogels. J Contr Rel 1996;39:47–55.

[17] De Smedt SC, Meyvis TKL, Demeester J, Van Oostveldt P, Blonk JCG, Hennink WE. Diffusion of macromolecules in dextran methacrylate solutions and gels as studied by confocal scanning laser microscopy. Macromolecules 1997;30:4863–70.

[18] Franssen O, Vandervennet L, Roders P, Hennink WE. Degradable dextran hydrogels: controlled release of a model protein from cylinders and microspheres. J Contr Rel 1999;60:211–21.

[19] Chiu HC, Wu AT, Lin YF. Synthesis and characterization of acrylic acid-containing dextran hydrogels. Polymer 2001;42:1471–9.

[20] Chiu HC, Lin YF, Hsu YH. Effects of acrylic acid on preparation and swelling properties of pH-sensitive dextran hydrogels. Biomaterials 2002;23:1103–12.

[21] Chiu HC, Hsu YH, Lin PJ. Synthesis of pH-sensitive inulin hydrogels and characterization of their swelling properties. J Biomed Mater Res 2002;61:146–52.

[22] Chiu HC, Lin YF, Hung SH. Equilibrium swelling of copolymerized acrylic acid–methacrylated dextran networks: effects of pH and neutral salt. Macromolecules 2002;35:5235–42.

[23] Errington E, Harding SE, Illum L, Schacht EH. Physico-chemical studies on diiodotyrosine dextran. Carbohydr Polym 1992;18:289–94.

[24] Cluff EF, Gladding EK, Praiser R. A new method for measuring the degree of crosslinking in elastomers. J Polym Sci 1960;45:341–5.

[25] Ulbrich K, Dusek K, Ilavsky M, Kopecek J. Preparation and properties of poly(*N*-butylmethacrylamide) networks. Eur Polym Sci 1978;14:45–9.

[26] Dusek K, Prins W. Structure and elasticity of non-crystalline polymer networks. Adv Polym Sci 1969;6:1–102.

[27] Ricka J, Tanaka T. Swelling of ionic gels: quantitative performance of the Donnan theory. Macromolecules 1984;17:2916–21.

[28] Firestone BA, Siegel RA. pH, salt, and buffer dependent swelling in ionizable copolymer gels: tests of the ideal Donnan equilibrium theory. J Biomater Sci Polym Ed 1994;5:433–50.

[29] Martin A. Physical pharmacy, 4th ed. Philadelphia, PA: Lea & Febiger; 1993.

[30] Helfferich F. Ion exchange. New York: McGraw-Hill; 1962.

[31] Eichenbaum GM, Kiser PF, Dobrynin AV, Simon SA, Needham D. Investigation of the swelling response and loading of ionic microgels with drugs and proteins: the dependence of cross-link density. Macromolecules 1999;32:4867–78.

[32] Atkins PW. Physical chemistry, 5th ed. Oxford: Oxford University Press; 1994.

[33] Flory PJ. Principles of polymer chemistry. Ithaca, NY: Cornell University Press; 1953.

[34] Horkay F, Tasaki I, Basser PJ. Osmotic swelling of polyacrylate hydrogels in physiological salt solutions. Biomacromolecules 2000;1:84–90.

[35] Treloar LRG. The physics of rubber elasticity, 3rd ed. Oxford: Oxford University Press; 1975.