Equilibrium Swelling of Copolymerized Acrylic Acid−Methacrylated Dextran Networks: Effects of pH and Neutral Salt

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ABSTRACT: In this study, pH-responsive dextran hydrogels were prepared by radical copolymerization of methacrylated dextran (MA-dextran) with acrylic acid (AAc) in aqueous solution, using ammonium peroxydisulfate and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine as an initiation system. The AAc content in hydrogels was determined quantitatively by FTIR. The swelling response of hydrogels to changes in pH/salt concentration was characterized by quantitative evaluation of ionic osmotic performance according to the Donnan equilibrium theory, taking account of the Debye−Hückel effect on the apparent acidity constant of AAc groups and the ionic binding of acrylate anions with counterions within hydrogel. A close-to-linear relation between the equilibrium swelling of hydrogels and the pH-induced ionic osmotic pressure is observed. The accompanied change of the polymer−solvent interaction parameter (χ) is obtained from free energy balance between ionic osmotic pressure, the elastic retractile force, and the interaction of polymer network with water molecules (the mixing contribution) and is governed by the concentration of fixed ionized AAc units within hydrogels. For hydrogels having varying AAc contents, the difference in swelling under identical osmotic pressure results from the variation in the effective network density of hydrogels. The neutral salt effect on the pH-induced swelling of the same hydrogel follows changes in the ionic osmotic pressure and the degree of dissociation of AAc units associated with the Debye−Hückel theory. The change of the degree of dissociation of AAc units in response to the ionic strength effect dominates and describes well the sensitivity of the swelling behavior of hydrogels to the ionic osmotic pressure.

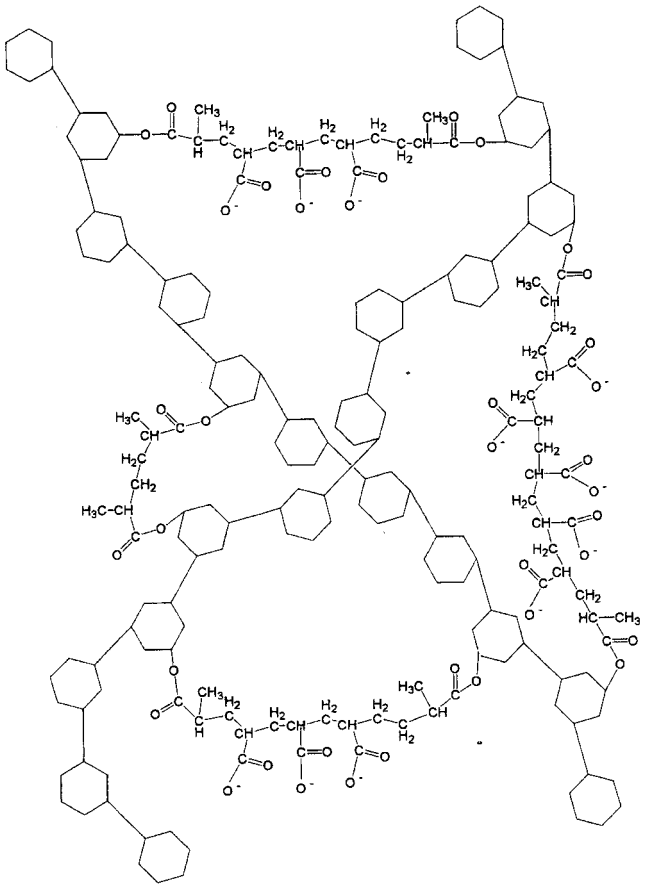
Introduction

Recently, the interest in dextran hydrogels has increased significantly owing to their potential applications in biochemical and biomedical fields. Dextran hydrogels are also frequently considered as a site-specific drug delivery system primarily due to their good tissue compatibility and enzymatic degradability at the desired sites. Several approaches exploited to prepare dextran hydrogels have been reported.[1−10] For example, van Dijk-Wolthuis et al. obtained dextran hydrogels by transesterification of glycidyl methacrylate (GMA) with dextran, followed by free radical polymerization of methacrylated dextran (MA-dextran) in aqueous solution. The polymerization was initiated by ammonium peroxydisulfate and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine.[3,4]

In our previous paper, we reported the effects of acrylic acid (AAc) on the preparation of dextran hydrogels and their swellings at pH 7.4.[11] Surprisingly somewhat, the chemical cross-linking density of polymerized MA-dextran networks was increased by the addition of AAc as a comonomer. The increase in the effective network density was primarily a consequence of an enhanced intermolecular covalent connection of MA moieties of MA-dextran molecules by the bridging effect of AAc (Scheme 1). Gel permeation chromatography also confirmed this by showing a significant increase of the molecular weight of the soluble cross-linked AAc-MA-dextran as the AAc concentration in copolymerization solution increased from 0.16 to 0.48 mol % with respect to the number of anhydroglucoside residues of MA-dextran.[11]

In this work, the pH/salt-triggered swelling response of copolymerized AAc-MA-dextran networks was analyzed by quantitative evaluations of the effective network density, the ionic osmotic pressure, and the

Scheme 1. Illustration of Connection of MA Moieties by AAc Bridging



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interaction of polymer network with water molecules. By taking account of the effects of the ionic strength associated with the Debye−Hückel theory and cationic exchange of fixed acrylate anions on the degree of dissociation of AAc units, the influence of the Donnan osmotic response on the swelling behavior was determined.

Experimental Section

Materials. Dextran (T-70) (Amersham-Pharmacia) was dried in vacuo for 72 h before use. Gel permeation chromatography (Column: Superose 6, calibrated with dextran standards (Polysciences); FPLC, Amersham-Pharmacia; Eluent: 0.1 M Tris buffer, pH 7.4, 0.02 wt % NaN_3; flow rate: 0.4 mL/min; RI detector, RI-930, Jasco) indicated that the number- and weight-average molecular weights were 61 000 and 106 000 g/mol, respectively. AAc (Lancaster) was vacuum-distilled before use. GMA, ammonium peroxydisulfate (APS), and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) were obtained from TCI, Showa and Lancaster Co., respectively, and used as received. Poly(sodium acrylate) from Aldrich Chemical Co. has a weight-average molecular weight of 15 000 g/mol. All other chemicals used were reagent grade. Deionized water used in polymerization and buffer preparation was obtained from Milli-Q Synthesis (Millipore). MA-dextran was obtained by transesterification of GMA with dextran, and the degree of substitution (DS) was determined by ^{1}H NMR as described elsewhere.[3] The DS is defined as the number of MA moieties per 100 anhydroglucoside residues.

Hydrogel Preparation. Hydrogels were obtained from radical copolymerization of MA-dextran with AAc in aqueous solution, using APS and TMEDA as an initiation system. MA-dextran (45 mg) was dissolved in borate buffer (0.3 mL, pH 8.5, *I* (ionic strength) 0.01) and APS (5 μmol in 0.1 mL of borate buffer) was added. The solution was extensively vortexed and purged with nitrogen for 30 min, followed by the addition of a prescribed amount of AAc and TMEDA (100 μmol) in borate buffer. The final concentration of MA-dextran was adjusted to 45 mg in 0.5 mL of aqueous solution and pH to 8.5. The polymerization was carried out at 25 °C for 72 h. Hydrogels were washed thoroughly in deionized water for 7 days and dried in vacuo.

FTIR Measurements. Hydrogels were placed in aqueous NaOH solution, and the pH was maintained at 8.5 for a full time period of 72 h. Hydrogels were then dried in vacuo at 50 °C for 72 h and subsequently ground with KBr and pressed into pellets. FTIR measurements (Spectrum One, Perkin-Elmer) were carried out by scanning each sample 16 times in triplicate in a wavenumber range from 4000 to 600 cm^{-1} with a resolution of 2 cm^{-1}. Calibration samples with varying mole ratios of AAc unit to MA moiety were prepared by dissolution of MA-dextrans with various levels of DS as determined by ^{1}H NMR and poly(sodium acrylate) in deionized water, followed by lyophilization. FTIR spectra were recorded for calibration samples in triplicate under identical conditions as described above. The absorbance peak areas at 1571 and 1721 cm^{-1}, assigned respectively to the asymmetrical stretching of carboxylate (sodium salt) of AAc and symmetrical stretching of carbonyl of MA-dextran,[3] were integrated. Irrespective of the DS of MA-dextran, a satisfactory calibration curve was obtained from the plot of the ratio of peak areas at 1571 and 1721 cm^{-1} vs the mole ratio of AAc unit to MA moiety in the range of AAc content used in this study. By placing hydrogels in aqueous NaOH solution, pH 8.5 for 72 h, full ionization of AAc units within hydrogels was confirmed by complete disappearance of the absorbance peak at 1730 cm^{-1} that was attributed originally to the carbonyl of AAc units. During the time period, the ester linkage of MA-dextrans remained intact under this condition as reported previously.[12,13] The AAc content in mol % is defined as the number of AAc units per 100 anhydroglucoside residues.

Swelling Measurements. Buffer solutions used for swelling tests were prepared from CH_3COOH/HCl or CH_3COOH/NaOH in the pH range from 3.0 to 6.0 and Tris/HCl from pH 6.5 to 7.4. The ionic strength (*I*) was adjusted to 0.005. Hydrogel (ca. 45 mg) was placed in buffer solution (200 mL) at 25 °C and constantly replaced with fresh buffer until no further changes in the swelling extent of hydrogels and pH of the external buffer solutions. The swelling ratio (*q*) herein is defined as the amount of water in grams that is uptaken by 1 g of dry gel. The swelling was also determined from buffer solutions in the presence of NaCl in the concentration range from 10^{-6} to 1.0 M. In calculation of the swelling extent in terms of the polymer volume fraction of hydrogels, the assumption of volume additivity was adopted and 1.61 g/cm^3 for the density of MA-dextran was employed.[14,15]

Cross-Linking Density Measurements. The effective network density of dextran hydrogel was evaluated by determining the modulus of elasticity in mechanical compression.[16−20] Hydrogels at the equilibrium swelling state from buffers with various pH values were cut into 1 cm diameter pieces. Equilibrium heights of swollen hydrogel under six different compressive stresses were determined in triplicate at 25 °C using a bench comparator (Ames 135). The rubberlike elastic behavior was ensured by complete recovery of hydrogels to their original heights after the removal of compressive stresses. The equilibrium modulus of elasticity (*G*) can be determined according to the following equation:[17−20]

$$F/A = -G(H - H^{-2}) \tag{1}$$

Here *F*/*A* is the compressive stress applied and *H* (height ratio) is defined as h/h_0, where *h* and h_0 are the equilibrium heights of deformed and original hydrogels, respectively. The value of *G* can be obtained from the slope of a linear plot of *F*/*A* vs $-(H - H^{-2})$. Under the assumptions of the affine network model and tetrafunctionality of effective junctions, the expression for the number of elastically effective chains of dextran hydrogel that was formed in solution phase is given by[17,18,21−24]

$$\nu_e = G/(RT{\nu_{2,s}}^{1/3}\langle\alpha\rangle_0^2) \tag{2}$$

In this equation, ν_e is the effective network density in mol/m^3, *R* the gas constant, *T* the absolute temperature, $\nu_{2,s}$ the polymer volume fraction of hydrogel at equilibrium swelling, and $\langle\alpha\rangle_0$ the isotropic dilation factor. The parameter $\langle\alpha\rangle_0$ can be approximated to ${\nu_{2,r}}^{1/3}$, where $\nu_{2,r}$ is the polymer volume fraction at the relaxed state.[23]

Results and Discussion

The polymerization yields of hydrogels were in the range from 80 to 85% as calculated from the ratio of the weight of dry gel and the weights of MA-dextran and AAc. Figure 1 illustrates the swelling response of dextran hydrogels (DS 5.7) with varying AAc contents to changes in pH of the external buffer solutions. Following the usual affine thermodynamic model, the swelling equilibria of hydrogels are primarily governed by an energy balance between the osmotic pressure within polymer network and elastic retractile force of the network structure. For neutral hydrogels, the osmotic pressure arises from energy changes in the dilution heat and the configurational entropy of polymer chains owing to the mixing interaction of disoriented polymer chains with solvent molecules. This mixing contribution in terms of the partial molar free energy of the solvent can be expressed quantitatively by the Flory−Huggins equation.[25] As hydrogel swells, the elastic reaction of network structure resulting from the change in the configurational entropy of polymer chains between cross-links militates against hydrogel swelling. This energy contribution can usually be derived from the statistical theory of rubber elasticity and is entirely

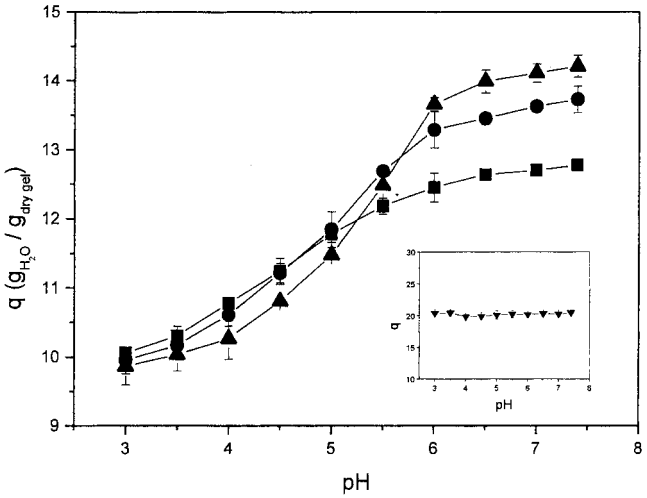

Figure 1. Equilibrium swelling of dextran hydrogels (DS 5.7) with AAc contents equal to (▼) 0, (■) 7.2, (●) 8.8 and (▲) 10.4 mol % as a function of pH of the external buffer solutions (I = 0.005). Error bar at each point represents the standard deviation of triplicate measurements.

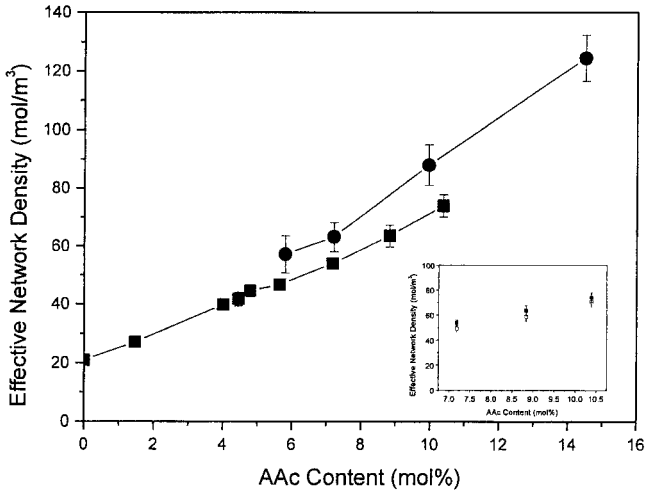

Figure 2. Effective network density of hydrogels with DS equal to (■) 5.7 and (●) 10.9 as a function of the AAc content at pH 7.4. The effective network density of hydrogels with DS 5.7 as a function of the AAc content at pH 3.5 (□) is shown in the inset. Error bars represent the standard deviation of triplicate measurements.

controlled by the cross-linking density of polymeric network that is set during the gel synthesis.[21,25] In this study, the swelling of dextran hydrogel without AAc is essentially independent of pH in the range from 3.0 to 7.4, indicating that the polymer−solvent interaction parameter (χ) of hydrogel in the void of AAc and its effective network density (ν_e) are both independent of pH.

A swelling response of dextran hydrogels with varying amounts of AAc units to changes in pH of the external buffer solutions was observed (Figure 1). In addition to the internal osmotic contribution obtained by the mixing interaction, a second internal osmotic pressure can also be generated by the accumulation of counterions within hydrogels owing to the presence of the fixed ionized acrylate groups.[26−28] The difference in chemical potentials of solvent molecules between the external and internal aqueous solutions of hydrogel results from a reduction of the translational entropy of mobile ions that are associated via ionic interaction with the fixed ionized groups in the network matrix.[29,30] However, the swelling of the AAc-containing hydrogels in the pH range used in this study is significantly reduced compared to that of hydrogels without AAc (Figure 1). The difference in swelling between the ionic and neutral hydrogels increases with decreasing pH of the external buffer solutions. At pH 3.0, where the ionic osmotic effect is negligible, ca. 2-fold difference in swelling is observed. The greatly reduced swelling of pH-responsive hydrogels is primarily a consequence of the increased cross-linking density of the networks based on copolymerization of MA-dextran and AAc (Figure 2). It was shown in our previous paper that copolymerization of MA-dextran with AAc results in an increased covalent connection of intermolecular MA moieties of MA-dextran molecules due to the chemical bridging of AAc units (Scheme 1).[11] Figure 2 also illustrates the effect of DS (5.7 and 10.9) of MA-dextran on the cross-linking density of hydrogels as compared to the bridging effect of AAc units. In addition, in the inset of Figure 2, little influence of external pH between 7.4 and 3.5 (I = 0.005) on the effective network density is observed. It is usually recognized that a dilution of the concentration of elastically effective chains of polymer network upon swelling results in decreases in the elastic modulus and the effective network density. However, the observations of the nonmonotonic dependence of elastic modulus on swelling of polymer network were reported previously.[19,31−33] The elastic deformation behavior of ionized polymer networks is affected by both the non-Gaussian behavior (finite extensibility) of effective chains and electrostatic interaction (accumulation of counterions and counterion condensation) within hydrogels.[31,32] Anbergen and Oppermann pointed out that, owing to a much higher intrinsic chain stiffness, polysaccharide networks can exhibit a non-Gaussian behavior upon swelling, causing an increase in the elastic modulus even at rather low degrees of swelling.[34] For the weakly charged dextran hydrogels in this study, the intrinsic stiffness and non-Gaussian behavior of elastically effective subchains may result in an opposed contribution to their dilution by swelling. However, further detail study will be required to clarify this issue.

As pH increases, ionization of AAc units occurs to a larger extent. Accompanied by an increase in the fixed charge density within hydrogel, the mobile counterion concentration within hydrogel increases concomitantly until the electrochemical potential of each free ion species between the internal and external aqueous solutions is the same and the local charge neutrality is reached. The ionic osmotic response generated by the accumulation of counterions within hydrogel can be determined by the difference between the total ion concentration inside and outside the hydrogel according to the van't Hoff equation. Similar to the Donnan equilibrium that describes quantitatively the distribution of diffusible ions between two solutions separated by a semipermeable membrane with a concentration of fixed charges on one side, the total ion concentration difference (ΔC_{total}) can be described as[26]

$$\Delta C_{total} = \sum(C'_i - C_i) = \sum C_i(\lambda^{z_i} - 1) \qquad (3)$$

Here C'_i and C_i are the concentrations of ion species i in aqueous solutions inside and outside the gel, respectively, z_i is the charge on the species i, and λ is the Donnan ratio. Following the principle of charge neutral-

ity within swollen hydrogel, the Donnan ratio (λ) can be obtained from the only real root of[26,35]

$$(1 - v_{2,s})\sum z_i C_i \lambda^{z_i} - \sigma_0 v_{2,s}\alpha = 0 \qquad (4)$$

Here σ_0 is the concentration of AAc units in dry gel and α the fraction of ionized AAc. Instead of the thermodynamic activity, the concentration of each ion species is used for the calculation of ionic osmotic pressure. The assumption is pertinent on the basis of the below observation that the difference of ion concentration between the internal and external solutions is relatively small as compared to the ion concentrations, and therefore, for each ion species, the activity coefficients in two different aqueous solutions are approximately equal.

However, in eq 4, the fraction of ionized AAc units (α) can be seriously influenced by the presence of salt within hydrogel in two respects. First, the apparent acidity constant (K'_{H^+}) of AAc units within hydrogel can significantly deviate from the intrinsic acidity constant (K_{H^+}) owing to the difference between the concentration and thermodynamic activity of AAc units. For this, the ionic strength effect on the apparent acidity constant of AAc units can be determined from the Debye−Hückel theory by the following equation:[36]

$$\mathrm{p}K'_{H^+} = \mathrm{p}K_{H^+} - \frac{0.51(2z_{AAc} - 1)\sqrt{I'}}{1 + \sqrt{I'}} \qquad (5)$$

where I' is the ionic strength within hydrogel and z_{AAc} the charge valence of ionized acrylate acid. The use of eq 5 is limited for ionic strengths up to 0.3 M. The ionic strength within hydrogel can be obtained by determining the concentration of each monovalent ion species (i.e., the void of multivalent ion in this study) within hydrogel as

$$C'_i = C_i \lambda^{z_i} \qquad (6)$$

Second, a highly swollen anionic hydrogel can usually be considered as a cationic exchanger, where, at high pH, the dissociation of protons from the fixed negative charges on polymer matrix leads to the partial binding with the mobile counterions.[37−39] The ionic binding of counterions (in this study, Na^+ and protonated Tris) with acrylate anions within hydrogel can result in a reduction in the degree of dissociation, although the binding strength is much lower than that of protons with acrylate anions. This effect can become more pronounced at a high salt concentration as we will soon encounter below. Taking into account the Debye−Hückel effect and the binding of counterions with acrylate anions within hydrogel, we derive an equation (eq 7) for the degree of dissociation of AAc units as

$$\alpha = K'_{H^+}K_{Na^+}K_{TrisH^+}/(K'_{H^+}K_{Na^+}K_{TrisH^+} + C_{H^+}\lambda K_{Na^+}K_{TrisH^+} + C_{Na^+}\lambda K'_{H^+}K_{TrisH^+} + C_{TrisH^+}\lambda K_{H^+}K_{Na^+}) \qquad (7)$$

Here K'_{H^+}, K_{Na^+}, and K_{TrisH^+} are the binding constants for protons, Na^+, and $TrisH^+$ with acrylate anions, respectively, and C_{H^+}, C_{Na^+}, and C_{TrisH^+} are their concentrations in the external buffer solution. The parameters used in this study are listed in Table 1, with a reasonable assumption that the Debye−Hückel effect on K_{Na^+} and K_{TrisH^+} is negligible. The Donnan ratio (λ), the total ion concentration difference (ΔC_{total}), the degree of dissociation of AAc units (α), and the ionic strength (I') within hydrogel can be concomitantly obtained by using Mathematica (version 4.0) to solve eqs 3−7 simultaneously. The relation of the internal ionic osmotic pressure ($\Pi_{ion} = RT\Delta C_{total}$) with external pH for hydrogels having varying concentrations of AAc units is shown in Figure 3.

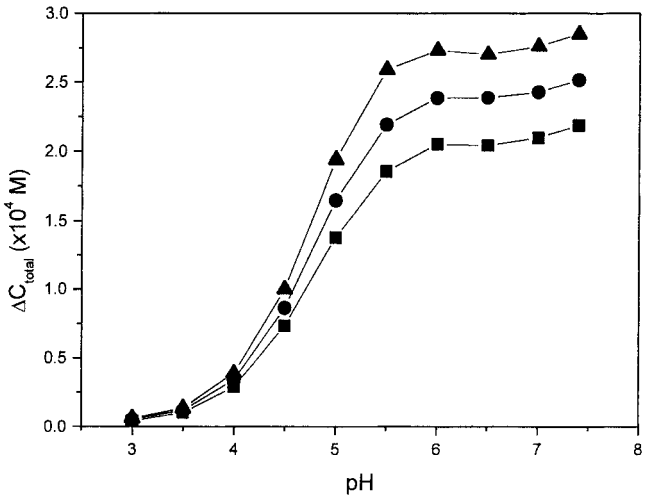

Figure 3. Effect of pH on the total ion concentration difference (ΔC_{total}) for hydrogels (DS 5.7) with AAc contents equal to (■) 7.2, (●) 8.8, and (▲) 10.4 mol %. The value of ΔC_{total} is calculated from the swelling data in Figure 1 by solving eqs 3−7 concomitantly.

Table 1. Values of Parameters Used for Calculation of the Degree of Dissociation of AAc Units from Eqs 5 and 7

parameter	value	source
K'_{H^+} (K_a) (AAc)	$10^{-4.25}$	ref 26
K_{Na^+}	0.25	ref 39
K_{TrisH^+}	0.20	ref 41
pK_a(Tris)	8.1	Merck Index, 12th ed.
pK_a(acetic acid)	4.7	Merck Index, 12th ed.

The fraction of ionized AAc units increases with increasing pH of the external solution. The internal pH that can be obtained from the Donnan ratio is somewhat higher than the external pH. The screening effect of the counterion concentration within hydrogel on the degree of dissociation of AAc is quantitatively expressed in eq 7. The ionic osmotic pressure is generated from the difference of the chemical potentials of water molecules outside and inside the hydrogel upon the accumulation of counterions within hydrogel. For dextran hydrogels having various amounts of AAc units, the ionic osmotic pressures are different under identical external pH condition. This is mainly a result of the varying amounts of ionized AAc in hydrogels. The variation of α for hydrogels with various AAc contents is rather small due to the relatively low counterion concentration within hydrogels (the screening effect). From the calculated results (data not shown here), the fraction of ionized AAc with AAc content equal to10.4 mol % is slightly lower than the other two (7.2 and 8.8 mol %).

As expected, the swelling increases with the increased ionic osmotic pressure. According to eq 4, in the case that the total ionic concentration of the external buffer solution is kept unchanged, the ionic osmotic pressure is governed by the concentration of ionized AAc units. In addition to the total AAc content, the ionized AAc concentration is also influenced slightly by the screening

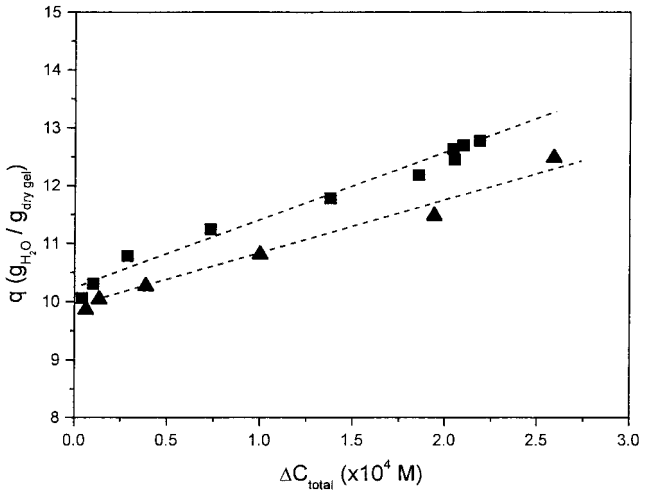

Figure 4. Relation of the pH-induced swelling (q) and ΔC_{total} for hydrogels (DS 5.7) with AAc contents equal to (■) 7.2 and (▲) 10.4 mol %.

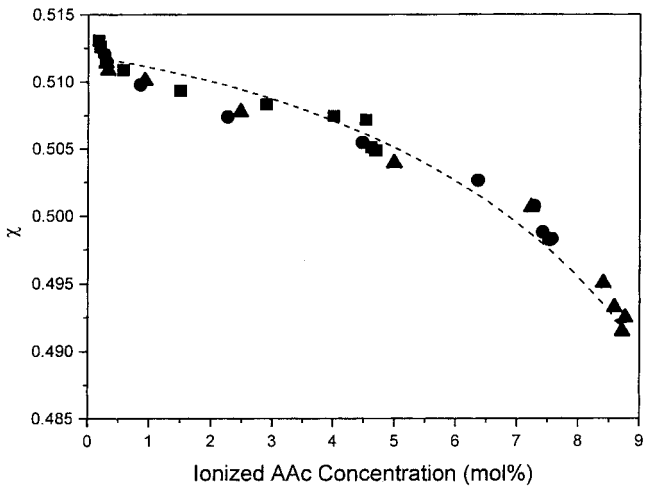

Figure 5. Relation of the polymer−solvent interaction parameter (χ) with the ionized AAc concentration of hydrogels (DS 5.7) with AAc contents equal to (■) 7.2, (●) 8.8, and (▲) 10.4 mol %.

effect and strongly by pH within hydrogel. Therefore, at low pH, the ionic osmotic pressure decreases owing to a low degree of dissociation of AAc units. Formation of hydrogen bond from the carboxyl (−COOH) groups has been usually used to explain the swelling behavior at low pH as shown in Figure 1. In this study, however, the increase of effective network density by copolymerization of MA-dextran with AAc must be considered. To justify this, the relation between the swelling of hydrogels having two different AAc contents (7.2 and 10.4 mol %) and ΔC_{total} (up to 2.6×10^{-4} M) was established. Figure 4 shows not only a monotonic increase in swelling with increasing ΔC_{total} but also a close-to-linear relationship between the swelling and ionic osmotic pressure for both hydrogels. The swelling of hydrogel with 7.2 mol % AAc units is higher than that with 10.4 mol % AAc under identical ionic osmotic pressure. Therefore, both changes in the effective network density and the interaction of polymer network with water molecules may be influential. While the effective cross-linking density can be directly obtained by the mechanical compression measurement, the polymer−solvent interaction parameter can be determined by the free energy balance of mixing, elastic retractile, and ionic osmotic responses as the swelling equilibrium of hydrogel is achieved. The chemical potential change due to the elastic reaction of network structure can be described by the following expression:[21,40]

$$\Delta\mu_{ela} = RTv_e V_1(v_{2,r}^{2/3} v_{2,s}^{1/3} - v_{2,s}/2) \qquad (8)$$

Here V_1 is the molar volume of water. The equation was derived from the rubberlike elasticity theory for polymer chains between cross-links that are introduced in solution phase. Therefore, the changes in the chemical potentials from these three contributions can be equated as

$$V_1 \sum C_i(\lambda^{z_i} - 1) = [\ln(1 - v_{2,s}) + v_{2,s} + \chi v_{2,s}^2] + v_e V_1(v_{2,r}^{2/3} v_{2,s}^{1/3} - v_{2,s}/2) \qquad (9)$$

The term on the left-hand side of eq 9 relates to the chemical potential difference in water molecules between two aqueous solutions acquired from the Donnan effect.[26,28] The first term on the right-hand members of eq 9 is derived from the Flory−Huggins equation for the chemical potential change upon the mixing of network structure with solvent, where χ is the interaction parameter between polymer and solvent.[25] The second term representing the change in elastic energy is directly obtained from eq 8. In eq 9, the Donnan ratio, λ, was obtained by utilizing the data in Figure 1 to solve eqs 3−7 together.

The results indicate that the change of the polymer−solvent interaction parameter for hydrogels with three different AAc contents (7.2, 8.8, and 10.4 mol %) under different external pH conditions follows the change in the ionized AAc concentration approximately in the same curve (Figure 5). The ionized AAc concentration was obtained from the degree of dissociation at which pH the χ value was determined. This strongly suggests that, irrespective of the total AAc content, the ionized AAc concentration is the main factor that controls the interaction of polymer network with water molecules. The influence of un-ionized AAc units in hydrogels is not significant, most likely owing to the very high extent of hydrogen bonding between the hydroxyl groups of dextran with water. Since the mixing and ionic osmotic responses are both governed by the ionized AAc concentration, it becomes evident that the difference in swelling between two series of hydrogels (AAc 7.2 and 10.4 mol %) shown in Figure 4 is primarily a consequence of the difference in the effective network density.

Figure 6 shows the dependence of the swelling of hydrogel (DS 5.7 and AAc 10.4 mol %) on salt concentration by addition of various amounts of NaCl in Tris buffer (pH 7.4, $I = 0.005$). The total ion concentration difference (ΔC_{total}) as a function of the NaCl concentration is also determined (Figure 6). Little change in ΔC_{total} is observed in the NaCl concentration range from 10^{-6} to 10^{-3} M owing to the presence of Tris with $I = 0.005$. Further increasing the NaCl concentration, the ionic osmotic responses is depressed. For the swelling behavior of a single type of hydrogel as illustrated in Figure 6, the total ion concentration difference is primarily determined by the fraction of ionized AAc units (α) and the ideal Donnan equilibrium effect. At pH 7.4, the Debye−Hückel performance within hydrogel on the degree of dissociation is insignificant, although the apparent acidity constant of AAc units in −log form

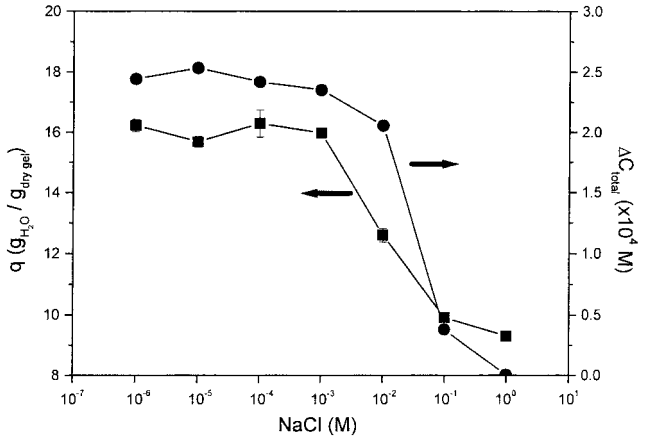

Figure 6. Equilibrium swelling (■) and ΔC_{total} (●) of hydrogels (DS 5.7 and AAc 10.4 mol %) at pH 7.4 as a function of the NaCl concentration of the external buffer solutions. Error bar at each point of swelling represents the standard deviation of triplicate measurements.

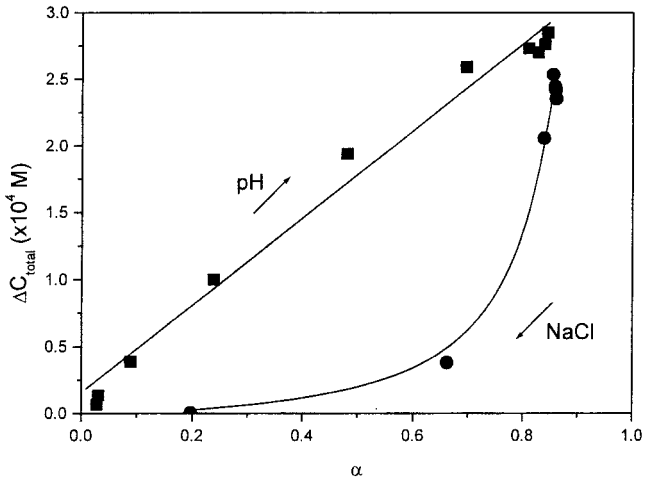

Figure 7. Relation between the total ion concentration difference (ΔC_{total}) and the degree of dissociation (α) of AAc units of hydrogels (DS 5.7 and AAc 10.4 mol %) in response to changes in pH (■) and NaCl concentration (●) of the external buffer solutions. Arrow symbols indicate the direction of the increases of pH and NaCl concentration. Data were obtained from Figures 3 and 6.

changes from 4.25 to 4.00 at 1.0 M of NaCl. On the other hand, the screening effect from the ionic binding of fixed acrylate anions with counterions at high concentrations of NaCl can reduce the degree of dissociation (α) of AAc (to ca. 20% at 1.0 M NaCl) and, therefore, the ionic osmotic response. In addition, the mixing contribution can also be reduced owing to the decreased degree of AAc ionization. More importantly, the total ion concentration difference can be dramatically reduced by the consequence of the ideal Donnan effect as illustrated in Figure 7. Figure 7 shows different changes in the total ion concentration difference in response to the variations in the degree of AAc ionization that are induced by changes in pH and salt concentration, respectively, and, therefore, illustrates the relation of the ionic osmotic pressure with NaCl concentration as described by the Donnan equilibrium theory. It is worthy to note that the effects of Debye−Hückel and ionic exchange have been taken into account in the calculation of α in Figure 7. As a result, a significant reduction in swelling is observed. The swelling from 1.0 M NaCl, pH 7.4 buffer solution (Figure 6) is even

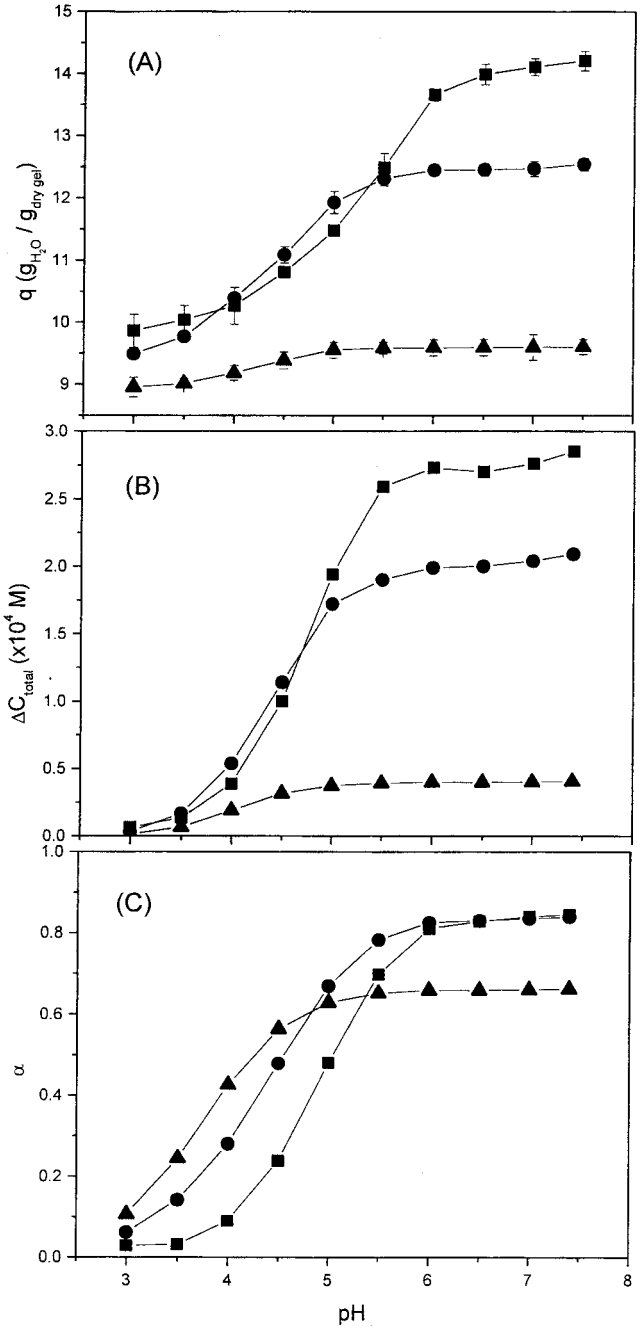

Figure 8. (A) Equilibrium swelling of hydrogels (DS 5.7 and AAc 10.4 mol %) as a function of pH of the external buffer solutions with NaCl concentrations equal to (■) 0, (●) 0.01, and (▲) 0.1 M. Error bar represents the standard deviation of triplicate measurements. (B) ΔC_{total} for the same hydrogel in (■) 0, (●) 0.01, and (▲) 0.1 M NaCl buffer solutions as a function of external pH. (C) The degree of dissociation (α) of AAc units in (■) 0, (●) 0.01, and (▲) 0.1 M NaCl buffer solutions as a function of external pH.

slightly lower than that of the same hydrogel (DS 5.7, AAc 10.4 mol %) at pH 3.0 in the absence of NaCl (Figure 1). The swelling is mainly an energy balance between only the mixing and elastic retractile responses.

The effect of the concomitant changes in pH and salt concentration on swelling of hydrogel (DS 5.7 and AAc 10.4 mol %) in buffer solution ($I = 0.005$) is shown in Figure 8A. Accordingly, the corresponding total ion concentration difference and the degree of dissociation are also determined (Figure 8B,C). It can be expected that the Debye−Hückel effect on the apparent dissocia-

tion constant (pK'_{H^+}) of AAc units becomes more pronounced with increasing salt concentration (eq 5). Therefore, at low pH, the fractions of ionized AAc units in 0.1 and 0.01 M NaCl buffer solutions are higher than that in the absence of NaCl (Figure 8C). At high pH, the Debye−Hückel effect on the fraction (α) of ionized AAc units decreases, and the ionic exchange effect becomes more predominant in controlling the degree of dissociation of AAc units in 0.1 and 0.01 M NaCl buffer solutions. In contrast to the fraction of ionized AAc units, the total ion concentration difference in 0.1 M NaCl buffer solution is significantly reduced as a direct consequence of the ideal Donnan effect as we have previously illustrated in Figure 7. The difference in swelling between hydrogels in 0 and 0.01 M NaCl buffer solution changes with pH. For the buffers in the pH range from 3.0 to 4.0, few AAc units in dextran hydrogels are ionized. Taking into account of the ideal Donnan effect, the change in ΔC_{total} for the hydrogel between two different salt concentrations (0 and 0.01 M) in this pH range becomes even smaller (Figure 8B). Despite this, on the basis of the thermodynamic theory as described in eq 9, we can still expect that the swelling of the hydrogel in 0.01 M NaCl buffer solution in this pH range is either identical or slightly higher than that in absence of NaCl. The discrepancy between the predicted and experimental swelling results will be explained below. In the pH range from 4.0 to 5.0, the increased fraction of ionized AAc units of hydrogel in 0.01 M NaCl buffer solution owing to the Debye−Hückel effect results in the increase in ionic osmotic pressure and mixing response. Therefore, the swelling in 0.01 M NaCl solution is somewhat higher than in the absence of NaCl. However, the ideal Donnan effect on the reduction of the ionic total concentration difference increases with pH. This leads to distinct profiles of ΔC_{total} as a function of pH for the hydrogel in 0 and 0.01 M NaCl buffer solutions in the pH range from 5.0 to 5.5. Since the degrees of dissociation of AAc at pH higher than 6.0 are essentially identical as an outcome of the Debye−Hückel and ionic binding effects, the difference in ΔC_{total} and swelling for 0 and 0.01 M NaCl is primarily a consequence of the ideal Donnan effect.

We have shown earlier the relation between the swelling of hydrogels and ΔC_{total} in Figure 4. The swelling performance for hydrogels with two different AAc contents (7.2 and 10.4 mol %) under identical osmotic pressures is different owing to changes in the effective network density of hydrogels caused by the effect of covalent bridging of AAc. The effect of salt concentration on the relation of the swelling of hydrogels (DS 5.7, AAc 10.4 mol %) with ΔC_{total} is illustrated in Figure 9. At values of ΔC_{total} greater than 0.5×10^{-4} M, the swelling of hydrogels in 0.01 M NaCl solution is higher than that without NaCl (0.0 M) under identical ionic osmotic pressure. This increased swelling of the hydrogel by the presence of 0.1 M NaCl is primarily a result of the change in the polymer−solvent interaction according to the energy balance at the swelling equilibria. For the same hydrogel, the polymer−solvent interaction parameter varies mainly with the degree of dissociation as illustrated in Figure 5. Therefore, to reach identical ionic osmotic pressure, the fraction of ionized AAc units in 0.1 M NaCl solution must be higher than that in the absence of NaCl as induced by either pH change or Debye−Hückel effect. As a consequence, an increase in the polymer−solvent interaction can

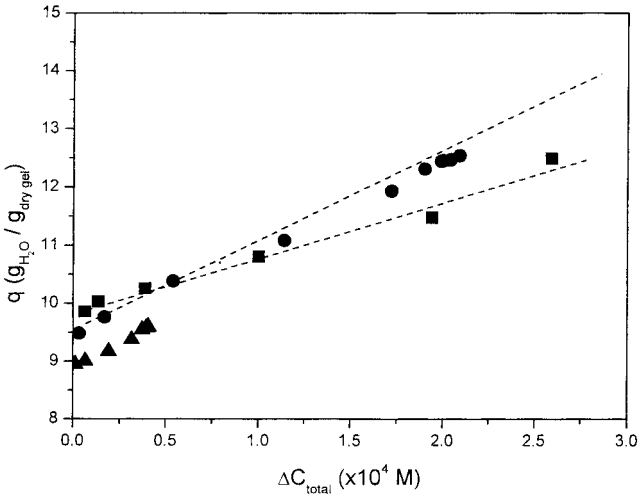

Figure 9. Relation between the pH-induced swelling and ΔC_{total} for hydrogels (DS 5.7, AAc 10.4 mol %) in (■) 0, (●) 0.01, and (▲) 0.1 M NaCl buffer solutions. Data were obtained from Figure 8.

enhance the response in swelling to ΔC_{total}. However, the preceding discussion cannot explain the swelling behaviors of the hydrogel in 0.1 and 0.01 M NaCl buffer solutions at ΔC_{total} less than 0.5×10^{-4} M. For the hydrogel in 0.01 M NaCl buffer, this result also reflects on the swelling lower than that in the absence of NaCl in the pH range from 3.0 to 4.0 (Figure 8), although, in 0.01 M NaCl buffer, the hydrogel aqueous system shows a slightly higher total ion concentration difference and fraction of ionized AAc units. In this pH range, most AAc groups are un-ionized, and the interaction between polymer network and solvent molecules subtly decreases in the 0.01 M NaCl aqueous solution. It is attributed to an additional "salting out" effect that reduces the water hydration from the polymer matrix and enhances the hydrophobic interaction. Owing to the increased hydrophobic interaction, the mixing enthalpy that militates against dilution and swelling, as represented by the term, $\chi v_{2,s}^2$, in the Flory−Huggins equation increases. In 0.1 M NaCl buffer solution, in addition to the removal of water hydration with polymer, the salting-out effect can further significantly reduce the electrostatic repulsion between approaching ionized AAc units. Therefore, significant deviation from the relation of χ with the ionized AAc concentration as illustrated in Figure 5 occurs in the pH range from 3.0 to 7.4.

Conclusions

In this study, dextran hydrogels were obtained by radical copolymerization of MA-dextran with AAc in aqueous solution, using APS and TMEDA as an initiation system. The swelling equilibria of hydrogels were analyzed by free energy balance between ionic osmotic, elastic retractile, and polymer−solvent mixing contributions. The internal ionic osmotic response to changes in pH and salt concentration was quantitatively determined according to the Donnan equilibrium theory, taking account of the Debye−Hückel effect on the apparent dissociation constant of AAc units and the ionic binding of acrylate anions with counterions.

The relation of the pH-induced swelling with ΔC_{total} was established. On the basis of the balance of free energy contributions at equilibrium swelling, the change in the polymer−solvent interaction parameter upon the

ionization of AAc was primarily governed by the concentration of ionized AAc units, irrespective of the total AAc content. The difference in swelling between hydrogels having varying AAc contents under identical ionic osmotic pressure was a result of the difference in the cross-linking density. The effect of salt concentration on the swelling of the same hydrogel resulted from the ideal Donnan effect and the degree of dissociation of AAc units that was influenced by the Debye−Hückel and ionic exchange contributions. The polymer−solvent interaction parameter changed primarily with the changes in the degree of dissociation owing to the Debye−Hückel effect in the presence of salt. The changes in the degree of dissociation of AAc units and total ion concentration difference obtained from the theoretical calculation explain well the relation of the swelling with ΔC_{total} for hydrogels from buffer solutions with different NaCl concentrations.

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References and Notes

(1) Edman, P.; Ekman, B.; Sjoholm, I. *J. Pharm. Sci.* **1980**, *69*, 838−842.
(2) Bronsted, H.; Hovgaard, L.; Simonsen, L. *S.T.P. Pharma Sci.* **1995**, *5*, 60−64.
(3) Van Dijk-Wolthuis, W. N. E.; Franssen, O.; Talsma, H.; Van Steenbergen, M. J.; Kettenes-Van Den Bosch, J. J.; Hennink, W. E. *Macromolecules* **1995**, *28*, 6317−6322.
(4) Van Dijk-Wolthuis, W. N. E.; Kettenes-Van Den Bosch, J. J.; Van Der Kerk-Van Hoof, A.; Hennink, W. E. *Macromolecules* **1997**, *30*, 3411−3413.
(5) Van Dijk-Wolthuis, W. N. E.; Tsang, S. K. Y.; Kettenes-Van Den Bosch, J. J.; Hennink, W. E. *Polymer* **1997**, *38*, 6235−6242.
(6) Chiu, H.-C.; Hsiue, G.-H.; Lee, Y.-P.; Hung, L.-W. *J. Biomater. Sci. Polym. Ed.* **1999**, *40*, 183−190.
(7) Kim, S. H.; Chu, C. C. *J. Biomed. Mater. Res.* **2000**, *49*, 517−527.
(8) Kim, S. H.; Won, C. Y.; Chu, C. C. *J. Biomed. Mater. Res.* **1999**, *46*, 160−170.
(9) Kim, S. H.; Won, C. Y.; Chu, C. C. *Carbohydr. Polym.* **1999**, *40*, 183−190.
(10) Chiu, H.-C.; Wu, A.-T.; Lin, Y.-F. *Polymer* **2001**, *42*, 1471−1479.
(11) Chiu, H.-C.; Lin, Y.-F.; Hsu, Y.-H. *Biomaterials* **2002**, *23*, 1103−1112.
(12) Van Dijk-Wolthuis, W. N. E.; Van Steenbergen, M. J.; Underberg, W. J. M.; Hennink, W. E. *J. Pharm. Sci.* **1997**, *86*, 413−417.
(13) Van Dijk-Wolthuis, W. N. E.; Hoogeboom, J. A. M.; Van Steenbergen, M. J.; Tsang, S. K. Y.; Hennink, W. E. *Macromolecules* **1997**, *30*, 4639−4645.
(14) Errington, E.; Harding, S. E.; Illum, L.; Schacht, E. H. *Carbohydr. Polym.* **1992**, *18*, 289−294.
(15) Hennink, W. E.; Talsma, H.; Borchert, J. C. H.; De Smedt, S. C.; Demeester, J. *J. Controlled Release* **1996**, *39*, 47−45.
(16) Cluff, E. F.; Gladding, E. K.; Praiser, R. *J. Polym. Sci.* **1960**, *45*, 341−345.
(17) Ulbrich, K.; Dusek, K.; Ilavsky, M.; Kopecek, J. *Eur. Polym. Sci.* **1978**, *14*, 45−49.
(18) Muniz, E. Z.; Geuskens, G. *Macromolecules* **2001**, *34*, 4480−4484.
(19) Zaroslov, Y. D.; Philippova, O. E.; Khokhlov, A. R. *Macromolecules* **1999**, *32*, 1508−1513.
(20) Lee, K. Y.; Rowley, J. A.; Eiselt, P.; Moy, E. M.; Bouhadir, K. H.; Mooney, D. J. *Macromolecules* **2000**, *33*, 4291−4294.
(21) Peppas, N. *Hydrogel in Medicine and Pharmacy*; CRC Press: Boca Raton, FL, 1986; Vol. I.
(22) Bae, Y. H.; Okano, T.; Kim, S. W. *J. Polym. Sci., Part B: Polym. Phys.* **1990**, *28*, 923−936.
(23) Dusek, K.; Prins, W. *Adv. Polym. Sci.* **1969**, *6*, 1−102.
(24) De Smedt, S. C.; Lauwers, A.; Demeester, J.; Van Steenbergen, M. J.; Hennink, W. E.; Roefs, S. P. F. M. *Macromolecules* **1995**, *28*, 5082−5088.
(25) Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, NY, 1953.
(26) Ricka, J.; Tanaka, T. *Macromolecules* **1984**, *17*, 2916−2921.
(27) Siegel, R. A.; Firestone, B. A. *Macromolecules* **1988**, *21*, 3254−3259.
(28) Brannon-Peppas, L.; Peppas, N. A. *Polym. Bull. (Berlin)* **1988**, *20*, 285−289.
(29) Rubinstein, M.; Colby, R.; Dobrynin, A.; Joanny, J. *Macromolecules* **1996**, *29*, 398−406.
(30) English, A. E.; Mafe, S.; Manzanares, J. A.; Yu, X.; Grosberg, A. Y.; Tanaka, T. *J. Chem. Phys.* **1996**, *104*, 8713−8720.
(31) Hasa, J.; Ilavsky, M.; Dusek, K. *J. Polym. Sci., Phys. Ed.* **1975**, *13*, 253−262.
(32) Bronsted, H.; Kopecek, J. *Biomaterials* **1991**, *12*, 584−592.
(33) Nisato, G.; Skouri, R.; Schosseler, F.; Munch, J.-P.; Candau, S. J. *Faraday Discuss.* **1995**, *101*, 133−146.
(34) Anbergen, U.; Oppermann, W. *Polymer* **1990**, *31*, 1854−1858.
(35) Firestone, B. A.; Siegel, R. A. *J. Biomater. Sci. Polym. Ed.* **1994**, *5*, 433−450.
(36) Martin, A. *Physical Pharmacy*, 4th ed.; Lea & Febiger: Philadelphia, PA, 1993.
(37) Helfferich, F. *Ion Exchange*; McGraw-Hill: New York, 1962.
(38) Eichenbaum, G. M.; Kiser, P. F.; Simon, S. A.; Needham, D. *Macromolecules* **1998**, *31*, 5084−5093.
(39) Eichenbaum, G. M.; Kiser, P. F.; Dobrynin, A. V.; Simon, S. A.; Needham, D. *Macromolecules* **1999**, *32*, 4867−4878.
(40) Brannon-Peppas, L.; Peppas, N. A. *Chem. Eng. Sci.* **1991**, *46*, 715−722.
(41) This value was obtained from the best-fitting result of eq 9 on the swelling data of Figure 1.

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