Swelling of Covalently Crosslinked Alginate Gels: Influence of Ionic Solutes and Nonpolar Solvents

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ABSTRACT: The equilibrium swelling volume of covalently crosslinked sodium alginate (a highly ionized stiff polymer network) gels has been studied as a function of electrolyte concentration, pH, and concentration of salts in ethanol–water mixtures. It is shown by numerical analysis of the Flory theory for swelling of gels that the ionic contribution to swelling seems to be the main determining factor for the swelling of these gels. Volume changes in aqueous solutions may be explained mainly by the ionic contribution to swelling. Reduced swelling and marked hysteresis are observed when the gels are exposed to low pH or cations that induce gelling in soluble Na–alginate. In contrast to predictions found in the literature, no discrete volume changes have been observed as a result of altering solvent composition. This discrepancy is attributed to the effect of the Donnan equilibrium and the high stiffness of the alginate chains. The gel volume changes in ethanol–water mixtures resemble the solubility of uncrosslinked alginates.

Introduction

We have previously reported on a new superswelling material with potential biomedical applications based on spherical gel beads of covalently crosslinked sodium alginate.[1,2] The gel beads, which can be prepared in the size range 5–4000 μm, are characterized by a very rapid reswelling from the dried state in water or in aqueous solutions (reswelling times of 0.5–3 min) with a swellability of 50–200 times their dry volume without losing their integrity or spherical form. The degree of swelling is not affected by neutral osmolytes, but swelling is somewhat reduced in physiological saline solution, as is expected for an ionic network. The volume decrease of the gels upon increase in ionic strength is, however, significantly lower (*i.e.*, the tolerance toward salt is significantly higher) than for other superswelling materials such as crosslinked polyacrylates.[2] In the present paper we have studied the swelling behavior of the crosslinked alginate beads as a function of concentration of gelling and nongelling ions, pH, and concentration of salts in ethanol–water mixtures. Such data are considered important for the utilization of this material as a water absorbent under various conditions. The object of the present work is further to use this gel system to obtain a better understanding of the swelling behavior of ionic gels in general.

Alginate Gels. Alginates are linear copolymers of α-L-guluronate and β-D-mannuronate. Their gelling properties arise from the cooperative binding of di- or trivalent cations—usually Ca^{2+}—between homopolymeric sequences of guluronate residues (termed "G-blocks").[3,4] This leads to a strong, thermostable gel with properties which are exploited in applications in the range from immobilization of cells and micoorganisms to restructured foods and pet foods.[5] Because of the high degree of crosslinking leading to comparatively short (and thus inflexible) elastic chains of the alginate gel, the swelling of Ca–alginate gels in salt solutions is usually limited as long as the calcium ions are bound in the network junctions.[6] This swelling has so far been considered to be small and with little practical significance except for some highly specialized applications as polyanion–polycation capsules for biomedical applications.[7] A pronounced increased swelling may be obtained by *O*-acetylation of the polymer.[8,9]

A fundamentally different gel system may be found in a covalently crosslinked sodium alginate gel. Here, the long, inflexible junction zones are substituted by small, discrete crosslinks distributed throughout the gel. This leads to a gel with considerably longer (and thus more flexible) elastic chains and with dramatically different swelling behavior.[1,2] This gel has been a useful model system for the study of alginate in the gel state where the Ca^{2+}–guluronate block interactions in the Ca–alginate gel may obscure the behavior of the chains between crosslinks.[10] A study of the swelling behavior of these gels may as well provide information about the swelling properties both of highly ionized stiff polymer networks and of alginate gels in general. The Na–alginate gel is produced by crosslinking a Ca–alginate gel with epichlorohydrin and subsequent removal of Ca^{2+} ions by treatment with a sequestering agent like EDTA.[2] The crosslinking of polysaccharides with epichlorohydrin has been known for a long time,[11] and the process is used extensively for the production of chromatography column materials.[12–16] The extent of the reaction and the numerous side reactions have also been studied,[17–21] whereas the physical properties of covalently crosslinked polysaccharide gels do not seem to have been studied to the same extent.

Theory

The basis for the theoretical treatment of the swelling of ionic gels is found in the works of Flory and contemporaries.[22] This theory has subsequently been applied with success by authors like Dušek and Patterson[23] and Tanaka and co-workers.[24–33] The swelling of ionic gels is a consequence of the equilibrium between osmotic pressure in the gel and the elastic reaction of the gel network. At equilibrium, the chemical potential of the solvent inside the gel equals the chemical potential of the solvent outside the gel

$$\Delta\mu_1 = \Delta\mu_{mix} + \Delta\mu_{ion} + \Delta\mu_{el} = 0 \quad (1)$$

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where, according to Flory[22]

$$\Delta\mu_{mix} = RT[\ln(1-\phi) + \phi + \chi\phi^2] \quad (2)$$

and

$$\Delta\mu_{el} = RTV_1^0\frac{\nu}{V_0}\left(\phi^{1/3} - \frac{1}{2}\phi\right) \quad (3)$$

where R is the gas constant, T absolute temperature, ϕ the volume fraction of polymer in the gel, χ the Flory–Huggins interaction parameter ($\chi < 0.5$ in good solvents, while $\chi > 0.5$ in bad solvents), V_1^0 the molar volume of the solvent, ν the molar number of crosslinked chains in the gel, and V_0 the volume of the gel when the network is formed.

The theory of swelling has been used to predict and analyze the volume changes of polymer gels upon changes in solvent composition.[23-33] In such treatments, the sum of the mixing term and the ionic term has been called the "swelling pressure" of the gel, Π_{osm}:

$$\Pi_{osm} = \frac{\Delta\mu_{mix} + \Delta\mu_{ion}}{V_1^0} \quad (4)$$

Tanaka and co-workers have used a modified expression for the elastic term, assuming that the network chains are in their random-walk configuration at a gel volume equal to the gel volume during crosslinking[25,31-33]

$$\Delta\mu_{el} = RTV_1^0\frac{\nu}{V_0}\left[\left(\frac{\phi}{\phi_0}\right)^{1/3} - \frac{1}{2}\frac{\phi}{\phi_0}\right] \quad (5)$$

and the expression used for the ionic term was given as

$$\Delta\mu_{ion} = fRTV_1^0\frac{\nu}{V^0}\left(\frac{\phi}{\phi_0}\right) \quad (6)$$

where f is the number of counterions per chain and ϕ_0 is the volume fraction of polymer in the gel when the crosslinks are introduced.

By a mathematical treatment of the formulas for equilibrium gel swelling (eqs 1, 2, 5, and 6), it is possible to obtain the so-called "reduced temperature", τ[25,32]

$$\tau = 1 - 2\chi = -\frac{\nu V_1^0}{V_0\phi^2}\left[(2f+1)\left(\frac{\phi}{\phi_0}\right) - \left(\frac{\phi}{\phi_0}\right)^{1/3}\right] + 1 + \frac{2}{\phi} + \frac{2\ln(1-\phi)}{\phi^2} \quad (7)$$

which gives the solvent goodness for $\Delta\mu_1 = 0$ as a function of gel swelling (ϕ/ϕ_0). One can readily see that $\tau > 0$ is equivalent to $\chi < 0.5$, *i.e.*, a good solvent, and $\tau < 0$ is equivalent to $\chi > 0.5$, *i.e.*, a poor solvent. This relation between τ and ϕ/ϕ_0 has been used for semiquantitative evaluations of discontinuous volume changes of polyacrylamide (PAAm) gels.[24-33]

In analyses of gel volume changes, it has been stated that the sole parameter determining whether the volume change is continuous or discontinuous is the parameter S, defined by[25]

$$S = \frac{\nu V_1^0}{V_0\phi_0^3}(2f+1)^4 = S_0(2f+1)^4 = \left(\frac{b}{a}\right)^3(2f+1)^4 \quad (8)$$

where b is the persistence length of the polymer and a the effective radius of the polymer chain. According to this treatment, a discontinuous phase transition depends critically on a sufficient chain stiffness or a sufficient amount of ionized groups.

In its original form eq 6 does not take into account the effect of the Donnan equilibrium and makes use only of the theory for osmotic pressure under ideal conditions. This is equivalent to regarding the mobile ions inside the gel as the molecules of an ideal gas exerting a "swelling pressure" on the gel.[26] The number of mobile ions inside the gel is assumed to be equal to the number of fixed charges on the polymer, irrespective of the ionic strength of the solution. The theory of Donnan equilibrium states that the higher the ionic concentration outside a semipermeable membrane with a polyelectrolyte inside, the lower is the difference in concentration of mobile ions between the two sides of the membrane. Because the gel chains are interlinked, the gel acts as if it provides its own semipermeable membrane. Equation 6 thus may not be suitable for predicting the swelling behavior of ionic gels as a function of ionic strength unless the parameter f is calculated as a function of external ionic strength, internal polymer concentration, and counterion condensation.

To allow for changes in swelling caused by altering the concentration of salts outside the gel, it is necessary to treat the ionic term, $\Delta\mu_{ion}$, as the effective difference in chemical potential of the solvent due to mobile ions inside the gel. This term is then given by

$$\Delta\mu_{ion} = RT[\ln(1 - X_{\text{mobile ions}}) - \ln(1 - X'_{\text{mobile ions}})] \approx -RTV_1^0\Delta C_{\text{mobile ions}} \quad (9)$$

where $X_{\text{mobile ions}}$ is the mole fraction of mobile ions inside the gel, $X'_{\text{mobile ions}}$ the mole fraction of mobile ions outside the gel and V_1^0 the molar volume of the solvent.[34] The effective osmolarity of mobile ions, $\Delta C_{\text{mobile ions}}$ (*i.e.*, the concentration difference of mobile ions between the gel and the surrounding liquid), is given by

$$\Delta C_{\text{mobile ions}} = (C_+ + C_-) - (C'_+ + C'_-) \quad (10)$$

where C_+ and C_- are the concentrations of positive and negative mobile ions, respectively, inside the gel and C'_+ and C'_- the concentrations outside the gel. The ion concentrations are determined by the Donnan equilibrium, which for anionic polymers in 1:1, 1:2, and 2:1 electrolytes is described by eq 11[34]

$$\begin{aligned} z_+C_+ &= z_-C_- + z_PC_P \\ z_+C'_+ &= z_-C'_- \\ \gamma_\pm^2 C_+^{z_-}C_-^{z_+} &= \gamma'^2_\pm C'^{z_-}_+ C'^{z_+}_- \end{aligned} \quad (11)$$

where z_+ and z_- are the absolute values of the valences of the mobile ions, z_P is the number of noncondensed charges per monomer residue, C_P is the concentration of polymer in the gel expressed as the molar concentration of monomer residues, and $\gamma_\pm$ and $\gamma'_\pm$ are the mean activity coefficients of the salt inside and outside the gel, respectively. The fraction of noncondensed ions per monomer residue, z_P, can be calculated from the Manning theory of counterion condensation[35,36]

$$\begin{aligned} z_P &= \theta z_P^0 \\ \theta &= (z_+\xi)^{-1},\ \xi > 1 \\ \theta &= 1,\ \xi < 1 \\ \xi &= \frac{e^2}{4\pi\epsilon kTb} \end{aligned} \quad (12)$$

where z_P^0 is the number of charges per monomer residue when counterion condensation is assumed not to take place (for this polymer: 1.0 at neutral pH), e is the electron change, ϵ the dielectric constant of the solvent, and b the average distance between fixed charges on the polymer.

For monovalent electrolytes, by assuming $\gamma_{\pm} = \gamma'_{\pm}$, one obtains two quadratic equations for C_+ and C_- which are easily solved. For 2:1 and 1:2 electrolytes, one gets third-order polynomial equations for C_+ and C_- which can be solved by numerical methods.

Correction terms have been added to the basic equations for gel swelling, introducing non-Gaussian behavior of the elastic chains and electrostatic interactions between fixed charges on the network.[37] Also, a modification of the mixing term $\Delta\mu_{mix}$ has been introduced to be able to predict lower critical solution temperatures as a function of pressure.[38,39] These modifications will not be considered here.

Experimental Section

Materials. Alginate extracted from *Laminaria hyperborea* stipes (LF 10/60) was obtained from Pronova Biopolymers A/S, Drammen, Norway. It had an intrinsic viscosity in 0.1 M NaCl of 6.3 dL/g, corresponding to a viscosity-average molecular weight of 190 000 g/mol, the content of guluronate residues (F_G) was 68%, and the average G-block length ($\bar{N}_{G>1}$) was 14.0, as determined by ^{1}H-NMR.[40] Ethanol was 96% by volume, epichlorohydrin was Merck p.s. grade, and salts were Merck p.a. grade.

Preparation of Covalently Crosslinked Na-Alginate Gels. Homogeneous Ca-alginate gel beads were made from a solution of 20 g/L of alginate as described by Skjåk-Bræk *et al.*[8,41] The water in the gel was exchanged with ethanol, and the gel beads were crosslinked in a suspension containing 4.3 mol/L epichlorohydrin, 0.14 moles/L NaOH, and 0.014 mol/L $CaCl_2$ in 60 vol % ethanol. The NaOH concentration was kept constant by titrating the system with 1 M NaOH in distilled water. The automatic titration system consisted of a Radiometer ABU 80 autoburette, a Radiometer TTT 60 titrator, and a Radiometer PHM 84 pH meter equipped with a G202B pH electrode and K711 reference electrode (double salt bridge with concentrated Li-acetate in the second salt bridge).[2] The crosslinking reaction was allowed to proceed for 4.5 h, after which the reaction was stopped by addition of 4 M HCl. At that point, 0.71 mL of NaOH solution had been used per mL of Ca-alginate gel beads. The crosslinked beads were washed first with 96% ethanol and then with distilled water, and the Ca^{2+} ions were removed by dialysis against 50 mM EDTA. After removal of Ca^{2+} ions, the gel beads were dialyzed extensively against distilled water to remove any salts. At the maximum gel volume, the concentration of polymer was 8.5 g/L, measured by taking a carefully measured weight of gel, drying it at 80 °C overnight, and weighing the dried polymer. Using the approach for estimating the crosslink density described earlier,[42] this corresponded to a mean elastic chain length of approximately 76 monomer residues. Although an inhomogeneity in crosslink distribution has been observed for Na-alginate cylinders (14-mm diameter × 15-mm height) prepared this way,[10] the small dimensions of the gel beads (1–2-mm diameter) should give a fairly rapid diffusion of crosslinker through the gel, ensuring a more homogeneous crosslink distribution.

Measurement of Gel Bead Volumes. All volume measurements were made by measuring the volume of a quantity of gel beads in measuring cylinders. The beads were dialyzed against the solution for 2 days, during which the dialysate was exchanged four times. The gel beads were transferred to measuring cylinders, and the suspension was allowed to settle for 30 min before the volume was measured.

Volume as a Function of Ionic Strength. Twenty-five mL of gel beads at their maximum volume (in distilled water) was taken out. A total of 400 mL of salt solution at each ionic strength was used for dialysis.

Volume as a Function of pH. As for the ionic strength measurements, 25 mL of beads in distilled water was taken out. A total of 400 mL of buffer was used as dialysate at each pH. Buffers at pH values from 5.0 to 2.0 were made, and the ionic strength was adjusted with NaCl to 0.1 and 1 M, respectively. For pH values from 5.0 to 3.5, acetate buffer was used, and for pH values from 3.5 to 1.5, glycine buffer was used. For measurement at pH 1 (ionic strength 1 M only), 1 M HCl was used.

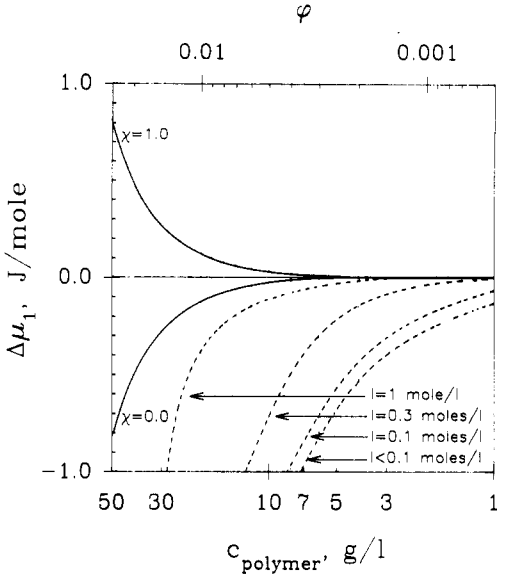

Figure 1. Numerical evaluation of the terms contribution to change in chemical potential for the solvent ($\Delta\mu_1$) in a Na-alginate gel as a function of polymer concentration, $c_{polymer}$, and of volume fraction of polymer, ϕ: - - -, ionic term (ideal Donnan term): —, mixing term. Specific volume of the polymer: 0.5 mL/g.

Volume as a Function of Ethanol Concentration. Fifty mL of gel beads in distilled water was taken out. A total of 800 mL of ethanol-water mixture was used for each ethanol concentration. The ethanol-water mixtures were made by mixing 96% ethanol with distilled water, and the ethanol concentration was measured by measuring the density of the solution. After measurement of the ethanol concentration, NaCl or LiCl was added to the appropriate concentration (NaCl: 0.001, 0.005, 0.01, 0.03 and 0.1 M; LiCl: 0.1 M).

Results and Discussion

Main Determining Factors for Swelling. By numerical analysis of the equations determining the swelling of the gel (eqs 2 and 9), it is seen that at ionic strengths below 1 mol/L the ionic term is the significant term contributing to the swelling pressure of the Na-alginate gel (Figure 1). The changes in swelling pressure due to the Donnan equilibrium ($\Delta\mu_{ion}$) were found to be at least 10 times the swelling pressure due to polymer-solvent mixing ($\Delta\mu_{mix}$) at the polymer concentrations and ionic strengths observed for this gel system, depending somewhat on the magnitude of the parameter χ (data not shown).

According to eq 9, the chemical potential difference due to the Donnan equilibrium is proportional to the concentration difference of mobile ions. This difference, $\Delta C_{mobile\ ions}$, can be calculated for a gel as a function of external salt concentration. The calculated concentration difference of ions for a 10 g/L gel in 1:1 (NaCl), 1:2 (Na_2SO_4), and 2:1 ($MgCl_2$) electrolytes is shown in Figure 2 (no counterion condensation assumed).

This can be compared to the swelling curves in the same three electrolytes (Figure 3). The good correlation between the curves is indicative evidence that the Donnan equilibrium is the main determining factor for swelling. It is also shown in a previous work[2] that moderate concentrations of nonionic solutes do not influence the swelling of these gels.

Volume as a Function of Salt Concentration. A marked volume decrease is observed when the salt

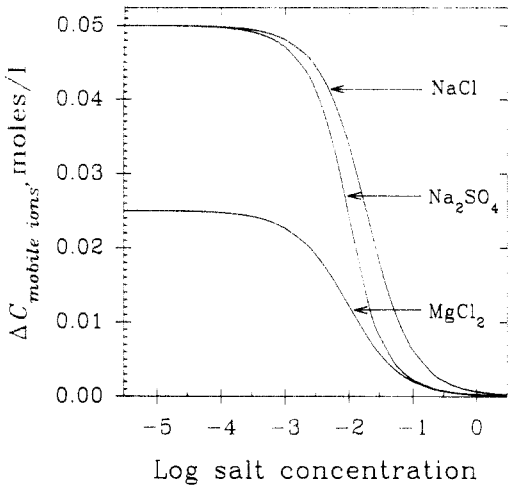

Figure 2. Difference in ion concentration between the inside and the outside of a 10 g/L Na-alginate gel (effective osmolarity in the gel) as a function of electrolyte concentration calculated using the theory of ideal Donnan equilibrium for a 1:1 (NaCl), 2:1 (Na_2SO_4), and 1:2 ($MgCl_2$) electrolyte. It is assumed that no counterion condensation takes place.

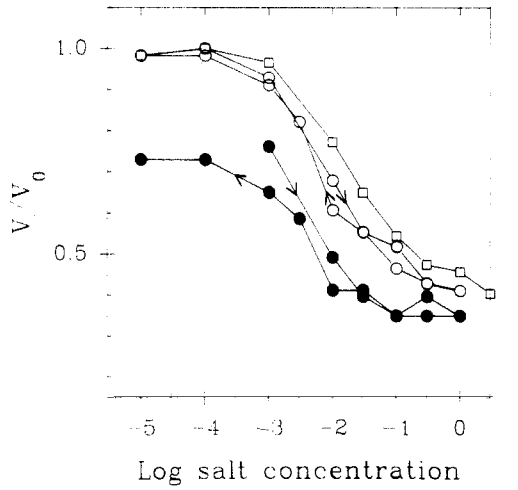

Figure 3. Volume changes of Na-alginate gel beads as a function of electrolyte concentrations: □, NaCl (1:1 electrolyte); ○, Na_2SO_4 (2:1 electrolyte); ●, $MgCl_2$ (1:2 electrolyte). V_0: volume of beads in distilled water.

concentration in the external solution is increased (Figures 4 and 5). This is in accordance with theory, since the Donnan equilibrium theory predicts that the difference in ionic concentration between the inside of the gel and the outside decreases when the concentration of salt in the surrounding liquid is increased. The marked hysteresis observed in the swelling curves for Ca^{2+}, Sr^{2+}, and Ba^{2+} arises from the fact that the alginate retains its ion-binding properties in the covalently crosslinked state, and additional crosslinks are introduced by the cooperative binding of the divalent cations in between homopolymeric sequences of guluronate residues. These crosslinks thereby add to the elastic term and prevent the reswelling of the beads when the ion concentration is lowered.

Volume as a Function of pH. The volume of the gels decreases as the pH of the surrounding liquid is lowered at constant ionic strength (Figure 6). The pK_a values of the uronate residues are in the range of 3.3–4.0, depending on the ionic strength.[43] As the polymer is protonated, the ion concentration difference is decreased (the term z_P in eq 11 decreases) and the gel shrinks. Alginates form acid gels by interactions between protonated homopolymeric sequences.[44] This is probably the reason for the observed hysteresis. The dissolution of the acid gel is a slow process,

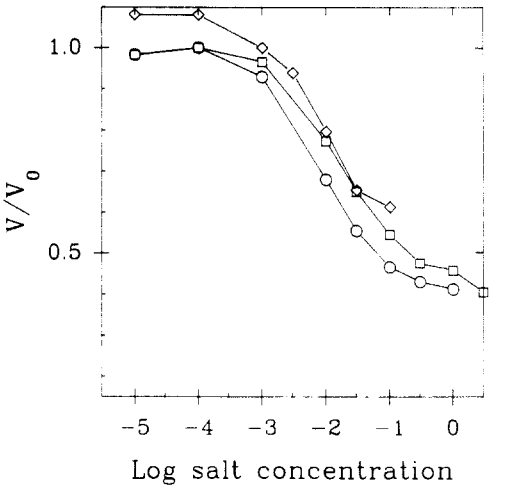

Figure 4. Volume changes of Na-alginate gel beads as a function of concentration of monovalent electrolytes: □, NaCl; ○, Na_2SO_4; ◇, tetramethylammonium chloride (TMA-Cl). V_0: volume of beads in distilled water.

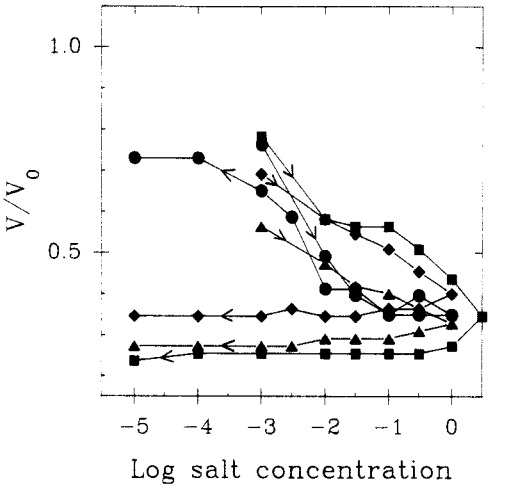

Figure 5. Volume changes of Na-alginate gel beads as a function of concentration of divalent (1:2) electrolytes: ●, $MgCl_2$; ■, $CaCl_2$; ♦, $SrCl_2$; ▲, $BaCl_2$. V_0: volume of beads in distilled water.

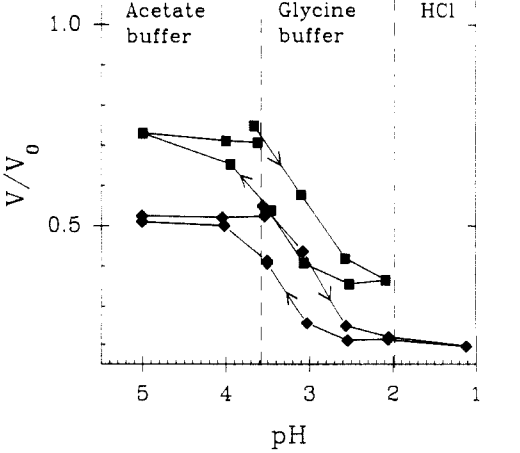

Figure 6. Volume changes of Na-alginate gel beads as a function of pH at constant ionic strength: ■, I = 0.01 M; ♦, I = 0.1 M. V_0: volume of beads in distilled water.

and some hysteresis in the swelling curves is therefore to be expected.

Volume as a Function of Ionic Swelling Pressure. Knowledge of the ion concentrations outside the gel and the concentration of polymer in the gel (from volume measurements) permits the calculation of ion concentra-

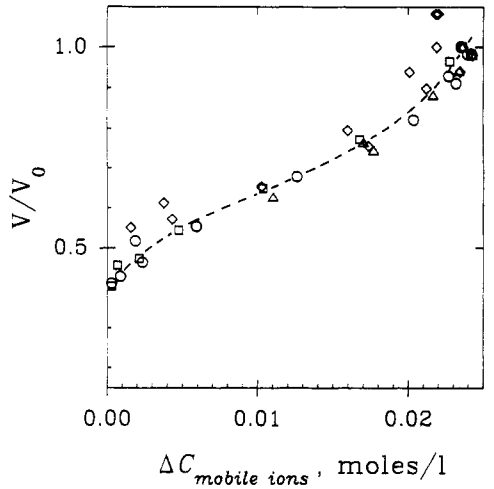

Figure 7. Volume changes of Na–alginate gel beads as a function of calculated effective osmolarity ($\Delta C_{\text{mobile ions}}$) in the gel beads for electrolytes of monovalent cations. Counterion condensation calculated using Manning theory: □, NaCl; ○, Na_2SO_4; ◇, TMA-Cl; △, tetraethylammonium chloride (TEA-Cl). V_0: volume of beads in distilled water. Dotted line indicates trend for sodium salts.

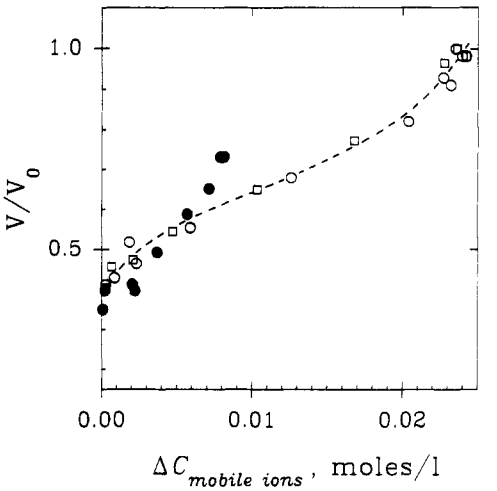

Figure 8. Volume changes of Na–alginate gel beads as a function of calculated effective osmolarity in the gel beads for different electrolytes. Counterion condensation calculated using Manning theory: □, NaCl (1:1 electrolyte); ○, Na_2SO_4 (2:1 electrolyte); ●, $MgCl_2$ (1:2 electrolyte. V_0: volume of beads in distilled water. Dotted line indicates trend for sodium salts.

tions inside the gel, assuming ideal Donnan equilibrium and counterion condensation as described by Manning.[35] The average distance between fixed charges, b, was estimated to be 0.43 nm when the measured value for counterion condensation of Na–alginate of 0.4 was used.[45] Figure 7 shows the swelling of these gels in monovalent electrolytes as a function of calculated effective ionic osmolarity. As can be seen from Figure 7, the swelling as a function of effective ionic osmolarity is approximately equal for all monovalent salts. This indicates that the difference in swelling behavior between these salts as observed in Figure 4 may be explained solely by the ideal Donnan equilibrium when counterion condensation is calculated according to the Manning theory.

The experimental data for TMACl and the sodium salts differ slightly, something which may be explained by the difficulty for the bulky tetramethylammonium ion to condense on the fixed charges on the polymer chains.

A comparison of the swelling as a function of calculated effective osmolarity, $\Delta C_{\text{mobile ions}}$, for three types of electrolytes (NaCl, Na_2SO_4, and $MgCl_2$) is given in Figure 8. From the figure, one can see that there is a quite good correlation between the data for the three types of electrolyte. A small, systematic deviation is observed for the $MgCl_2$ data and may be attributed to ion-specific effects.

The gel swelling data for the salts of different divalent cations show roughly the same concentration dependence. The swelling data for these salts do not fit as well as for the monovalent salts, and a large hysteresis is observed (Figure 9). The deviations observed for divalent cations may be attributed to one or more of the factors listed below: (i) The Donnan equilibrium is calculated assuming only one kind of counterion in the solutions. The gels were in the sodium form at low ionic strengths when the experiments started, and both the presence of residual Na^+ ions and the dissociation of water would lead to monovalent ionic species that are not accounted for. The ratio of mono- to divalent ions inside the gel was not estimated. (ii) Most of the divalent ions (Ca^{2+}, Sr^{2+}, and Ba^{2+}) interact strongly with the alginate gel, introducing additional crosslinks which strongly influence the elastic and swelling behavior of the gel. The ionic crosslinks introduced at low volumes may prevent the reswelling of

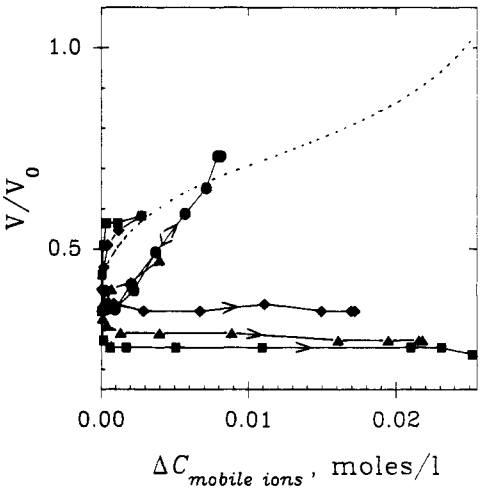

Figure 9. Volume changes of Na–alginate gel beads as a function of calculated effective osmolarity in the gel beads for electrolytes of divalent cations. Counterion condensation calculated using Manning theory: ●, $MgCl_2$; ■, $CaCl_2$; ♦, $SrCl_2$; ▲, $BaCl_2$. V_0: volume of beads in distilled water. Dotted line indicates trend for sodium salt.

the gel when decreasing the ion concentration outside the gel. (iii) The counterion condensation is calculated assuming that this condensation is concentration-independent. One cannot exclude the possibility of an ion-specific, concentration-dependent degree of ion condensation which would affect the calculated ion concentration difference.

Knowledge of the pH and the pK_a values for the monomers permitted the calculation of the Donnan equilibrium in the pH experiments. The pH inside the gel was assumed to be equal to the pH outside the gel, and the pK_a values of the monomer residues were assumed to be equal to the pK_a values of the free monomers (3.65 for guluronate, 3.38 for mannuronate[43]). As a consequence of the Donnan equilibrium, the pH inside the gel is not necessarily exactly equal to the pH outside the gel. However, the data did not permit any explicit calculation of the proton concentration inside the gel, thus the assumption of equal pH. The degree of counterion condensation will vary with pH. As the pH is lowered and reaches values around the pK_a values of the monomer residues, a protonation of the polymer will take place, and

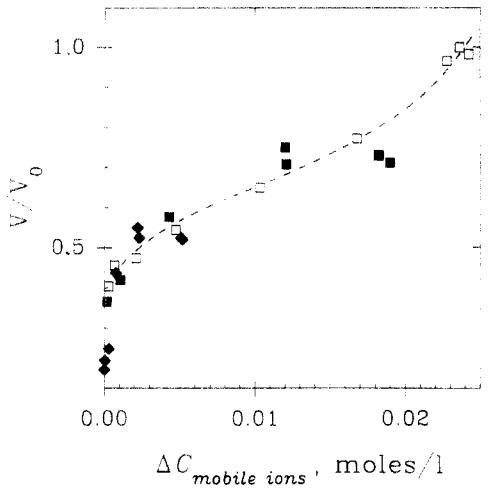

Figure 10. Volume changes of Na–alginate gel beads when varying pH at constant ionic strength as a function of calculated effective osmolarity in the gel beads. Counterion condensation calculated using Manning theory: ■, variable pH, fixed $I = 0.01$ M; ♦, variable pH, fixed $I = 0.1$ M; □, fixed pH = 7, variable ionic strength. V_0: volume of beads in distilled water. Dotted line indicates trend for sodium salts.

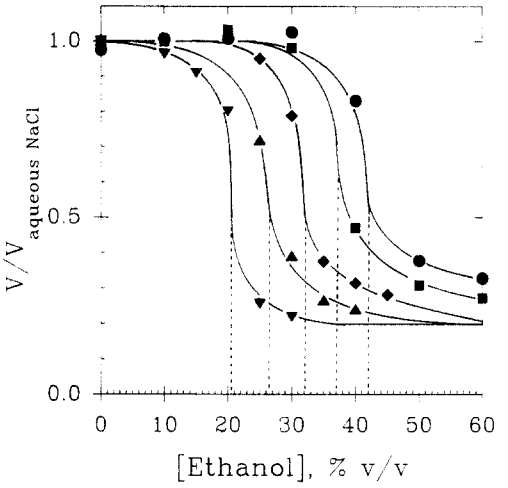

Figure 11. Volume changes of Na–alginate gel beads as a function of ethanol concentration at constant ionic strength: ●, $I = 0.001$ M; ■, $I = 0.005$ M; ♦, $I = 0.01$ M; ▲, $I = 0.03$ M; ▼, $I = 0.1$ M. $V_{\text{aqueous NaCl}}$: volume of beads in aqueous NaCl, no ethanol.

the average distance between fixed charges, b, will increase. The fraction of dissociated counterions per monomer residue (z_P) will then be a function of pH, both because of the acid properties of the polymer and the variable counterion condensation. By taking this into considerations, the effective osmolarity inside the gel can be calculated for the pH experiments as well. Figure 10 shows the swelling of the gels in buffer at constant ionic strength as a function of calculated effective osmolarity ($\Delta C_{\text{mobile ions}}$), and it shows that there is a good correlation between the volume changes obtained by varying the pH at constant ionic strength (*i.e.*, the fraction of dissociated counterions per monomer residue, z) and those obtained by varying the concentration of salt. At pH values well below the pK_a values for the uronic acids, segment–segment interactions between uncharged homopolymeric blocks also decreases the swelling (Figure 10). This can be regarded as analogous to alginic acid gel formation, leading to additional crosslinks in the gels like the swelling in solutions of divalent cations. Again, the relatively good correlation between data for the ionic strength experiments, and the pH experiments indicates that the Donnan equilibrium is the main determining factor for swelling also under these conditions.

Volume as a Function of Concentration of Ethanol and Salts. The gel volume as a function of ethanol concentration at different ionic strengths is shown in Figure 11.

The difference in ion concentration inside and outside the gel was calculated also for these experiments, assuming a solvent-dependent ion condensation of the polymer (Manning condensation). A plot of swelling vs the calculated ion concentration difference is given in Figure 12. From this figure, it is seen that the calculated ion concentration difference for the shrunken gels is unreasonably high; *i.e.*, the volume decrease of the gels is larger than could be expected from a pure Donnan equilibrium/ion condensation theory. To explain the volume changes as caused by the polymer–solvent interaction parameter (χ) would require values for χ that decrease with increasing ethanol concentration until the onset of volume decrease. After the onset of volume decrease, χ values would have to increase to the range of 4–8 to explain the observed volume decrease (data not shown).

The analogy between a polymer network (a gel) and a single molecule has been pointed out previously,[22,46] and the analogy between the volume decrease of the gels upon solvent changes and the precipitation of uncrosslinked alginate[47] is striking. In both cases, there is an abrupt change from a "dissolved", voluminous state to a "solid", compact state. It was therefore of interest to investigate whether the volume decrease of these alginate gels showed similarities to the precipitation of alginates by mixtures of ethanol, water, and salts. The precipitation of ionic polysaccharides has been studied by Smidsrød and Haug,[47] using a solubility product approach analogous to the precipitation of simple salts. For precipitation of simple salts, we have the equilibrium

$$aA^{n+} + xX^{m-} \rightleftarrows A_aX_x$$

leading to the solubility product $K_{sp} = [A^{n+}]^a[X^{m-}]^x$. Likewise, one may treat the precipitation of an ionic polymer as the same kind of equilibrium.

$$P^{n-} + nM^+ \rightleftarrows PM_n$$

This is a very simplistic treatment, where the precipitation of the polymer is assumed to take place when the concentration product of the polymer exceeds the solubility product, K_{sp}. According to the Debye–Hückel theory, the solubility product of a monovalent salt in two solvents with different dielectric constants is related by the formula[47]

$$RT \ln \frac{K_{sp,1}}{K_{sp,2}} = RT \ln \frac{f_2}{f_1} = A\left(\frac{1}{\epsilon_2} - \frac{1}{\epsilon_1}\right) \qquad (13)$$

where K_{sp} is the solubility product of the salt with the activity coefficient f in the solvent with the dielectric constant ϵ, A is a constant, and subscripts 1 and 2 refer to the two solvents. Such a precipitation could also be viewed as a condensation of mobile ions onto the polymer, thus dramatically reducing the ionic contribution to the swelling. The "neutral" alginate gel may then contract due to segment–segment interactions as explained in a first approximation by the Flory–Huggins mixing term, $\Delta\mu_{mix}$.

Analogous to the precipitation of alginates in ethanol–water mixtures, the ethanol concentration needed for a gel collapse decreases with increasing ionic strength (Figure 11). To investigate whether or not the gel collapse was analogous to the precipitation of alginates, a plot of the

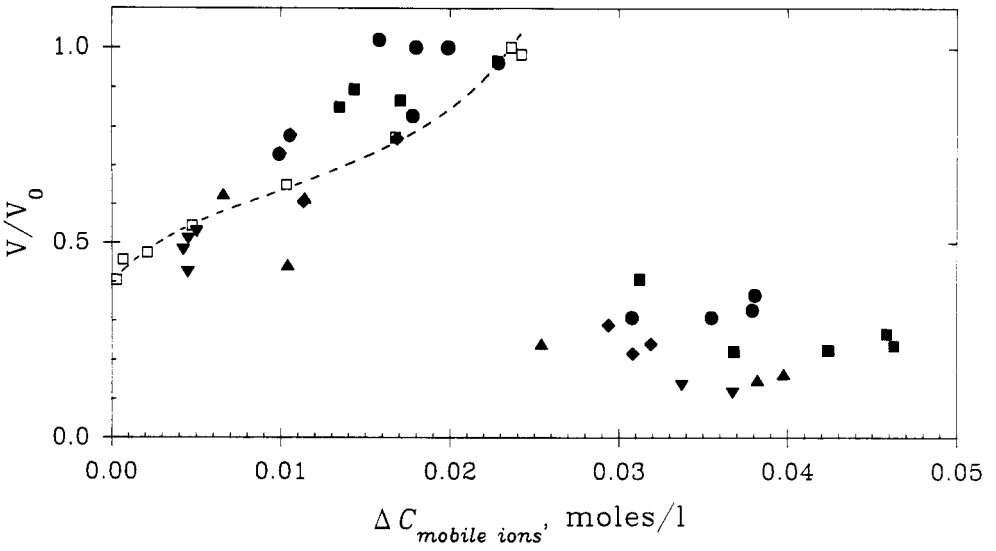

Figure 12. Volume changes of Na-alginate gel beads as a function of calculated effective osmolarity in the gel beads when varying the ethanol concentration: ●, 0.001 M NaCl; ■, 0.005 M NaCl; ♦, 0.01 M NaCl; ▲, 0.03 M NaCl; ▼, 0.1 M NaCl; □, swelling in aqueous NaCl. Counterion condensation is assumed to follow the Manning theory, with dielectric constant of the solvent linearly dependent on the weight concentration of ethanol in the solvent. V_0: volume of beads in distilled water.

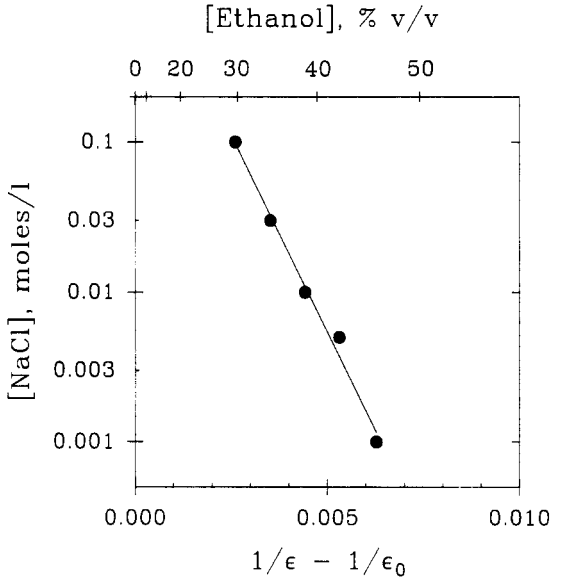

Figure 13. Na^+ concentration vs $(1/\epsilon - 1/\epsilon_0)$ and ethanol concentration at conditions for 50% volume decrease: ϵ, dielectric constant of the ethanol-water mixture; ϵ_0, dielectric constant of water.

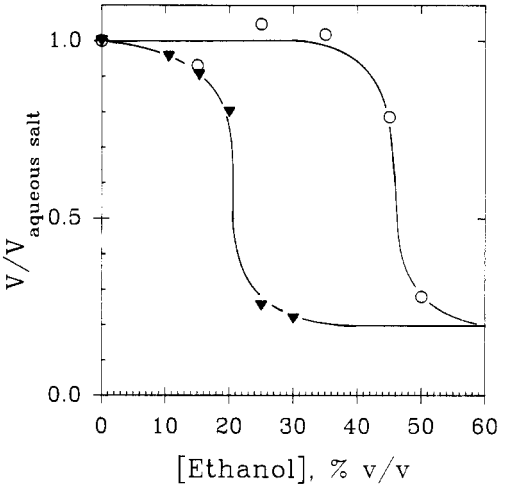

Figure 14. Volume changes of Na-alginate gel beads as a function of ethanol concentration at constant ionic strength: ▼, 0.1 M NaCl; ▽, 0.1 M LiCl. $V_{\text{aqueous NaCl}}$: volume of beads in aqueous NaCl, no ethanol.

ionic strength vs $1/\epsilon - 1/\epsilon_0$, where ϵ is the dielectric constant of the ethanol-water mixture and ϵ_0 is the dielectric constant of water, was made as shown in Figure 13. According to eq 13, the logarithm of the ratio between the solubility products should be linearly related to $1/\epsilon - 1/\epsilon_0$.

The linear relationship between the critical sodium concentration and $1/\epsilon - 1/\epsilon_0$ suggests that these volume decreases may be regarded as a precipitation of alginate. In solubility studies of ionic polysaccharides, specific cation effects have been observed.[47] To investigate if different cations could induce different behavior as in the precipitation of polysaccharides, we measured the volume of the gels as a function of ethanol concentration in 0.1 M LiCl. It is seen (Figure 14) that the behavior of the gels is different in LiCl solutions than in NaCl solutions, again suggesting that the volume decrease very well may be viewed as a precipitation reaction for this polymer system. Cation effects could influence both the solubility constant of the polymer, K_{sp}, and the polymer–solvent interactions (seen in the magnitude of the Flory–Huggins parameter χ), two phenomena which probably are interrelated. A more systematic study of ion-specific effects could provide further information about ionic effects on solvent goodness for these polymers.

Unlike the volume changes observed for PAAm gels upon changes in solvent composition,[32] these alginate gels show volume changes which appear to be continuous (Figure 11). It has been stated[25] that if the elastic chains in a gel have a sufficient stiffness or a sufficient amount of ionization, discrete volume transitions should be observed. As alginate indeed is a stiff polymer[48] with a high degree of ionization, this suggests that discrete volume changes should be expected. The discrepancy between this theory and our experimental results is attributed to the effect of the Donnan equilibrium and the large stiffness of the polymer chains. It has been shown[42] that the elasticity of Na-alginate gels is approximately 2–10 times that for a rubber-elastic network. Similar results have been obtained for other biopolymer networks.[49]

It can be demonstrated that a high stiffness of the alginate chains may suppress the discontinuous volume changes reported for PAAm gels. The "reduced temperature", τ, at zero osmotic pressure can be calculated as a

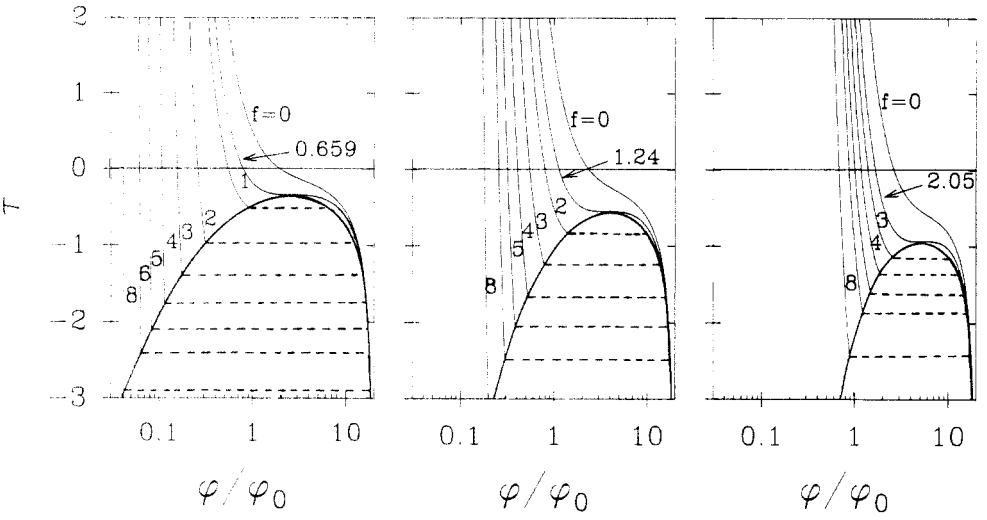

Figure 15. Correlation between solvent goodness expressed through the "reduced temperature" τ and swelling of a gel at $\Delta\mu_1 = 0$, for various values of effective network ionization, f, using eq 7. Calculated with $S_0 = 10$ and $\phi_0 = 0.05$. Correlation calculated using rubber-like front factors of 0.5 (left), 1.5 (middle), and 5 (right), respectively. Arrowed values correspond to the critical point.

function of swelling.[32] Figure 15 shows the calculated "reduced temperature" as a function of gel swelling for networks with rubber–elastic front factors of 0.5, 1.5, and 5. As can be seen from the figure, an increase in the elastic term may suppress the discontinuous volume change of the gel. It has also been showed that in a highly ionized network the effect of the Donnan equilibrium suppresses the discontinuous volume change observed in partially ionized PAAm gels.[37]

In an earlier work by Tanaka,[46] an analogy has been drawn from a hydrogel to a single polymer. Such a correlation is indicative of the similarity between the solvent-free energy for a gel network and for one single molecule, which was pointed out already by Flory.[22] Here, we report on an analogy between phenomena observed for uncrosslinked alginate and phenomena observed for crosslinked alginate gels. This is another indication of the principal similarity between bel networks and single polymer molecules in terms of their physical chemistry. It is hoped that the physical chemistry of polymer networks which has been applied by numerous people over the years also may be extended to provide explanations for phenomena like the precipitation of ionic polysaccharides.

Conclusions

It has been shown that the swelling behavior of covalently crosslinked Na–alginate (a highly ionized stiff polymer network) gel beads can be described using the theory of ideal Donnan equilibrium taking counterion condensation into consideration. A numerical analysis of the effects which determine the swelling of these gels in aqueous solutions indicates that the influence of the "goodness" of the solvent for the polymer (*i.e.*, the Flory–Huggins parameter χ) is negligible compared to the Donnan equilibrium. The swelling behavior in 1:1, 1:2, and 2:1 electrolytes and in acidic media may be explained mainly by the ionic contribution to swelling. At low pH and in solutions of gelling ions like Ca^{2+}, Sr^{2+}, or Ba^{2+}, the introduction of physical junctions causing reduced volume and large hysteresis effects is evident. The large volume decrease at ethanol concentrations above a certain value seems to be analogous to a cation-dependent precipitation of the polymer. The unexpected continuous volume change of these gels upon changes in solvent composition are attributed to the stiffness of the polymer chains and the effect of the Donnan equilibrium. It has also been shown that an increase in the elastic term in the equations for gel swelling may suppress the discontinuous volume changes observed for synthetic gels.

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