Role of Hydrogen Bonding and Hydrophobic Interaction in the Volume Collapse of a Poly(ethylenimine) Gel

Etsuo Kokufuta,*,† Hironori Suzuki,† Ryo Yoshida,† Kazunori Yamada,‡ Mitsuo Hirata,‡ and Fumitake Kaneko‡

Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305, Japan, and Department of Industrial Chemistry, College of Industrial Technology, Nihon University, Narashino, Chiba 275, Japan

Received August 12, 1997. In Final Form: December 1, 1997

A cationic polyelectrolyte gel was prepared through the cross-linking of linear poly(ethylenimine) (LPEI) with ethylene glycol diglycidyl ether. The cross-linking degree was ca. 12% by means of elemental analysis. A pH change cycle (3 to 12 and vice versa) brought about a discontinuous transition near pH 5.9 (swelling) and pH 10.7 (deswelling); thus, a large hysteresis appeared in the swelling curves. In contrast, the gel underwent a continuous swelling−deswelling change without a hysteresis during the cyclical change of NaCl concentration from 0 to 0.1 M and vice versa. A careful analysis of the changes in pH of an aqueous gel dispersion during titration with NaOH followed by a back titration with HCl demonstrated that the origin of the hysteresis in the swelling curves was due to a hysteresis in the deprotonation−protonation process. The formation through hydrogen bonding of a stable structure between the LPEI chain segments in the network and water molecules as the solvent is considered to be the reason for the hysteresis in the deprotonation−protonation process. However, this does not account for the gel undergoing discontinuous transitions in the protonation and deprotonation processes. We therefore assumed that the transition takes place when the repulsive force due to the $-NH_2^+-$ and $-NH^+<$ groups overcomes the hydrogen bonding as the attractive force or vice versa. To support this assumption, the effects of urea and anionic surfactants were investigated. Urea facilitated the swelling of the gel at higher pH levels during the protonation process, which suggests that urea inhibits the formation of the hydrogen bond under consideration. On the other hand, anionic surfactants such as sodium dodecyl sulfate (SDS) effectively collapsed the gel due not only to the neutralization of the cationic charges but also to hydrophobic interaction; the diameter of the gels was about half that of the gel collapsed at pH > 10.7. However, urea exhibited no influence on the SDS-induced gel collapse, thus indicating that urea inhibits the formation of hydrogen bonds but does not affect the hydrophobic interaction.

Introduction

In the formation of gels through the cross-linking of polymers, various properties of individual polymers become visible on a macroscopic scale. Among these, the volume phase transition of gels is one of the most important phenomena allowing us to explore the principles underlying the molecular interactions in synthetic and biological polymers. The first experiment to demonstrate that gels undergo a volume phase transition was reported by Tanaka[1] who discovered the discontinuous volume change of a covalently cross-linked acrylamide (AAm) gel in an acetone−water mixture when varying the temperature or composition of the mixture. Tanaka then attempted to account for his experimental results using the Flory−Huggins equation. After that, several studies attempted to theoretically account for the phase transition in terms other than those of the Flory−Huggins theory. For example, Otake *et al.*[2] proposed a theoretical model that takes hydrophobic interaction into account to explain the thermally induced discontinuous volume collapse of hydrogels, while Prausnitz *et al.*[3] employed a lattice model and tried to explain the swelling curves of gels consisting of AAm with [(methacrylamide)propyl]trimethylammonium chloride.[4]

Although the development of theories on the volume phase transition is of scientific interest, a more general and simple explanation on molecular grounds is necessary in order to apply gels to the technological field. We have therefore tried to account for the volume phase transition by hypothesizing a balance between the repulsive and attractive forces within the cross-linked polymers in the networks which arise from a combination of four intermolecular forces: ionic, hydrophobic, van der Waals, and hydrogen bonding.[5] When a repulsive force, usually electrostatic in nature, overcomes an attractive force such as hydrogen bonding or the hydrophobic interaction between the network chains, gel volume should increase discontinuously in some cases and continuously in others. The variables triggering the transition influence these intermolecular forces and thereby the balanced state of the attractive and repulsive forces. This concept has helped us to design gels during the course of the construction of "functional" immobilized biocatalysts,[6,7] in particular, the construction of "biochemo-mechanical systems"[8] which convert the energy arising from bio-

* To whom correspondence should be addressed.
† University of Tsukuba.
‡ Nihon University.

(1) Tanaka, T. *Phys. Rev Lett.* **1978**, *40*, 820.
(2) Otake, K.; Inomata, H.; Konno, M.; Saito, S. *J. Chem. Phys.* **1989**, *91*, 1345.
(3) Prange, M. M.; Hooper, H. H.; Prausnitz, J. M. *AIChE J.* **1989**, *35*, 803.
(4) Hooper, H. H.; Baker, J. P.; Blanch, H. B.; Prausnitz, J. M. *Macromolecules* **1990**, *23*, 1096.
(5) Ilmain, F.; Tanaka, T.; Kokufuta, E. *Nature* **1991**, *349*, 400.
(6) Kokufuta, E. *Prog. Polym. Sci.* **1992**, *17*, 647.
(7) Kokufuta, E. *Adv. Polym. Sci.* **1993**, *110*, 159.
(8) Kokufuta, E.; Matsukawa, K.; Ebihara, T.; Matsuda, K. In *Macroion Characterization: From Dilute Solutions to Complex Fluids*; Schmitz, K. S., Ed.; American Chemical Society: Washington, DC, 1994; Chapter 39.

S0743-7463(97)00910-4 CCC: $15.00
Published on Web 01/22/1998

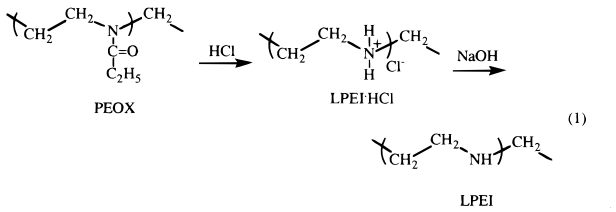
chemical changes such as enzyme reactions into mechanical work through the volume phase transition of gels containing immobilized biocatalysts.

Several recent studies dealing with polyelectrolyte gels have attempted to account for observed volume collapses by considering which of the previously mentioned four intermolecular forces is the dominant one; e.g., see refs 9−13 . In the reports on these studies, however, very little experimental evidence was given for the attractive forces resulting in the gel collapse. The purpose of the present study is to demonstrate that hydrogen bonding and hydrophobic interaction play an important role in the volume collapse of polyelectrolyte gels. The effects of urea and sodium alkyl sulfate or sulfonates on the pH dependence of the swelling degree were thus carefully examined in an attempt to come up with a simple but effective way to demonstrate the role of hydrogen bonding and hydrophobic interaction in the volume collapse of polyelectrolyte gels.

Poly(ethylenimine) (PEI) is a representative polybase with either a linear or a branched polymer structure; the former is more often abbreviated as LPEI and the latter as BPEI. The acid hydrolysis of poly(ethyloxazoline) (PEOX) gives rise to LPEI,[14] while BPEI can be obtained through the ring-opening polymerization of ethylenimine.[15] In this study, we selected LPEI in the preparation of the polyelectrolyte gel because of the following advantages: (i) The deprotonation was fully studied as a function of pH by means of potentiometric[16,17] and calorimetric titrations with strong bases such as NaOH;[18] (ii) the polymer precipitates to form crystalline hydrates from alkaline solutions (pH > 9) through hydrogen bonding between the −NH− groups and water molecules;[17,19] (iii) X-ray structure analysis[19] has demonstrated that such hydrates consist of alternately stacked layers of polymers and water molecules in the crystallized state; (iv) in addition, our preliminary experiments revealed that a gel of LPEI can be obtained without difficulty via cross-linking with ethylene glycol diglycidyl ether (EGDGE).

Experimental Section

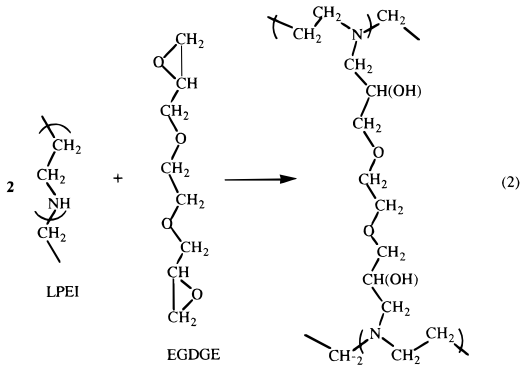
Preparation of LPEI. LPEI was obtained from PEOX with $M_w \sim 2 \times 10^5$ (a commercial product from Nihon Shokubai Co., Tokyo, Japan) by the acid hydrolysis with HCl, followed by the deacidification with NaOH (see eq 1). Freshly distilled carbonate-free water was used as the solvent. The reaction and purification procedures were the same as those reported by Warakomski et al.,[14] except for the use of HCl instead of H_2SO_4 in the acid hydrolysis. Since LPEI has been reported to be remarkably hygroscopic,[19] the purified polymer was heated over phosphorus pentaoxide at 105 °C in vacuo until a constant weight was reached; therefore, the thermogravimetric analysis showed that the sample is an anhydrous polymer. The structure was identified by ^{13}C NMR and the degree of hydrolysis was determined by elemental analysis.[20] Anal. Calcd for $(C_2H_5N)_n$: C, 55.77; H, 11.70; N, 32.52. Found: C, 56.25; H, 11.09; N, 31.86.



(1)

The hydrochloride salt of LPEI, namely, LPEI·HCl, was prepared through the neutralization of the polybase with HCl. LPEI (10 g) was dissolved in a 100 mL solution containing HCl in amounts equimolar with the nitrogen of the polybase, slowly heated to dryness at 100 °C, and heated further over phosphorus pentaoxide at 105 °C in vacuo until a constant weight was reached. The thermogravimetric analysis was also used in this case for identifying an anhydride structure of LPEI·HCl. Anal. Calcd for $(C_2H_6NCl)_n$: C, 30.21; H, 7.60; N, 17.61. Found: C, 30.30; H, 7.82; N, 17.70.

Preparation of gels. Gels were prepared by heating an aqueous solution (50 mL; pH = 8.2) containing 5.0 g of LPEI and 3.95 g of EGDGE at 60 °C for 12 h (see eq 2).



(2)

The molar ratio of

in EGDGE to −NH− in LPEI was then adjusted to 1:5; this ratio was determined from the results of preliminary experiments. The gelation was performed in test tubes into which glass capillaries with inner diameters of 0.3 mm had previously been inserted. After the gelation was completed, the gel mass was taken out of the test tubes together with the capillaries. Thus, two types of gel samples were available from the same preparation: cylindrical gels for the swelling experiment and powdered gels for the potentiometric titration.

A fine gel rod was taken out of the capillary and cut into cylinders approximately 2 mm in length. The obtained gel samples were purified by repeated swelling and shrinking procedures, respectively performed in aqueous HCl and NaOH solutions (0.1 M each). Finally, the gels were thoroughly washed with a large amount of pure water. The purification was completed when total organic carbon (TOC) analysis with a Beckman TOC analyzer (Model 915 B) showed that there was no detectable TOC in the washings (see ref 21 for TOC analysis). The purified gels were stored in water before use.

(9) Annaka, M.; Tanaka, T. *Nature* **1992**, *335*, 430.
(10) Annaka, M.; Tanaka, T.; Osada, Y. *Macromolecules* **1992**, *25*, 4826.
(11) Kokufuta, E.; Tanaka, T.; Ito, S.; Hirasa, O.; Fujishige, S.; Yamauchi, A. *Phase Transitions* **1993**, *44*, 217.
(12) Hirata, M.; Yamada, K.; Matsuda, K.; Kokufuta, E. In *Macroion Characterization: From Dilute Solutions to Complex Fluids*; Schmitz, K. S., Ed.; American Chemical Society: Washington, DC, 1994; Chapter 37.
(13) Philippova, O. E.; Hourdet, D.; Audebert, R.; Khokhlov, A. R. *Macromolecules* **1996**, 29, 2822.
(14) (a) Saegusa, T.; Ikeda, H.; Fujii, H. *Polym. J.* **1972**, *3*, 35. (b) Warakomski, J. M.; Thill, B. P. *J. Polym. Sci., Polym. Chem. Ed.* **1990**, *28*, 3551.
(15) Davis L. E. In *Water-Soluble Resins*; Davidson, R. L., Sittig, M., Eds.; Van Nostrand Reinhold Co.: New York, Cincinnati, Toronto, London, Melbourne, 1968; Chapter 11.
(16) Kobayashi, S.; Hiroishi, K.; Tokunoh, M.; Saegusa, T. *Macromolecules* **1987**, *20*, 1496.
(17) Weyts, K. F.; Goethals, E. *J. Makromol. Chem., Rapid Commun.* **1989**, *10*, 299.
(18) Lewis, E. A.; Barkley J.; Pierre, T. S. *Macromolecules* **1981**, *14*, 546.
(19) Chatani, Y.; Tadokoro, H.; Saegusa, T.; Ikeda, H. *Macromolecules* **1981**, *14*, 315
(20) The elemental analyses of all the samples used here were carefully performed by experts since the polymers and gels of LPEI in the form of a free base or an HCl salt are remarkably hygroscopic.
(21) Kokufuta, E.; Fujii, S.; Hirai, Y.; Nakamura, I. *Polymer* **1982**, *23*, 452.

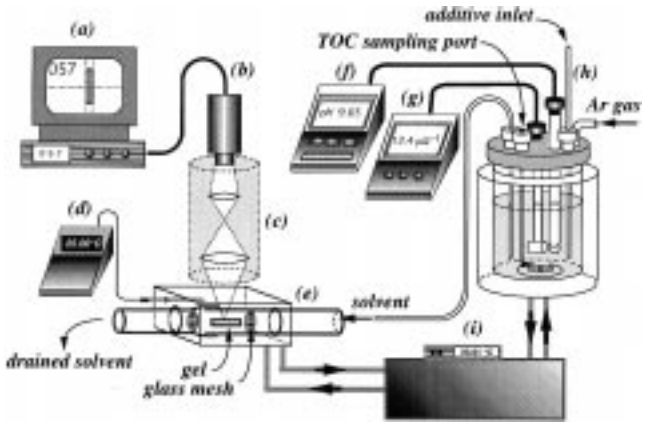


Figure 1. Setup for the measurements of the swelling ratio: (a) image analyzer; (b) CCD camera; (c) microscope; (d) thermometer; (e) measuring cell; (f) pH meter; (g) conductivity meter; (h) aqueous phase supply system (APSS); (i) temperature control system (TCS).

In the preparation of the powdered gels, the gel mass was ground, passed through a screen with a mesh size of 1 mm, and purified in the same manner as the cylindrical gel. The gel was then converted into the base form (LPEI gel) followed by the salt form (LPEI·HCl gel). In the case of the LPEI gel, the purified gel powder was incubated in 1 M NaOH solution at 60 °C for 5 h, thoroughly washed with freshly distilled carbonate-free water, lyophilized for 1 day, and finally heated over phosphorus pentaoxide at 105 °C in vacuo until a constant weight was reached. Anal. C, 54.71; H, 9.23; N, 25.91. The LPEI·HCl gel was prepared via the neutralization of the LPEI gel with HCl in amounts equimolar with total nitrogen of the gels in the system, the lyophilization and the desiccation were carried out under the same conditions as the LPEI gel. Anal. C, 32.65; H, 5.48; N, 15.56. The thermogravimetric analysis showed that both LPEI and LPEI·HCl gels were obtained in an anhydrous form. The former gel was used in the estimation of the cross-linking degree, while the latter was subjected to potentiometric titration.

Swelling measurements. We used our own setup in the measurements of the swelling ratio under various concentrations of the following additives in the outer medium surrounding the gel: HCl, NaOH, NaCl, urea, and three kinds of anionic surfactants (sodium butylsulfonate, SBS; sodium octylsulfonate, SOS; sodium dodecyl sulfate, SDS). Our instrument consists of four different parts (see Figure 1): an aqueous phase supply system (APSS) equipped with a pH meter, a conductivity meter, a TOC sampling port, and an additive inlet; a microscope (Olympus CK2-TRP-1) with a CCD camera and an image-analyzer; a water-jacketed separable measuring cell with glass meshes; and a temperature control system (TCS). A section of the cylindrical gel was inserted into the glass cell and an aqueous solution with the desired additive concentration was continuously supplied around the gel sample from the APSS by means of Ar gas pressure. The surfactant concentration was accurately regulated by means of conductivity measurements (at low surfactant concentration ranges) or TOC analyses (at high surfactant concentration ranges). The conductometric measurements were also used for regulating the NaCl concentration, while the urea concentration was adjusted by means of TOC. Calibration curves showing the changes in conductivity and/or TOC with the additive concentrations were used in all the measurements. The attainment of an equilibrated gel diameter was evaluated by careful measurements in which the additive concentration was made to fluctuate several times in ±3% around a setting used for determining the diameter of the gel sample. The temperature was controlled to within a range of ±0.1 °C using TCS with water circulating around both the APSS and the measuring cell.

Viscosity Measurements. The reduced viscosity (η_{sp}/C) of salt-free and 0.1 M NaCl solutions containing a 0.5 g/dL of LPEI·HCl was determined at 20 ± 0.01 °C as a function of pH using an Ubbelohde viscometer with a flow time of 237 s for water at 20 °C. The LPEI·HCl solution (100 mL) was first adjusted to pH ~ 3 with a very small volume of a carbonate-free 6 M NaOH solution, incubated at 20 °C for 1 day, and subjected to the viscosity measurement. This series of procedures was repeated until the cycle in which the pH was raised to 9 and returned to 3 was complete. When the TOC analysis was applied, the change in the polymer concentration during such a pH change cycle was less than 2%. On the other hand, the NaCl concentration was increased to 3.2 mM at the end of the cycle as estimated from amounts of NaOH and HCl used in the pH adjustment; this value was negligible in the salt-containing system but not in the salt-free system.

Potentiometric Titrations. Both the polymer solution and the gel dispersion were used in the pH titration. Three-time distilled carbonate-free water was used as the solvent. The polymer solution was prepared by dissolving 0.238 g of LPEI·HCl into 30 mL of the solvent. In the case of the gel, two samples were prepared by dispersing 0.273 g of powdered LPEI·HCl gels and 0.162 g of powdered LPEI gels in the same solvent volume. The molar concentration based on the ionizable groups (−NH_2Cl− for the polymer, −NH_2Cl− plus −NHCl< for the LPEI·HCl gel, and −NH− plus −NH< for the LPEI gel) was 0.1 M for each sample. To avoid a change in the sample concentration during the titration, an aqueous NaOH or HCl solution with a 3 M concentration was used as the titrant. LPEI·HCl was titrated with the NaOH at 20 ± 0.1 °C under nitrogen using a computer-controlled automatic titration apparatus (Hirama Model ATC-3). The titration of the LPEI·HCl gel was performed with NaOH followed by HCl, while the LPEI gel was titrated with HCl. The same titration apparatus was used, but a batchwise titration was carried out to record the time-dependent pH change after the addition of the titrant. When the pH reached a steady value (within a change of ±0.01 pH unit over a monitoring period of 10 min), the next addition of the titrant was automatically carried out by an on-line computer system. The sample was stirred at 70 rpm during the titration. The electrodes were calibrated by using phosphate and potassium acid phthalate buffers.

Results and Discussion

Characterization of the LPEI Gel by Elemental Analysis. For the purpose of this study, it is important to determine the amounts of −NH− (or −NH_2Cl−) and −N< (or −NHCl<) in the gel. In principle, the elemental analysis makes it possible to determine these quantities from the carbon and/or nitrogen contents. Taking into account 100 monomer units of the EGDGE-cross-linked LPEI chains (base form) according to the reaction in eq 2, the contents (in w/w %) of nitrogen (C_N) and carbon (C_C) may be expressed as follows:

$$C_N = \frac{100 m_N}{M_1(100 - x) + M_2 x} \times 100 \qquad (3)$$

$$C_C = \frac{[2(100 - x) + 6x] m_C}{M_1(100 - x) + M_2 x} \times 100 \qquad (4)$$

Here, m_N (14.01) and m_C (12.01) respectively denote the atomic masses of nitrogen and carbon, M_1 (43.97 for C_2H_5N) and M_2 (138.17 for $C_6H_{12}O_2N$) respectively are the molar masses of un-cross-linked and cross-linked monomer units of the polymers in the network, and x is the number of cross-linking points per 100 monomer units.[22] Since the elemental analysis for the LPEI gel gave C_N = 25.91% and C_C = 54.71% (see Experimental Section), we may estimate $x \sim 12$ for our gel sample.

The moles of nitrogen are equivalent to those of the ionizable groups in the gel; thus, the charge densities (in mol/g) for the gels in the forms of free base (D_b) and HCl salt (D_s) may be determined by eqs 5 and 6,

(22) The number of cross-linking points is equivalent to that of the −N< or −NHCl< groups.

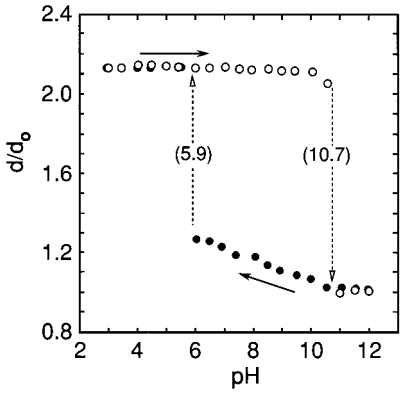


Figure 2. Normalized equilibrium diameters (d/d_0) of the LPEI gel in aqueous solution at 20 °C as a function of pH. Open symbols denote an increase in pH from 3 to 12, whereas closed symbols show a decrease in pH from 12 to 3. The values in parentheses indicate the pH at the phase transition point.

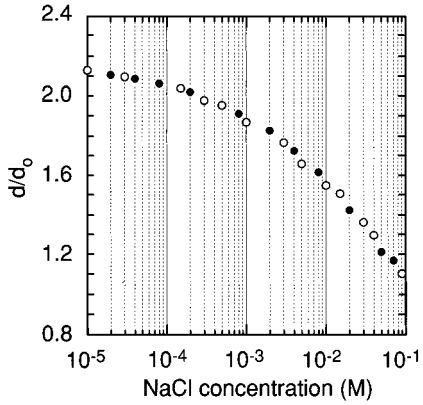


Figure 3. Change in the normalized equilibrium gel diameters (d/d_0) with NaCl concentration at pH 3 and at 20 °C. Open symbols denote an increase in the concentration from 0 to 0.1M, whereas closed symbols show a decrease in the concentration from 0.1 to 0 M.

respectively.

$$D_b = \frac{100}{M_1(100 - x) + M_2 x} \quad (5)$$

$$D_s = \frac{100}{(M_1 + M_3)(100 - x) + (M_2 + M_3)x} \quad (6)$$

where M_3 (36.40) is the molecular weight of HCl and $x = 12$. Since $C_N = 25.91\%$ for the LPEI gel and 15.56% for the LPEI·HCl gel, we obtain $D_b = 18.5$ mmol/g and $D_s = 11.0$ mmol/g.

Effects of pH and NaCl Concentration on the Swelling Degree. The gel sample was inserted into a glass cell together with 0.001 M HCl. After this, the outer medium, which was maintained at 20 ± 0.1 °C and adjusted to a desired pH within the range of 3 to 12 using various concentrations of HCl (pH < 6.7) or carbonate-free NaOH (pH > 6.7), was made to flow slowly through the cell until the diameter of the gel reached equilibrium under the given conditions. Figure 2 shows pH-dependent changes in the normalized equilibrium gel diameter (d/d_0). The normalization of each observed equilibrium diameter (d) was then performed with the inner diameter (d_0) of the capillary used in the gel preparation. During a pH change cycle (3 to 12 followed by 12 to 3), the gel underwent a discontinuous volume phase transition near pH 10.7 (collapse) and pH 5.9 (swelling); thus, a large hysteresis appeared in the swelling curve.

The effect of NaCl concentration on the gel diameter was studied at pH 3 at which the gel was in a fully swollen state. As can be seen from Figure 3, an increase in the NaCl concentration brought about a monotonic gel collapse without transition. The gel diameter at 0.1 M NaCl concentration was almost half that in an NaCl-free solution at pH 3; this value was close to the fully collapsed diameter in Figure 2 (i.e., pH > 10.7). No hysteresis appeared in a concentration change cycle of NaCl.

It is true that pH and NaCl concentration influence the ionization state of polyions. If we assume that these factors affect the state of ionization of the network in a polyelectrolyte gel and thereby its swelling degree is altered, the ionization state of a PEI gel in a 0.1 M NaCl solution and that in a salt-free solution at pH ~ 10.7 seem to be almost the same because there is little difference between the d/d_0 ratios determined in both solutions. However, a large difference was observed in the deswelling of the gel when increasing the pH and NaCl concentration. At present, to our knowledge, there is no theory which fully accounts for this difference on the basis of a mathematical model.

We will now attempt to explain on molecular grounds the observed swelling−deswelling characteristics of the LPEI gel, especially the pH-induced change, in terms of a balance between the repulsive and attractive forces within the cross-linked polymers in the network. Previous studies[16,17] have reported that a highly deprotonated LPEI forms crystalline hydrates in its alkaline solutions (pH > 9). The existence of two distinct hydrates, sesquihydrate with a unit cell containing 8 monomeric units and 12 water molecules, and dihydrate with a unit cell containing 4 monomeric units and 8 water molecules, has been reported on the basis of the X-ray structure analysis. The crystals of both hydrates consist of alternatingly stacked layers of polymers and water molecules arranged parallel to the *bc* plane. As shown in Figure 4, three types of hydrogen bonds (N–H···O, O–H···O, and O–H···N) play a major role in the stabilization of the crystal lattices. In the case of the cross-linked LPEI, i.e., the gel, it should not form such crystalline hydrates, but several parts of the polymer chain segments in the network would form a stable structure like that in Figure 4, at pH > 10.7 at which both the −NH− and −N< groups are highly deprotonated. Therefore, it is at this pH that the attractive force overcomes the repulsive electrostatic force. We consider hydrogen bonding to be the attractive force at work and assume that the gels which have collapsed due to hydrogen bonding do not swell again until significant numbers of the −NH− and −N< groups are protonated as the pH decreases; this would account for the large hysteresis in the swelling curves. On the other hand, an increase in NaCl concentration may eliminate the positive charges of the network as the repulsive electrostatic force, but it fails to form such a hydrogen-bond-stabilized structure because of the protonated ionizable groups ($-NH_2^+-$ and $-NH^+<$); therefore, the gel collapses monotonically.

pH Dependence of Viscosity for the Polymer. Since we expected a similarity between the pH-induced changes in gel volume and polymer conformation, we thus measured the reduced viscosity of LPEI·HCl in salt-free and 0.1 M NaCl solutions as a function of pH. Kobayashi et al.[16] have already studied the pH dependence of the reduced viscosity for LPEI in a 1 M KCl solution at 24.5 °C and reported that an increase in pH from 2.9 to 8.8 brought about a viscosity fall at pH < 6 but an increase at pH > 8 after passing through a minimum value around pH 6.5. As can be seen from Figure 5, however, our measurements showed a monotonic decrease in the

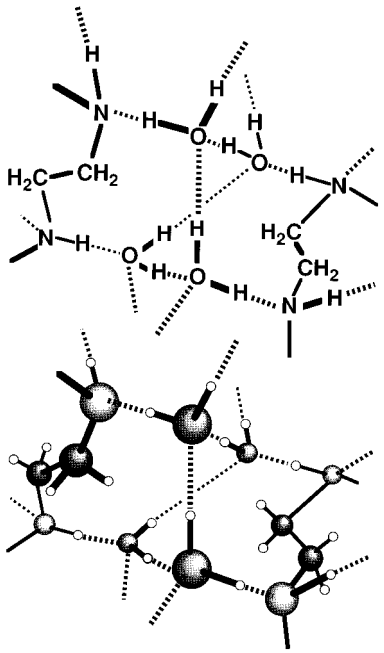


Figure 4. Schematic illustration for three types of hydrogen bonds in a dihydrate of LPEI. The structural formula (top) and the corresponding atomic arrangement (bottom) were illustrated by reference to Figures 4, 6, and 7 in ref 16. Each H_2O oxygen atom is bound to four hydrogen atoms; two of them are covalently bonded and the remaining two are hydrogen bridged from neighboring water molecules or NH groups. Such a hydrogen-bond-stabilized structure would form in highly deprotonated LPEI chain segments in the network.

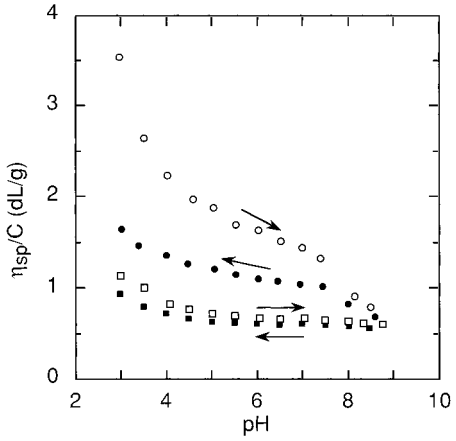


Figure 5. pH-induced changes in reduced viscosities (η_{sp}/C) of salt-free solution (open and closed circles) and 0.1 M NaCl solution (open and closed squares) of LPEI at 20 °C. Open symbols denote an increase in pH, whereas closed symbols are a decrease in pH.

viscosity with increasing pH from 2.9 to 8.8. At pH > 9, it was very difficult to measure the viscosity, especially for the salt-containing system, because more often the sample became turbid due to the formation of hydrates of highly deprotonated LPEI via polymer−polymer association. Kobayashi et al. have considered such an association as one possibility leading to an increase in the viscosity at pH > 8, but they also mentioned that another reason was the stretching of the LPEI chain due to repulsion between lone-pair electrons of nitrogen atoms in the polymer and Cl^- and/or OH^- ions in the medium.[23]

The most striking feature in the viscosity curves is the appearance of a hysteresis during the pH change cycle (from 2.9 to 8.8 and vice versa). Since the hysteresis was also observed even in the presence of 0.1 M NaCl, it is not due to an increase in ionic strength during the pH change cycle. One might assume that a very slow alteration in the polymer conformation under a given pH may cause a hysteresis. Although each of the sample solutions was allowed to stand for 1 day with mild stirring after the pH adjustment, we cannot conclude that there is no kinetic effect in an infinite period. However, the appearance of the hysteresis in both the viscosity and swelling curves seems to be due to the same reason as described in the previous section. Even when the solution basicity is not high enough to result in crystalline hydrates of LPEI, the deprotonation of a large portion of the ionizable groups could lead to the collapse of the polymer chain through "intrapolymer" hydrogen bonding with the aid of water molecules.[24] Therefore, the successive protonation of such a collapsed polymer becomes increasingly difficult; as a result, we observed that the viscosity during the pH decrease is lower than that during the pH increase.

Potentiometric Titration Curves. Investigation of the protonation−deprotonation process by means of potentiometric titration could provide a key to understanding the pH-dependent changes in the gel volume and polymer conformation. Three previous studies[16−18] have dealt with the potentiometric titrations of aqueous polymer solutions; the results are outlined as follows: (i) Protons were dissociated from LPEI·HCl with increasing difficulty, in particular at the protonation >50%, due to their strong interaction with neighboring charged ammonium ions, i.e., the nearest neighbor interaction originally proposed by Katchalsky et al.[25] (ii) It was not possible to achieve 100% protonation even at pH ∼ 2, but an approximately 70% degree of protonation gave an "apparent end point" of titration. (iii) At less than 10% of the protonation the polymer precipitated to form crystalline hydrates. With respect to the formation of crystalline hydrates through hydrogen bonding, Weyts and Goethals[17] reported that aqueous salt-free solutions containing 0.1 M LPEI·HX (X = Cl^-, $HCOO^-$, and CH_3COO^-) exhibited an unusual pH change when the titration proceeded until 10% of the protonation (i.e., pH ∼ 9.4); that is, the solution pH increased due to the addition of a strong base until 10% protonation, but further addition of a very slight amount of the base brought about a decrease in pH by ca. 0.7 unit accompanied by the appearance of solution turbidity. This phenomenon was then interpreted as follows: Once the crystallization starts, the enthalpy of crystallization provides energy to expel more protons of LPEI·HX into the solution; this promotes the formation of crystalline hydrates via interpolymer hydrogen bonding with the aid of water molecules and results in an increase of the fraction of protonated ammonium groups on the LPEI remaining in the solution; therefore, these in turn lead to a decrease in the solution pH. For this reason, no previous studies have attempted to carry out a back-titration with strong acids after titrating LPEI·HX with strong bases, although it would provide important information in order to understand the hysteresis observed in the viscosity curves.

In the case of the gel, on the other hand, the back-titration with an acid for the sample dispersion, which has already been titrated with a base until an equivalent point, should be possible since precipitation does not need to be taken into consideration. In contrast to the polymer,

(23) Since it is reasonable to consider that lone-pair electrons of nitrogen atoms in the −NH− groups are solvated by coordination to water molecules as the solvent, we cannot understand why such lone-pair electrons serve as the anion.

(24) Interpolymer hydrogen bonding was not the case since we did not observe a rise in viscosity under the conditions used here.

(25) Katchalsky, A.; Mazura, J.; Spitnik, P. *J. Polym. Sci.* **1957**, *23*, 513.

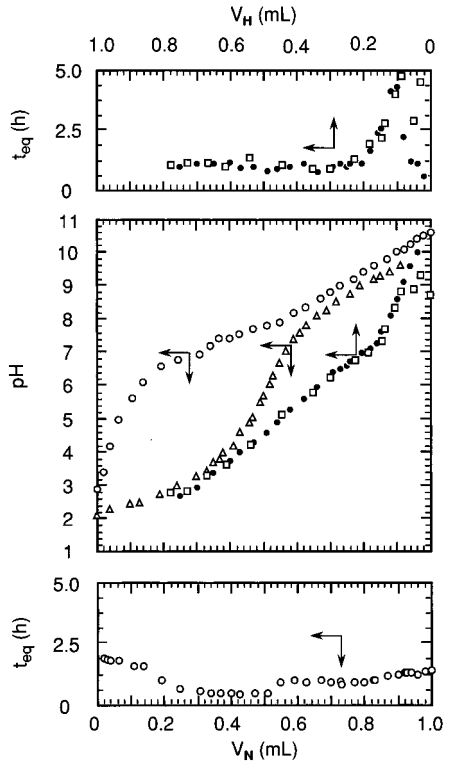


Figure 6. Change in pH during titration of LPEI·HCl with NaOH (open triangles) as well as pH changes during titration of LPEI·HCl gel with NaOH (open circles) followed by back-titration with HCl (closed circles). Also shown in this figure is the titration curve (open squares) with HCl of LPEI gel in the form of free base. The curves of t_{eq} vs V_N (open circles) and V_H (closed circles and open squares) were given to indicate the time (t_{eq}) required for the establishment of pH equilibrium at each stage of the titration if unit volume of the NaOH or HCl titrant were added into the titration system. Titration conditions were as follows: titrant concentration, 3 M for each; sample concentration, 0.1 M for each (by moles of the $-NH_2Cl-$ groups in the polymer, the $-NH_2Cl-$ plus $-NHCl<$ groups in LPEI·HCl gel, and the $-NH-$ plus $-N<$ groups in LPEI gel); sample size, 30 mL for each; 20 °C.

however, there are several difficulties in the analysis of the titration data, for example, how to estimate the "real" acid−base equilibrium within the gel phase from the pH measurements of the outer solution. So far, no study has dealt with the potentiometric titrations of polyelectrolyte gels. We nevertheless employed the potentiometric titration with NaOH for the aqueous dispersion of powdered LPEI·HCl gels, followed by a back-titration with HCl; through both titrations we intended to clarify the origin of the hysteresis in the swelling curve.

Figure 6 shows the titration curves in which the pH of the outer medium was plotted against the volumes of NaOH (V_N) and HCl (V_H). Also shown in Figure 6 for the purpose of comparison is the titration curve of the polymer (LPEI·HCl) with NaOH. In the titrations of the gel, the time (t_{eq}) required for the establishment of equilibrium pH calculated on the basis of 1 mL of titrant was measured at each stage of the titration and plotted against V_N and V_H. In all the titration curves, both sample and titrant concentrations were precisely controlled on the basis of the results of the elemental analysis in order to complete the stoichiometrical neutralization of all the titratable groups with 1 mL of NaOH or HCl titrant; i.e., 1 mL of NaOH or HCl being equivalent to moles of the $-NH_2^+-$ plus $-NH^+<$ groups or the $-NH-$ plus $-N<$ groups. A comparison of the three titration curves in Figure 6 provides us with some significant information: (i) The pH of the gel dispersion is higher than that of the polymer solution over all the stages of titration with NaOH, especially in the range of $V_N < 0.5$ mL at which, as demonstrated in a previous study,[18] the nearest neighbor interaction does not have a strong influence on the deprotonation reaction of LPEI·HX (i.e., $-NH_2^+- \rightarrow -NH- + H^+$).[26] (ii) At the onset of the back-titration of the gel with HCl, a very rapid decrease in pH is observed and the curve exhibits an infliction point at $V_H \sim 0.1$ mL. (iii) This is not due to the titration with HCl of the remaining NaOH in excess in the system because a very slow attainment of acid−base equilibrium was observed from the t_{eq} vs V_H curve at $V_H < 0.15$ mL. (iv) In the back-titration, the initial pH level (2.9) of the original gel dispersion is reached even at $V_H = 0.71$ mL.

Special attention ought to be paid to results (ii) and (iv) with regard to our present purpose. Our gel contains two kinds of amines as the ionizable groups, $-NH-$ and $-N<$ in the form of a free base; the contents are 88 mol % for the former and 12 mol % for the latter by means of elemental analysis. It is generally believed that pK_a (i.e., $-\log K_a$) for tertiary alkylammonium salts is larger than 10; e.g., p$K_a = 10.64$ for $(CH_3CH_2)_3N$·HCl at 25 °C. Taking this into account, we may predict that the $-N<$ groups in the network are preferentially protonated at $V_H < 0.1$ mL: $-N< + H^+ \rightarrow -NH^+<$. In fact, the amount of the titrated groups in this range is about 10 mol % of the total ionizable groups in the system, the value of which is very close to the content of the $-N<$ groups.

If the protonation of the $-N<$ groups could be terminated at $V_H \sim 0.1$ mL, the stage of the titration at $V_H > 0.1$ mL may be related to the reaction $-NH- + H^+ \rightarrow -NH_2^+-$. As mentioned above, however, this reaction appears to go essentially to completion at $V_H \sim 0.7$ mL; this corresponds with the 68% degree of protonation for a total of the $-NH-$ groups because the $-NH-$ content = 88%. Therefore, the reaction does not follow a 1:1 stoichiometry. However, this is not surprising when we consider that a highly deprotonated LPEI gel contains a large number of $-NH-$ groups whose nitrogen atoms are stabilized with bound water molecules via hydrogen bonding and resistant to the coordination (protonation) with the protons added. The same interpretation has been applied to the viscosity curves, and even in the gel system a structure as shown in Figure 4 may form within chain segments of the networks. As a result, the potentiometric titration clearly demonstrated that the origin of the hysteresis in the swelling−deswelling process is due to the hysteresis in the deprotonation−protonation process; in other words, a large difficulty in the protonation caused by hydrogen bonding as shown in Figure 4. However, this does not account for the gel discontinuously swelling in the protonation process or discontinuously collapsing in the deprotonation process. We thus have to consider that the gel undergoes a transition when the repulsive force due to the $-NH_2^+-$ and $-NH^+<$ groups overcomes the hydrogen bonding as the attractive force or vice versa.

By taking the above into account, we can explain why the $-N<$ groups were preferentially protonated in the titration with HCl, but the preferential deprotonation did not take place in the titration with NaOH. In the protonation process, the $-NH-$ groups behave as a very

(26) At the protonation degree <50% there is little influence from the nearest neighbor interaction on the dissociation of protons from the polymer, but there is a strong influence in the case of the gel because cross-linking should facilitate "intramolecular" interactions between the ionizable groups bound to the network. Therefore, we assumed that the cross-linking of the polymer chains was responsible for this result.

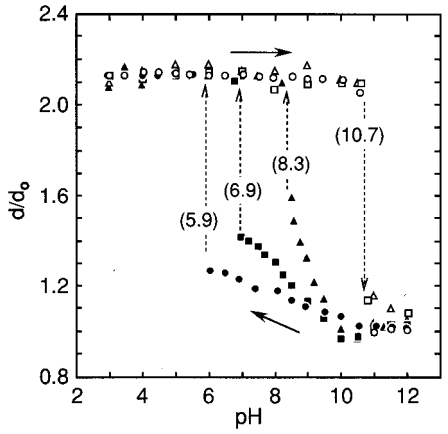


Figure 7. pH-induced changes of normalized equilibrium gel diameters (d/d_0) at 20 °C in aqueous solutions containing different concentrations of urea: 0 M (circles); 1 M (squares); 3 M (triangles). Open symbols denote an increase in pH from 3 to 12, whereas closed symbols show a decrease in pH from 12 to 3. The values in parentheses indicate the pH at the phase transition point.

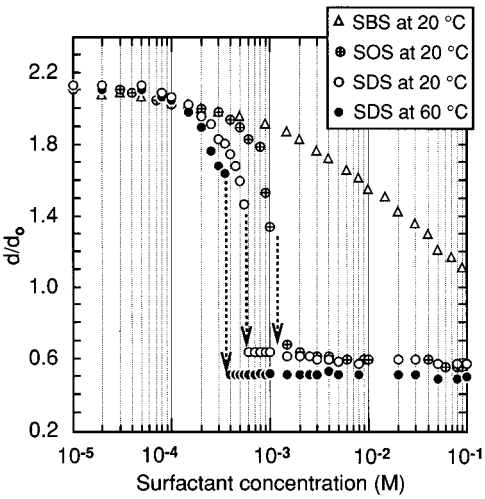


Figure 8. Changes of normalized equilibrium gel diameters (d/d_0) in aqueous pH 3 solutions containing different surfactants at 20 and 60 °C. The kinds of surfactants and the temperatures for the measurements are shown in the figure.

weak base due to the previously mentioned stabilization effect, while the $-N<$ groups as a strong base preferentially accept protons to form the tertiary alkylammonium ions. At the onset of the titration with NaOH, the $-NH_2^+-$ groups may be taken as a strong acid since at $V_N = 0$ pH = 2.1 for LPEI·HCl and 2.9 for LPEI·HCl gel. Thus, in the range of $V_N < 0.1$, the dissociation of protons from the $-NH_2^+-$ groups overlaps with that from the $-NH^+<$ groups, leading to a titration curve without an inflection point.

Our titration data have indicated that there was a hysteresis in the deprotonation−protonation process. However, we should consider that the titration was performed in the absence of supporting electrolytes such as NaCl. Consequently, the ionic strength of the system increased during the course of titration; for example, at $V_N = 1.0$ mL the ionic strength became 0.1 M due to NaCl resulted from the reaction $-NH_2Cl- + NaOH \rightarrow -NH- + NaCl + H_2O$. Thus, a titration which is not accompanied by such a salt formation is desired. Taking this into account, we performed the titration with 3 M HCl of an aqueous dispersion containing the LPEI gel (base form). The result obtained was overplotted in Figure 6 (see open squares). At $V_N < 0.03$ mL the titration curve for the LPEI gel was different from the back-titration curve for the salt-form gel, perhaps due to a difficulty in the protonation of the $-N<$ groups (this may be conceivable when the gel sample in the base form was directly subjected to the titration). At $V_N > 0.05$ mL, however, there was a good agreement between both titration curves, indicating that NaCl resulted from the titration of the LPEI·HCl gel with NaOH and accumulated in the system little influences the back-titration.

Effect of Urea on the pH-Induced Volume Transition. Urea has been frequently employed in the biochemical field as a means to identify hydrogen bonds since it is generally believed that urea can break up intra- or intermolecular hydrogen bonding of proteins in aqueous systems. We thus studied the effects of urea on the pH dependence of the gel diameter (Figure 7) and found that urea facilitates the swelling of the gels at higher pH levels during the protonation process, even at urea concentrations one-fourth or one-eighth lower than the most commonly employed concentration (8 M) in the denaturation of proteins.

It has long been believed that urea disrupts the cluster structure of water molecules,[27] i.e., "structure breaking effect". Thus, it appears that urea may inhibit the formation of a structure (as shown in Figure 4) stabilized through the hydrogen bonds with water molecules. In the presence of urea, therefore, both the acceleration of the protonation (i.e., ionization) and the weakening of the attractive interaction between the cross-linked chains take place at the same time during the protonation process. As a result, a pH at which the gel swells discontinuously should shift to an alkaline pH side; in other words, a tendency for the hysteresis to disappear can be observed when adding urea.

Effects of Anionic Surfactants on the Swelling Degree. Urea, being a water structure breaker, would weaken the hydrophobic interaction between solute molecules as reported in several previous studies; e.g., see ref 28 . It is thus necessary for us to examine the effects of urea on the hydrophobic interaction in the present gel system. However, this is a considerably difficult problem which, to our knowledge, has not yet been dealt with by any researchers in the field of polyelectrolyte gels. The main reason is the lack of information about whether hydrophobic interaction plays a role in the volume collapse of usual polyelectrolyte gels with a lot of hydrophilic ionizable groups, such as the LPEI gels in question. As a novel approach in order to overcome this difficulty, we decided to examine the swelling curves of the LPEI gel as a function of the concentration of anionic surfactants in the presence and absence of urea.

Our previous study[13] demonstrated that the binding of SDS anions to the charged ammonium ions of EGDGE-cross-linked branched poly(ethylenime) gels brings about a dramatic volume collapse due not only to the neutralization of the charges but also to hydrophobic interaction. As shown in Figure 8, this is the case in the LPEI gel system. SBS without definitive critical micelle concentration (C_{mc}) in aqueous solutions exhibited the same effect

(27) For previous studies showing that urea acts as a water structure breaker, see the introduction of the following articles: (a) Kuharski, R. A.; Rossky, P. J. *J. Am. Chem. Soc.* **1984**, *106*, 5786. (b) Tanaka, H.; Touhara, H.; Nakanishi, K.; Watanabe, N. *J. Chem. Phys.* **1984**, *80*, 5170. However, we should note that both articles deal with an molecular dynamics simulation of a dilute aqueous urea solution and report that urea has little effect on water structure under a infinitely low concentration.

(28) Dubin, P.; Stauss, U. P. *J. Phys. Chem.* **1973**, *11*, 1427.

on the gel diameter as NaCl (see Figure 3). However, SOS ($C_{mc} \sim 130$ mM) and SDS ($C_{mc} \sim 8.3$ mM) showed remarkable and strong effects on the volume phase transition of the LPEI gel: (i) The concentration at which the transition takes place is lower for SDS than for SOS because the former is stronger than the latter with respect to hydrophobicity. (ii) The SDS concentration bringing about the transition was lower at 60 °C than that of 20 °C, the result of which agrees with the common knowledge that a rise in temperature enhances the hydrophobic interaction. (iii) In addition, the gel diameter was reduced to 26% with SOS and SDS at 20 °C and to 23% with SDS at 60 °C, these values are about half compared to the diameter of a fully deprotonated gel at pH > 10.7, clearly indicating that the hydrophobic interaction may act as an attractive force in the gel collapse.

In a previous study[29] we suggested that the binding of surfactants such as SDS to LPEI gels occurs mainly in the region in the near vicinity of the gel surface but that the molecules fail to penetrate into the core of the gel phase. A Donnan potential, generated due to an excess of surfactant anions around the gel surface, would make further diffusion of the surfactants with anionic charges more difficult. From the results in Figure 8, however, the surfactant−LPEI gel system may be regarded as a well-defined polyelectrolyte gel whose volume collapse takes place via hydrophobic interaction as an attractive force. We thus measured the d/d_0 of the gel samples in aqueous solutions containing different amounts of SDS in the presence and absence of urea; the pH of the solutions was adjusted to 3.0 so as to maintain a completely swollen state for the gels. As can be seen from Figure 9, urea has no influence on the SDS-induced discontinuous volume collapse for the LPEI gel, which indicates that urea does not contribute to weakening the hydrophobic interaction, at least in our model system. Therefore, we may say that the results shown in Figure 7 are due to the breaking up of hydrogen bonds by urea molecules.

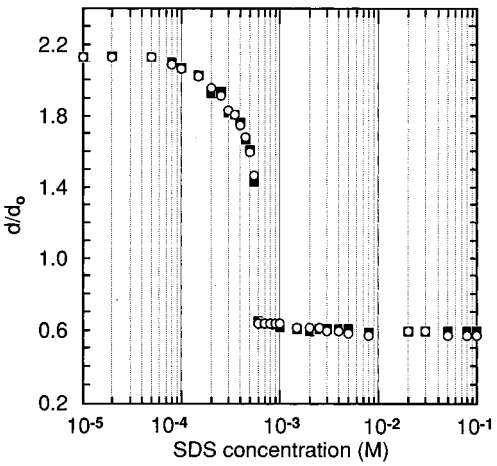


Figure 9. Effect of urea on SDS-induced changes in normalized equilibrium gel diameters in aqueous pH 3 solutions at 20 °C. Closed squares and open circles respectively show the curves in the presence and absence of 3 M urea.

Conclusions

An understanding on molecular grounds of the volume phase transition in polyelectrolyte gel systems has been attempted by hypothesizing a balance between the repulsive and attractive forces within the cross-linked polymers in the network. Since the repulsive force is electrostatic in nature in the case of polyelectrolytes, we were interested in elucidating what attractive forces are at work in the gel collapse. The swelling−deswelling behavior of a cationic polyelectrolyte gel prepared via the cross-linking of LPEI with EGDGE was thus studied. The results obtained are summarized as follows: (i) A pH change cycle (3 to 12 and vice versa) brought about a discontinuous transition near pH 5.9 (swelling) and pH 10.7 (deswelling); therefore, a large hysteresis appeared in the swelling−deswelling process. (ii) The gel underwent a continuous swelling−deswelling change without a hysteresis during a cyclical change of NaCl concentration from 0 to 0.1 M and vice versa. (iii) A hysteresis was also observed in the viscometric measurements of aqueous LPEI solutions in the absence and presence of NaCl when increasing the solution pH followed by a decrease in pH. (iv) The potentiometric titrations showed that there is a large difficulty in the protonation of the −NH− and −N< groups bound to the polymers in the network. (v) A cyclical change of pH in the presence of 1 or 3 M urea exhibited a tendency for the hysteresis in the swelling−deswelling process to disappear. (vi) Anionic surfactants such as SOS and SDS effectively collapsed the gel, the diameter of which became about half that of the gel collapsed in an alkaline solution at pH > 10.7. (vii) Urea has no influence on the SDS-induced gel collapse. On the basis of these results, as well as by reference to literature on X-ray structure analysis for crystalline hydrates of LPEI, a detailed discussion was given about the roles of hydrogen bonding and hydrophobic interaction as the attractive force in the gel collapse.

Acknowledgment. We wish to thank the Analytical Center of Tsukuba University for the elemental analyses of the polymer and gel samples. This research was supported by a grant to E. K. from the Ministry of Education of Japan (No. 08558092).

LA9709103

(29) Kokufuta, E.; Suzuki, H.; Yoshida, R.; Kaneko, K.; Yamada, K.; Hirata, M. *Colloid Surf., A*, in press.