pH-Sensitive Shrinking of a Dextran Sulfate/Chitosan Complex Gel and Its Promotion Effect on the Release of Polymeric Substances

TAKAHARU SAKIYAMA, HAJIME TAKATA, TAKEHIRO TOGA, KAZUHIRO NAKANISHI

Department of Bioscience and Biotechnology, Faculty of Engineering, Okayama University, 3-1-1 Tsushima-naka, Okayama 700-8530, Japan

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ABSTRACT: A pH-sensitive gel was prepared by polyelectrolyte complex formation between dextran sulfate and chitosan. When the complex gel contained more amino groups than sulfate groups, it shrank pronouncedly at around pH 7 in NaCl solutions of various concentrations, probably because of the deionization of protonated amino groups remaining free from electrostatic interaction with the sulfate groups. Using the complex gel loaded with dextran by absorption, releasing behaviors were studied under various conditions. It was shown that shrinking of the complex gel had a promotion effect on the release of dextran. In 170 m*M* NaCl solution, the complex gel released dextran more rapidly at pH 8 than at pH 2, because the degree of shrinking was greater at pH 8. Thus, the promotion effect of the complex gel on the release of dextran was pH dependent, though the release rates at the two pHs became closer as the average molecular weight of dextran loaded was lowered. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 81: 667–674, 2001

Key words: polyelectrolyte complex gel; pH-sensitive swelling; Donnan equilibrium; release promotion

INTRODUCTION

Polymer gels with weakly ionizable functional groups are known to undergo volume change in response to environmental pH changes.[1–3] This is due to the variation in ionization degree of the functional groups fixed on the polymer chains, and has been explained quantitatively on the basis of Donnan equilibrium.[4] The volume change of pH-sensitive polymer gels is of interest from the viewpoint of controlled release of drugs. Two modes are known for the modulation of release rate by the volume change of polymer gels used as drug carriers. One is the promotion of drug release through the squeezing effect caused by shrinking of carrier gels.[5] The other is the suppression of drug release due to dense shell formation as a result of shrinking of carrier gels.[6] If a polymer gel is designed to shrink in the vicinity of neutral pH, it may be used as a drug carrier for oral delivery because the gastrointestinal tract has a pH variation across the neutral pH. For such an oral application, not only the performance of volume change but also the safety to the human body should be taken into consideration. From this context, natural polymers may be preferred as the gel material, although most of the published works on pH-sensitive polymer gels have featured synthetic polymers so far.

Polyelectrolyte complex formation is a way to prepare pH-sensitive complex gels from natural polymers.[7–9] Previously we showed that a poly-

Correspondence to: T. Sakiyama.

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electrolyte complex gel prepared from dextran sulfate and chitosan changed its volume pronouncedly in the vicinity of pH 10.[9] Chitosan, having amino groups, acts as a polycation at low pHs and hence forms a polyelectrolyte complex with a polyanion, dextran sulfate. The net charge on the polymer chains is an essential factor determining the volume of a polyelectrolyte complex gel.[10] The complex gel with equivalent numbers of amino and sulfate groups has no net charge at acidic pHs. In a range of alkaline pH, the net charge becomes negative due to the change in ionization degree of the amino groups. This makes the complex gel swell. The volume of the complex gel in the most swollen state was about 300 times as large as that in the shrunken state. However, the volume change occurred only in an alkaline pH range.

In this article, we report a dextran sulfate/chitosan complex gel (DS/CH gel) which shrank pronouncedly in the vicinity of neutral pH under various ionic strengths. Such a complex gel was available by selecting an appropriate molar ratio of amino group to sulfate group. Furthermore, release experiments were performed at different pHs using the DS/CH gel loaded with a model substance, dextran, to discuss the effect of the shrinking on the release rate.

EXPERIMENTAL

Materials

Chitosan (Chitosan 8B) was a product of Katokichi Co., Ltd. (Kagawa, Japan). Degree of *N*-acetylation of the chitosan was 18.1%. Viscosity of 0.5% solution of the chitosan in 0.2*M* acetic acid was 0.257 Pa · s at 20°C. Dextran sulfate sodium salt (average molecular weight: 5×10^5) was purchased from Pharmacia Biotech (Uppsala, Sweden). Dextran preparations with different average molecular weights (11,000, 67,000, and 200,000–300,000) were purchased from Sigma Chemical Co. (St. Louis, MO) or Wako Pure Chemical Industries Ltd. (Osaka, Japan). Other materials were of reagent grade.

Preparation of Polyelectrolyte Complex Gel Beads

Chitosan and dextran sulfate were dissolved in 2% acetic acid and deionized water, respectively. To avoid the aggregation of the two oppositely charged polyelectrolytes, one-tenth part of NaCl was added to each solution. Both solutions were heated in boiling water to decrease their viscosity, and then mixed with each other. In most cases, final concentrations of chitosan and dextran sulfate were 2.25 and 1.5%, respectively. This composition corresponds to a molar ratio of sulfate group to amino group (referred to as S/N ratio hereafter) of 0.70. In some preparations, the final concentration of dextran sulfate was varied to change the S/N ratio of the mixture. The mixture was centrifuged at 3000 rpm for 10 min to remove air bubbles, and dropped into a toluene/chloroform (5 : 3) mixture kept at 0°C. Droplets formed in the solvent mixture were carefully transferred into a large volume of deionized water. The droplets gelled during this desalting process. The spherical gel beads thus obtained were further rinsed in deionized water for at least 2 days. The diameter of the gel beads was about 4 mm.

Swelling Experiments

Swelling experiments were performed in NaCl solutions of different concentrations. In a 50-mL tissue culture flask, a DS/CH gel bead was immersed in 30 mL of a NaCl solution, pH of which was preliminarily adjusted to a specified value by adding dilute NaOH or HCl solution. The flask was purged with N_2 gas, sealed to avoid the dissolution of CO_2, and incubated at 30°C. At appropriate intervals, the diameter of the gel, d, was measured until the size of the gel ceased to change. The equilibrium swelling ratio was defined as the ratio of the equilibrium volume to the initial one, and calculated as $(d_e/d_i)^3$, where d_e and d_i are the equilibrium and initial diameters, respectively.

Estimation of pH Values Inside the Complex Gel Beads

Information on pH inside a DS/CH gel bead is important to understand its swelling equilibrium. Here the internal pH values at swelling equilibria were estimated by the method described in our previous article.[9]

In a polyelectrolyte complex gel having sulfate and amino groups, the charge balance equation is as follows:

$$\sum_i Z_i C_i^G - \alpha_S C_S^G/X + \alpha_N C_N^G/X = 0 \qquad (1)$$

where C_i^G is the concentration of mobile ion i in the gel, Z_i, the charge on ion i, C_S^G and C_N^G, the initial concentrations of sulfate and amino groups

in the gel, respectively, α_S and α_N, degrees of ionization of sulfate and amino groups, respectively, and X, the equilibrium swelling ratio. The degree of ionization of sulfate groups, α_S, can be assumed as unity unless pH is extremely low. The degree of ionization of amino groups, α_N, is related to the dissociation constant of protonated amino groups, K_N, as follows:

$$K_N = C_H^G(1 - \alpha_N)/\alpha_N \quad (2)$$

where C_H^G is the concentration of proton (hydronium ion) in the gel. Assuming the Donnan potential created by the fixed charge of those functional groups, mobile ions are distributed between the gel and the external solution as described in the following equation.

$$C_i^G/C_i^S = K^{Z_i} \quad (3)$$

where C_i^S is the concentration of mobile ion i in the solution. The Donnan ratio, K, which depends on such external conditions as pH and ionic strength, is identical for all ion species. Activity coefficients of ions are assumed to be unity for simplicity in eq. (3). Using eqs. (2) and (3), eq. (1) can be expressed in terms of the external concentrations of mobile ion species as follows:

$$\sum_i Z_i K^{Z_i} C_i^S - C_S^G/X + KC_H^S C_N^G/\{(KC_H^S + K_N)X\} = 0 \quad (4)$$

We obtained the value of Donnan ratio, K, from eq. (4) with experimental values for C_i^S, C_S^G, C_N^G, and X. In the calculation, we assumed that the dissociation constant of protonated amino groups in DS/CH gel was equal to that of protonated amino groups in chitosan, and hence $pK_N = -\log K_N = 6.3$.[11] With the value of K obtained, the value of pH inside the gel was calculated as $-\log C_H^G = -\log(KC_H^S)$.

Release Experiments

To load the DS/CH gel beads with dextran, 2 g of the gel beads were immersed in 10 mL of an aqueous solution of 1% dextran at 30°C for 24 h. About 1 g of the dextran-loaded gel beads thus obtained were weighed and put into a test solution at 30°C. As the test solution, 170 m*M* NaCl solution containing either 10 m*M* HCl (pH 2) or 20 m*M* Tris-HCl (pH 8) was used. In some experiments, NaCl solutions of different concentrations were also used as the test solution without pH adjustment. The suspension of the gel beads was incubated at 30°C with continuous stirring. Small portions of the solution (0.1 mL each) were withdrawn at appropriate intervals during the incubation to measure the concentration of dextran by the phenol-sulfuric acid method. After 24 h of the incubation, the weight of the gel beads was measured again. The ratio of the final weight to the initial one, w_e/w_i, was taken as a measure of the volume change of the gel beads during the release experiment.

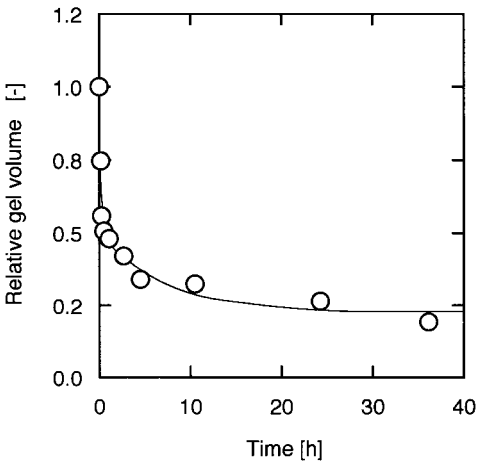

Figure 1 Shrinking of a DS/CH gel bead (S/N = 0.70) immersed in 170 m*M* NaCl solution at pH 8. The initial diameter of the gel bead was 4.2 mm.

RESULTS AND DISCUSSION

Swelling Characteristics of DS/CH Gel Beads

Figure 1 shows a typical course of volume change observed for a DS/CH gel bead (S/N = 0.70). In this case, the gel bead shrank in 170 m*M* NaCl solution, pH of which was adjusted to 8. Although a major part of the volume change occurred in a few hours from the beginning, it took no less than 24 h for the gel bead to reach an equilibrium state.

Figure 2 shows pH dependence of the equilibrium swelling ratio of the DS/CH gel (S/N = 0.70). In the absence of NaCl, the DS/CH gel swelled at around pH 10 and shrank at pHs between 6 and 9. No volume change was observed at pHs below 4 and above 10.5. When NaCl concentration increased, the equilibrium swelling ratio at pHs below 4 decreased because of the increase in osmotic pressure of the ambient solution. The equilibrium swelling ratio at around pH 10 also de-

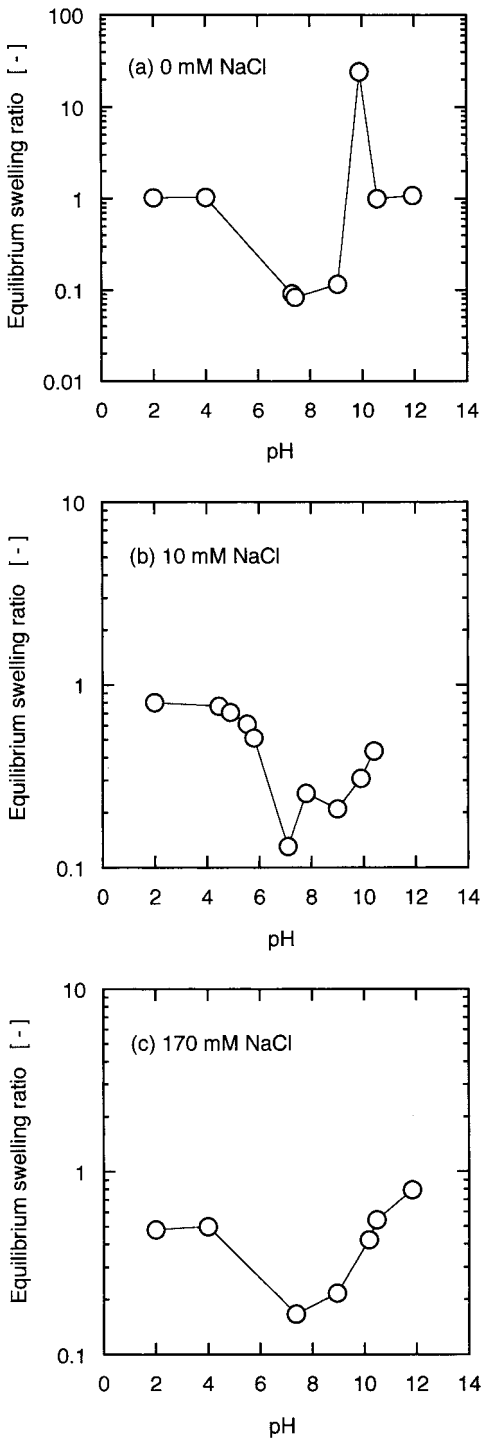

Figure 2 Effect of pH on the equilibrium swelling ratio of the DS/CH gel (S/N = 0.70) under different NaCl concentrations.

creased with the increase in NaCl concentration of the ambient solution. However, the equilibrium volume of the DS/CH gel was significantly small in the range of pH between 6 and 9 even in 170 m*M* NaCl solution.

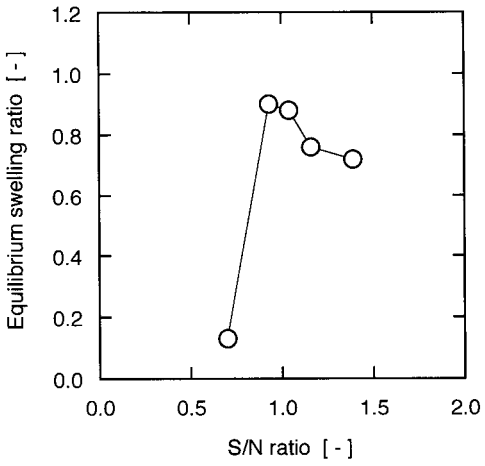

Figure 3 Effect of the S/N ratio on the equilibrium swelling ratio at pH 7 in the presence of 10 m*M* NaCl.

Figure 3 shows the effect of the S/N ratio on the equilibrium swelling ratio in 10 m*M* NaCl solution at pH 7. DS/CH gels with excess sulfate groups (S/N $>$ 1) showed little volume change at pH 7. Considerable shrinking was observed only for the DS/CH gel with excess amino groups. Thus, the shrinking in a neutral pH region can be ascribed to the presence of excess amino groups in DS/CH gels.

Assuming the Donnan equilibrium, the pH value inside the DS/CH gel (S/N = 0.70) was estimated for each swelling experiment performed in 10 m*M* NaCl. The results are shown in Figure 4 as a function of external pH. In general, the internal pH value increased with the increase

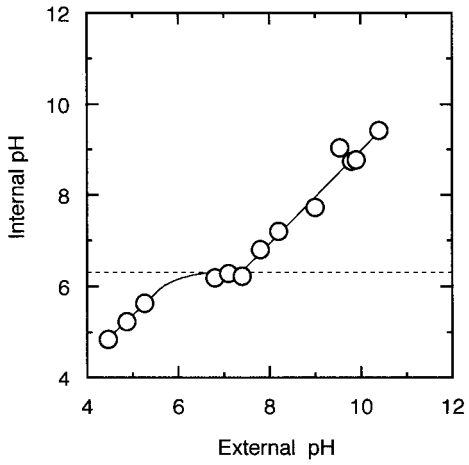

Figure 4 Values of internal pH estimated for the DS/CH gel (S/N = 0.70) immersed in 10 m*M* NaCl solutions of various pH values. The broken line represents the value of pK_N (6.3), where K_N is the dissociation constant for protonated amino groups of chitosan.

in the external pH value. However, the increase in internal pH was much more gradual in the range of external pH between 6 to 7.4 than in any other pH ranges. Furthermore, the internal pH values in this range of external pH were very close to 6.3, the pK_N value for protonated amino groups of chitosan. This suggests that the neutralization of protonated amino groups in the DS/CH gel occurred in this pH range.

Considering the above results, mechanism of pH-sensitive swelling of the DS/CH gel with excess amino groups is explained as illustrated in Figure 5. In the initial state, the sulfate groups in the DS/CH gel are negatively charged and bound electrostatically to protonated amino groups. Because the DS/CH gel has more amino groups than sulfate groups, part of the protonated amino groups remain free from electrostatic interaction with sulfate groups. Thus, the DS/CH gel has net positive charge initially. The presence of counterions for the excess protonated amino groups contributes to osmotic pressure of the DS/CH gel and keeps it in a swollen state. When the DS/CH gel is immersed in a solution of neutral pH, the protonated amino groups remaining free from electrostatic interaction with sulfate groups are deionized because their pK_N value is 6.3.[11] Thus, the net charge decreases, which makes the DS/CH gel shrink. The protonated amino groups bound electrostatically to the sulfate groups may be hard to be deionized because of the presence of negatively charged sulfate groups in their close neighborhood. At higher pHs, even those protonated amino groups can get deionized. Thus, the DS/CH gel becomes negatively charged and swells in a solution of low ionic strength at alkaline pHs.

The DS/CH gel prepared in this study shrank in the vicinity of the neutral pH. The point for

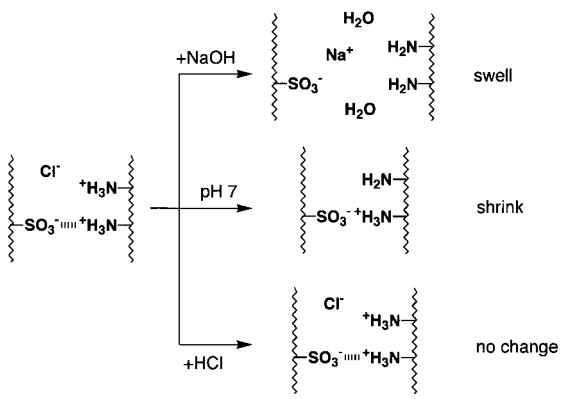

Figure 5 Mechanism for swelling and shrinking of the DS/CH gel with excess amino groups.

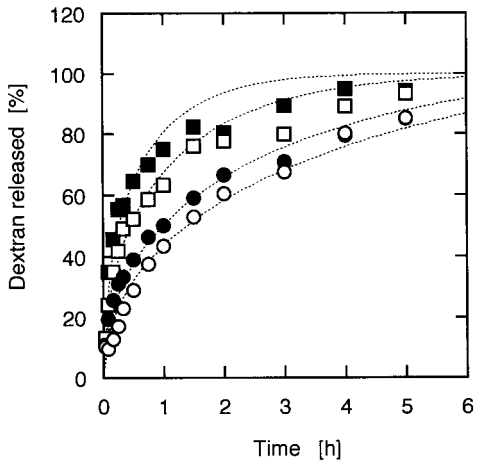

Figure 6 Release profiles of dextran (MW 67,000) from the DS/CH gel (S/N = 0.70) immersed in aqueous NaCl solutions of different concentrations. NaCl concentrations: ○, 0 m*M*; ●, 1.7 m*M*; □, 17 m*M*; ■, 170 m*M*. The dotted curves represent the calculated results with eq. (5) fitted to the initial part of each set of experimental data.

obtaining such a complex gel is to make the molar concentration of the amino group higher than that of the sulfate group. A polyelectrolyte complex can also be obtained by spontaneous precipitation if solutions of oppositely charged polyelectrolytes are mixed without any salt addition. However, it is difficult to control the complex composition by the precipitation method. The preparation procedure used in this study has an advantage to obtaining polyelectrolyte complexes with desired compositions.

Release of Dextran from DS/CH Gel Beads

We used the DS/CH gel at an S/N ratio of 0.70 for the release experiments because it shrank pronouncedly in the vicinity of neutral pH. To study the effect of shrinking of the DS/CH gel on the release of substances loaded, release experiments in aqueous NaCl solutions of different concentrations were performed without a pH adjustment. As a model substance, dextran with the average molecular weight of 67,000 was loaded by immersing the gel beads in an aqueous solution of the dextran. At the end of the loading, concentrations of the dextran inside and outside the gel beads were almost equal (data not shown), suggesting that there was no specific interaction between the dextran and the DS/CH gel. Figure 6 shows the courses of the dextran release from the DS/CH gel immersed in NaCl solutions of different concentrations. The release of dextran be-

Table I Apparent Diffusion Coefficients of Dextran (MW 67,000) Released from the DS/CH Gel (S/N = 0.70) under Various NaCl Concentrations

NaCl [mM]	w_e/w_i[a] [−]	Apparent Diffusion Coefficient, D [10^{-10} m^2/s]
0	1.03	0.16
1.7	0.86	0.29
17	0.51	0.70
170	0.31	1.2

[a] w_e/w_i represents the ratio of the final weight of the gel beads to the initial one.

came more rapid with the increase in NaCl concentration. Ratios of gel weights before and after the release experiment, w_e/w_i, are listed in Table I to show the degrees of shrinking. No shrinking was observed during the release experiment in the absence of NaCl. A high NaCl concentration resulted in a high degree of shrinking because of a high osmotic pressure. Thus, shrinking of the DS/CH gel promoted the release of dextran.

To compare the release rates, a diffusion equation was tried to fit the experimental results. Diffusion of a solute from sphere of a constant radius r into a well-stirred ambient solution can be described by the following equation.[12]

$$\frac{C_t}{C_\infty} = 1 - \sum_{n=1}^{\infty} \frac{6\alpha(\alpha+1)}{9 + 9\alpha + \alpha^2 q_n^2} \exp\left(-Dq_n^2 \frac{t}{r^2}\right) \tag{5}$$

where C_t and C_∞ are the concentrations of solute in the ambient solution at time t and at equilibrium, respectively, α, the ratio of the amount of solute in the ambient solution to that in the gel at equilibrium, D, diffusion coefficient in the gel, and q_n, the nth positive root of the following equation.

$$\tan q_n = \frac{3q_n}{3 + \alpha q_n^2} \tag{6}$$

The dotted curves illustrated in Figure 6 represent the calculated results with eq. (5) using the best-fit values of apparent diffusion coefficient, D. Equation (5) did not fit the entire profile of the experimental data, especially at high NaCl concentrations, probably because of shrinking of the gel beads. Using the initial parts of the experimental data ($C_t/C_\infty < 0.8$), the best-fit values of D were obtained as listed in Table I. Taking the initial mean radius of the gel beads as the value

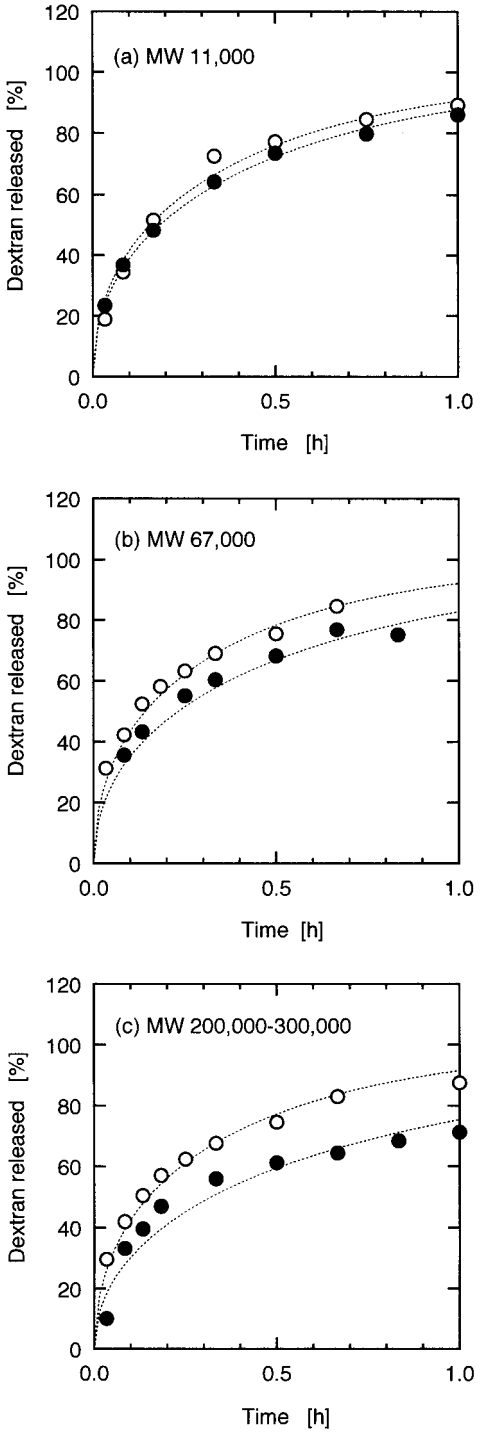

Figure 7 Release profiles of dextrans with different average molecular weights from the DS/CH gel (S/N = 0.70) immersed in 170 mM NaCl solutions at pH 2 (●) and at pH 8 (○). The dotted curves represent the calculated results with eq. (5) fitted to the initial part of each set of experimental data.

Table II Apparent Diffusion Coefficients of Dextrans with Different Average Molecular Weights Released from the DS/CH Gel (S/N = 0.70) Immersed in 170 m*M* NaCl Solution at pHs 2 and 8

		Apparent Diffusion Coefficient, D [10^{-10} m^2/s]		
pH	w_e/w_i[a] [−]	MW 11,000	MW 67,000	MW 200,000–300,000
2	0.28	2.2	1.7	1.2
8	0.11	2.6	2.8	2.6

[a] w_e/w_i represents the ratio of the final weight of the gel beads to the initial one.

of r, the initial courses of dextran release were well approximated by eq. (5) despite of decreasing radius of the gel beads. In the final stage of the release courses, deviation between the experimental and calculated values was observed especially at high NaCl concentrations, indicating retardation of dextran diffusion in the gel beads. This was probably due to formation of dense layer through shrinking. According to a correlation reported in literature,[13] the diffusion coefficient of dextran in water at 30°C is estimated to be 0.44×10^{-10} m^2/s assuming the molecular weight to be 67,000. It is reasonable that the value of D in the absence of NaCl was smaller than this value, considering the effect of steric hindrance of a gel matrix. As shown in Table I, the value of D increased with the increase in NaCl concentration and in the shrinking degree. The value of D in the presence of 170 m*M* NaCl was about eight times as large as that in the absence of NaCl. Thus, the shrinking of the DS/CH gel was shown to have a promotion effect on the release of dextran.

Release experiments using the DS/CH gel (S/N = 0.70) were performed at different pHs in the presence of 170 m*M* NaCl. Figure 7 shows the courses of release experiments for dextrans with different average molecular weights performed at pHs 2 and 8. The dotted curves illustrated in Figure 7 represent the calculated results with eq. (5) fitted to the initial parts of experimental data. The initial mean radius of the gel beads was taken as the value of r in each experiment. The values of D obtained are listed in Table II together with the values of w_e/w_i at the both pHs. Although the DS/CH gel shrank at the both pHs due to high osmotic pressure of the ambient solution, the degree of shrinking was higher at pH 8 than at pH 2. For the dextran with an average molecular weight of 67,000, the values of D at the both pHs exceeded 0.16×10^{-10} m^2/s, the D value obtained for gel beads immersed in deionized water (Table I). Thus, the shrinking of the DS/CH gel promoted the release of dextran at the both pHs. The release profiles at the two pHs were similar for the dextran with an average molecular weight of 11,000. However, the dextrans with higher average molecular weights were released more rapidly at pH 8 than at pH 2 in accordance with the degree of shrinking. The difference between D values at the two pHs became larger, although not drastically, as the average molecular weight of the dextran increased. Thus, for dextrans with high average molecular weights, the promotion effect caused by shrinking of the DS/CH gel was larger at pH 8 than at pH 2.

As can be seen from Table II, the D value was approximately constant at pH 8 irrespective of average molecular weight of dextran, whereas the D value obtained at pH 2 decreased with increasing average molecular weight. This suggests that the promotion effect caused by shrinking of the DS/CH gel was dominant in the kinetics of dextran release at pH 8. Contribution of molecular diffusion may be significant at pH 2, especially for the dextran with an average molecular weight of 11,000. According to a correlation reported in literature,[13] the diffusion coefficient of dextran in water at 30°C is estimated to be 1.1×10^{-10} m^2/s assuming the molecular weight to be 11,000. This value of the diffusion coefficient amounts to a half of the D value obtained at pH 2, suggesting significance of molecular diffusion in the release kinetics of dextran with an average molecular weight of 11,000.

CONCLUSIONS

A DS/CH gel containing more amino groups than sulfate groups was shown to shrink in the vicinity of neutral pH. The shrinking behavior was well characterized in terms of Donnan equilibrium. The analysis indicated that the shrinking in the vicinity of neutral pH was ascribed to the deionization of the protonated amino groups remaining free from electrostatic interaction with the sulfate

groups. Shrinking of the DS/CH gel beads had a promotion effect on the release of dextran, which had been loaded into the gel beads by absorption. The promotion effect was pH dependent for dextrans with high molecular weights: the release was more rapid at pH 8 than at pH 2 because of the higher degree of shrinking at pH 8. However, the release rates at the two pHs became closer as the average molecular weight of dextran was lowered.

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