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Concentration of Proteinaceous Solutions with Superabsorbent Hydrogels

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ABSTRACT

A process is investigated quantitatively for concentrating dilute aqueous bovine serum albumin solutions with superabsorbent crosslinked hydrogels. In the process, the gel swells by absorbing water only while macromolecules are excluded, thus producing a concentrated retentate. The concentration of the retentate is about 5.8 times larger than that of the feed for a dosage of dry gels of 0.63% by weight. The dynamic behavior of the concentration process is described by using the equation of motion of the gel network on the basis of the kinetics of the swelling of the gel. The pore size of the hydrogen controlling the sieving property is estimated from measurements of the permeation rate of water through the compressed, packed bed of the swelling gels, known as the compression–permeability test. It is demonstrated that slight stirring during the concentration process is accompanied by a rapid removal of the BSA filter cake on the gel surface, leading to efficient concentration.

INTRODUCTION

It is generally recognized that separation processes of proteinaceous solutions play a vital role in the fields of biotechnology, biomedicine, and food and beverage processing. Traditionally, a wide variety of separation techniques are used for concentrating the dilute proteinaceous solutions. One promising technique involves the use of hydrophilic polymer gels. In the technique, dry gel particles are added to a dilute aqueous solution containing biological macromolecules. The gel swells by absorbing water only; i.e., it leaves macromolecules outside the particles. This technique would be desirable for large-scale operation because it may provide a rapid, inexpensive method of concentrating dilute aqueous solutions. Moreover, the method does not require any special device, and it is espe-

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cially effective for the concentration of labile solutes such as proteins because it is a mild operation. Such a process was originally developed by Flodin et al. (1). Since then, the process has been further refined by a number of researchers (2–6).

However, the water-absorbing capacity of the gel used in previous works (1–6) was relatively poor. In recent years, superabsorbent cross-linked hydrogels which display a drastic change in volume by absorbing several hundred times their own weight of water while still remaining insoluble have been developed, and they are mainly employed in sanitary supplies such as baby diapers. The use of such superabsorbent hydrogels should be beneficial for concentrating dilute proteinaceous solutions efficiently.

Concentration of proteinaceous solutions with hydrogels is regarded as a kind of ultrafiltration process. In this separation process, the available driving force of separation is the osmotic pressure difference between the inside and outside of the gel. The network of the gel acts as the membrane which separates the protein solutes from the solvent. Thus, the relationship between the molecular size and shape of the solute and the pore size of the network of the hydrogel is of central importance in determining the sieving property.

The objectives of this investigation are to describe quantitatively the concentration behavior of proteinaceous solutions with superabsorbent hydrogels. The kinetics of the concentration process is examined by describing the swelling behavior of the gels. A method has been exploited to determine the pore size of the network of the gel. Furthermore, the effects of stirring are especially examined as one of many factors contributing to the concentration property.

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EXPERIMENTAL

The superabsorbent gel used in this research is a commercially available spherical gel (Dia Wet A III, Mitsubishi Petrochemical Corp.) of cross-linked sodium polyacrylate. After the dry gel particles were sieved by using a Rotap sieve shaker, the gels with a mean particle diameter of 238 μm were employed in the experiments. The biological macrosolute studied was bovine serum albumin (BSA) (Fraction V powder, Katayama Chemical Ind. Corp.) with a molecular weight of ~67,000 Dalton and an isoelectric/isoionic pH of ~5.1. The solutions were prepared by dissolving a weighed amount of BSA powder in a specified weight of water prepared by a ultrapure water system for laboratory use (Puric-R, Olgano Corp.) with gentle agitation for a sufficient time (~2 hours for all runs) to insure homogeneity at a solute concentration of 5×10^{-4} by weight.

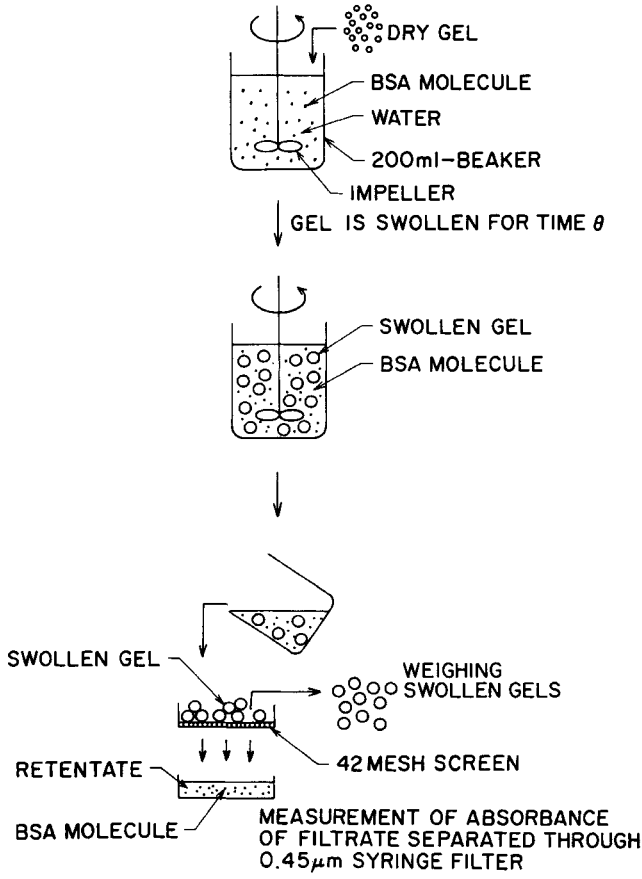

FIG. 1 Schematic diagram of concentration process.

The experimental procedure is shown schematically in Fig. 1. A known amount of the dry gels is added to a known amount of feed solution (about 100 g) in a 200-mL beaker, and they are stirred for various time periods under a constant impeller rotational speed using the standard jar test apparatus that accommodates an impeller of the turbine type (Irie Shokai Corp.). The gel swells by absorbing water selectively while large molecules are prevented from entering the gel phase. As a consequence, the solution is gradually concentrated. The swollen gel particles are separated from the concentrated solution with a 42 mesh stainless steel screen. The retentate is then filtered through a syringe filter with a pore size of 0.45 μm in order to remove any impurities. The solute concentrations of the

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filtrate are determined spectrophotometrically by reading the absorbance at a wavelength of 280 nm. The concentration ratio s/s_0, defined as the ratio of the concentration of the concentrate to that of the feed, can be calculated. The total amount of swollen gel particles is measured with an electronic balance. The mass of water absorbed by the gels can be calculated by subtracting the mass of the dry gels from that of the swollen gels. If the gel absorbs water only, then the concentration ratio s/s_0 can be evaluated from

$$s/s_0 = W/(W - W_w) \tag{1}$$

where W is the mass of the feed, and W_w is the mass of water absorbed by the gels.

EFFECT OF DOSAGE OF GEL

Figure 2 shows a plot of the concentration ratio s/s_0 with respect to the mass fraction s_{g0} of the dry gels added to the solution. The swelling time θ is 20 min, and this time is enough to reach the equilibrium condition,

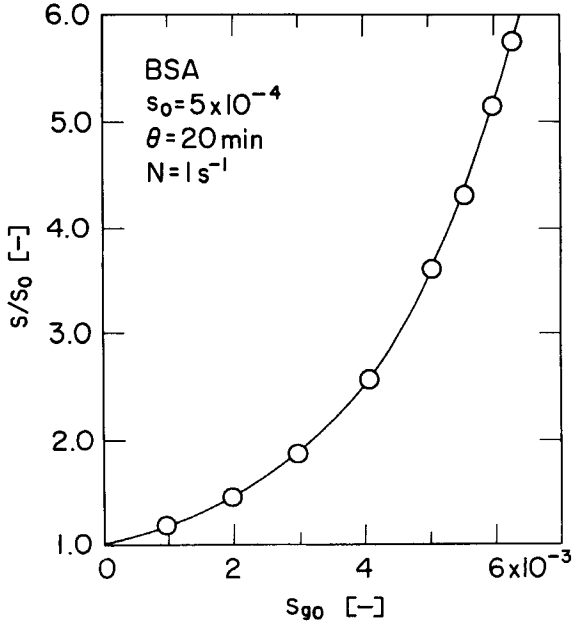

FIG. 2 Effect of dosage of gel on concentration ratio.

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as mentioned below. The degree of the increase in the concentration ratio is observed to become more marked with an increase in the dosage of the gels. As an example, an increase in the concentration of the solutions by a factor of about 5.8 was attained for the dosage of the gels of 0.63% by weight. If the amount of the gels is significant enough to absorb all the solvent in the solutions, the concentration ratio approaches infinity theoretically. However, in practice, as the dosage of the gels increases, separation efficiency becomes low because of the difficulty in separating the small amount of the concentrate entrained among the swollen gel particles.

Assuming that the gel absorbs water only, the mass W_w of water absorbed by the gels can be calculated from the variation of the absorbance of the solution by using Eq. (1). In Fig. 3 the quantity W_w is plotted against the mass W_{g0} of the dry gels. The results show that the gel absorbed water, swelling about 160 times its dry weight, if the amount of gel is not too large. The plot is a concave curve. It appears that swelling of the gel is reduced by mutual contact among the gel particles as W_{g0} increases.

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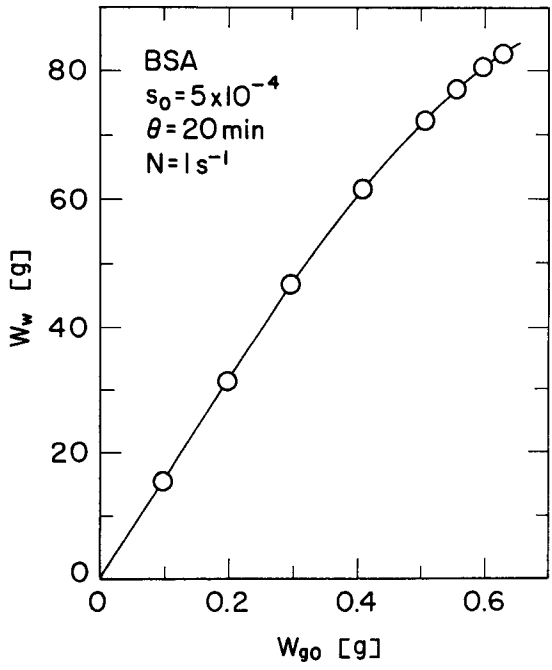

FIG. 3 Effect of dosage of gel on mass of water absorbed by gel.

KINETICS OF CONCENTRATION PROCESS

In the concentration process, the gel absorbs water, producing the concentrated solution. Therefore, it is very important to describe the kinetics of a change of state of the gel in order to establish the optimum operation time required for the concentration process to reach steady-state.

The variations of the concentration ratio s/s_0 with time are shown in Fig. 4. The real concentration ratio obtained from the variation of the absorbance of the solution is compared with that evaluated from the variation of the mass of the gel particles assuming that the gel absorbs water only. Figure 4 indicates that the gel used in this research reaches the equilibrium condition at ~8 minutes. The concentration ratio based on the mass of the gel particles was consistently overestimated until the gels reached the equilibrium condition. This is primarily because the gel swells while water among the gels is removed. However, both values are identical in equilibrium within experimental error. Therefore, it seems reasonable to assume that BSA molecules are almost completely excluded from the gel. The time-dependent behavior of the mass W_w of water absorbed by

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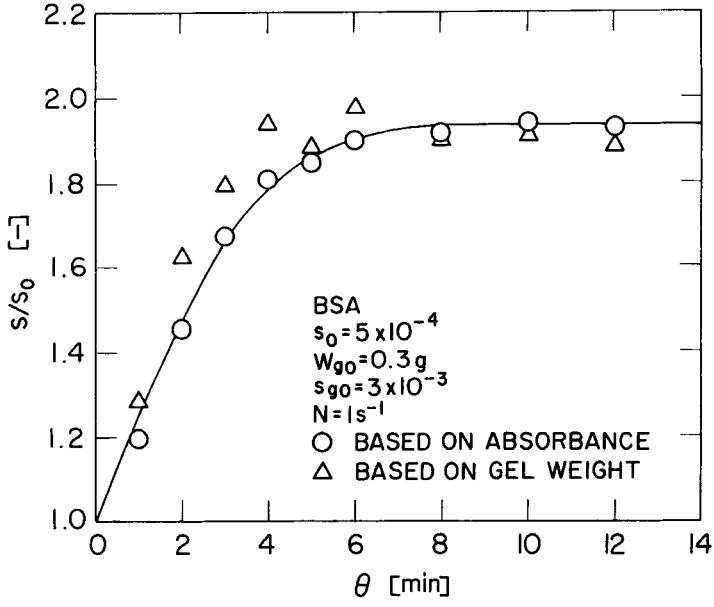

FIG. 4 Time-dependent behavior of concentration ratio.

the gels is shown in Fig. 5 on the basis of the experimental results shown in Fig. 4.

The swelling behavior of the gel in the BSA solution and in pure water is compared in Fig. 6. The data values were identical within the limits of experimental accuracy, indicating that the presence of BSA molecules has a negligible influence on both the swelling rate and the swelling capacity of the gel.

Tanaka et al. (7–10) recently presented a theory describing the kinetics of the swelling of gel. They imagined that a gel consists of a crosslinked polymer network immersed in a fluid. In order to describe the kinetics of the swelling of the spherical gel, they introduced a displacement vector $u(r, \theta)$ which represents the displacement of a point r in the fiber network from its final equilibrium location at time θ. The magnitude of the displacement may be expressed as

$$\frac{\partial u}{\partial \theta} = D\frac{\partial}{\partial r}\left\{\frac{1}{r^2}\left[\frac{\partial}{\partial r}(r^2 u)\right]\right\} \tag{2}$$

where D is the diffusion coefficient of the gel, and it is defined as the ratio of the bulk modulus K of the polymer network to the friction coefficient

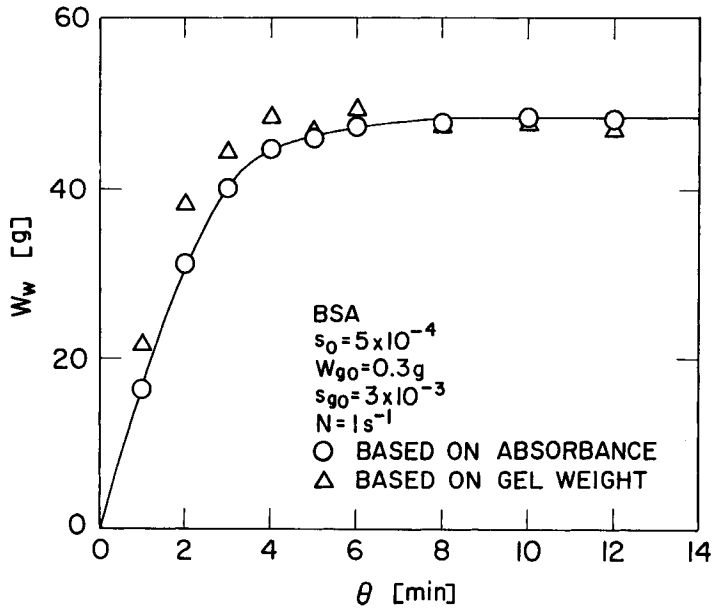

FIG. 5 Time-dependent behavior of mass of water absorbed by gel.

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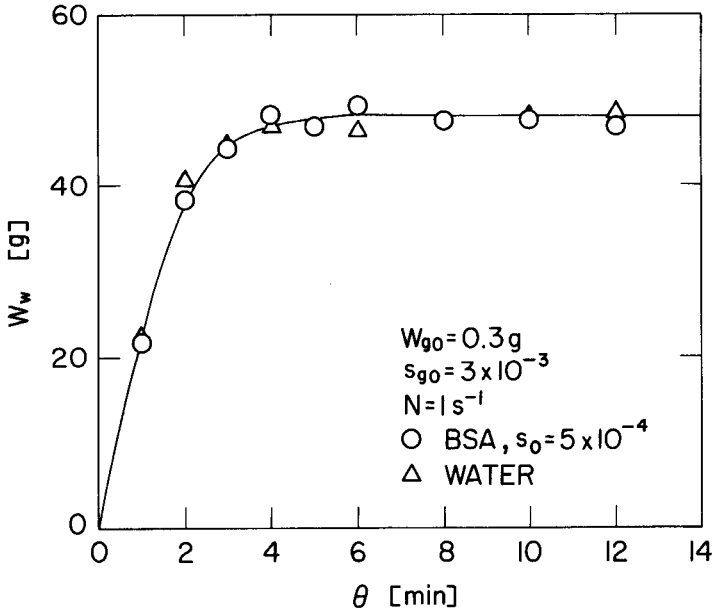

FIG. 6 Effect of solution on mass of water absorbed by gel.

f between the network and the solvent. The boundary condition and the initial condition may be respectively given by

$$\sigma_{rr} = (K/r^2)[\partial(r^2u)/\partial r] = 0 \quad \text{at} \quad r = a \tag{3}$$

$$u(r, 0) = \Delta a_0(r/a) \quad \text{at} \quad \theta = 0 \tag{4}$$

where σ_{rr} is the stress in the radial direction, a is the final radius of the gel sphere in equilibrium with the surrounding solvent, and Δa_0 is the total increase in the radius of the sphere in the entire swelling process. On the basis of the solutions of Eqs. (2)–(4), the variation of the radius of the gel with time during the swelling process can be completely described by

$$\frac{\Delta a(\theta)}{\Delta a_0} = \frac{6}{\pi^2} \sum n^{-2} \exp(-n^2\theta/\theta_s) \tag{5}$$

where $\Delta a(\theta)$ is the increase in the radius of the gel from time θ to equilibrium, and θ_s is the characteristic time of swelling, defined as

$$\theta_s = a^2/D \tag{6}$$

It is apparent from Eq. (5) that the ratio $\Delta a(\theta)/\Delta a_0$ is a function solely of θ/θ_s. This implies that if time θ is scaled by θ_s, the swelling pattern is

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always the same for any spherical gel. Furthermore, examination of Eqs. (5) and (6) reveals that the swelling rate is proportional to the diffusion coefficient D of the network and to the reciprocal square of the gel radius a. As a result, smaller gels reach steady-state more quickly.

In order to facilitate the measurements in the concentration process with the gel, it is convenient to discuss the variations of the net mass of the gel particles rather than the variations of the radius of each gel (11). Thus, on the condition that $[\Delta W_0 - \Delta W(\theta)] \gg W_{g0}$, rewriting the left-hand side of Eq. (5) yields

$$\frac{(\Delta W_0)^{1/3} - (\Delta W_0 - \Delta W(\theta))^{1/3}}{(\Delta W_0)^{1/3} - (\rho W_{g0}/\rho_g)^{1/3}} (\equiv M) = \frac{6}{\pi^2} \sum n^{-2} \exp(-n^2\theta/\theta_s) \quad (7)$$

where $\Delta W(\theta)$ is the increase in the mass of the gel from time θ to equilibrium, ΔW_0 is the total increase in the mass of the gel in the entire concentration process, ρ is the density of the solvent (water), and ρ_g is the density of the dry gel. The derivation of the above equation is based on the assumption that the gel particles are spheres of uniform size.

The quantity M, defined in Eq. (7), is plotted against time θ on a semilog scale as shown in Fig. 7. The curve becomes substantially linear except

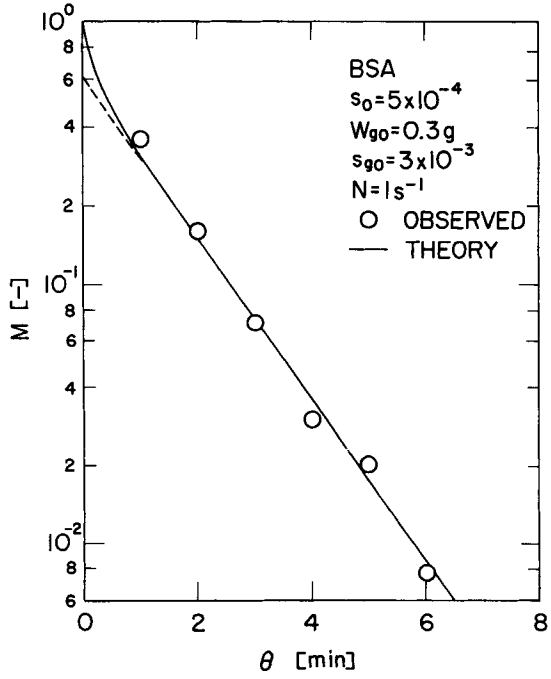

FIG. 7 Plot based upon Eq. (7).

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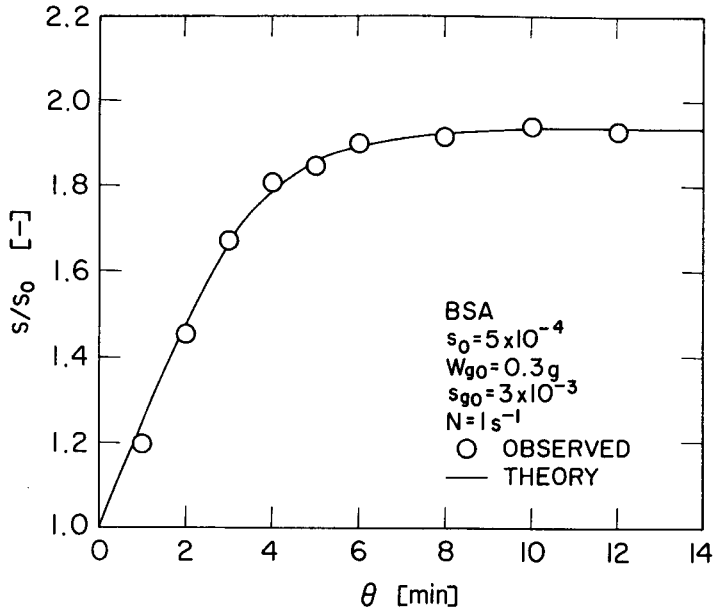

FIG. 8 Estimation of time-dependent behavior of concentration ratio.

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for the initial period of the concentration process, in accordance with the fact that, for $\theta/\theta_s > 0.25$, Eq. (7) can be approximated by

$$M = \frac{6}{\pi^2} \exp(-\theta/\theta_s) \tag{8}$$

Hence, it is possible to determine θ_s directly from the slope of the line. In Fig. 8, the experimental values of the concentration ratio s/s_0 are compared with the calculations based on Eq. (7) using the value of θ_s.

PORE SIZE OF NETWORK OF GEL

From a comparison between the variation of the absorbance of the solution and the variation of the mass of the gel particles during the concentration experiment, it is concluded that the gel absorbs water only while the BSA molecules are excluded. The mechanism of separation can be elucidated by imagining that the hydrogel is an expanded mesh. Small molecules, such as water, can easily penetrate the gaps in the mesh, but macromolecules, such as BSA, cannot enter. The relationship between the molecular size and shape of the solute and the pore size of the hydrogel

is likely to be the principal determinant of this size-selective separation. Therefore, it is necessary to know the pore size of the gel network in order to evaluate the ability of the gel to sieve the macromolecules.

A variety of methods have been developed for determining the pore size of the gel network (5, 12). In this paper, an innovative method is proposed. In this method, the pore size is determined on the basis of the data of the permeation rate of water through the compressed bed of gel particles. These data may be obtained by using the compression–permeability cell (13–17). The apparatus employed essentially consists of a container in which the swollen gel particles can be placed and subjected to vertical loading, as shown in Fig. 9. In the swollen state, the gel is placed between perforated plates with filter papers and kept saturated with water while the load is applied. At the instant the load is added, the stress is applied to both the gel particles and the liquid within the interstices. The liquid is free to flow through the porous plates, resulting in a consolidation or shrinking of the gel particles. The instant the swelling pressure of the gel particles becomes equal to the applied pressure, the liquid ceases to flow and the consolidation reaches equilibration. The equilibrium value of the porosity ϵ can be determined from the measurements of the equilibrium

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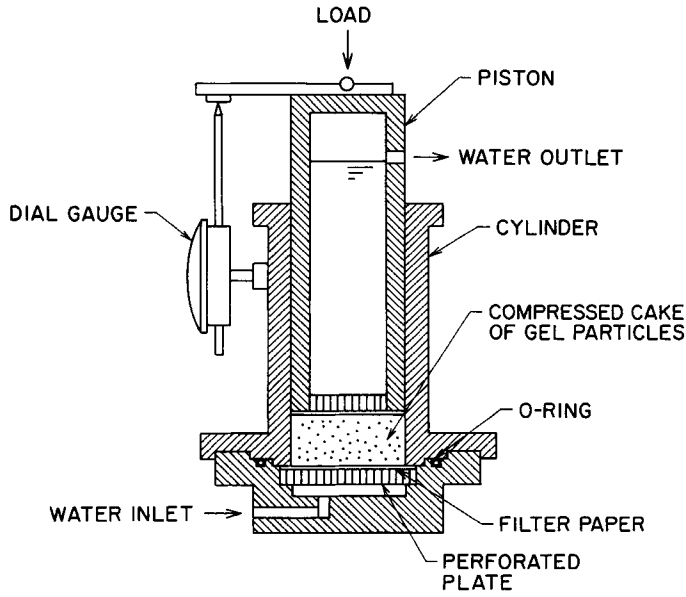

FIG. 9 Compression–permeability cell.

thickness L of the compressed cake layer of the gel particles by

$$\epsilon = 1 - W_0/(L\rho_g) \tag{9}$$

where W_0 is the mass of the dry gel particles of the entire cake per unit area. The water is allowed to flow through the compressed cake under a relatively small pressure p. The specific flow resistance α may be calculated by

$$\alpha = p/(\mu u_1 W_0) \tag{10}$$

where μ is the viscosity of water and u_1 is the permeation rate.

The relation between the specific flow resistance α and the porosity ϵ measured under various load conditions, known as compression–permeability data, is shown in Fig. 10. Interestingly, the compression process of the randomly packed bed composed of gel particles may be divided into two stages (18). They can be represented by straight lines connecting

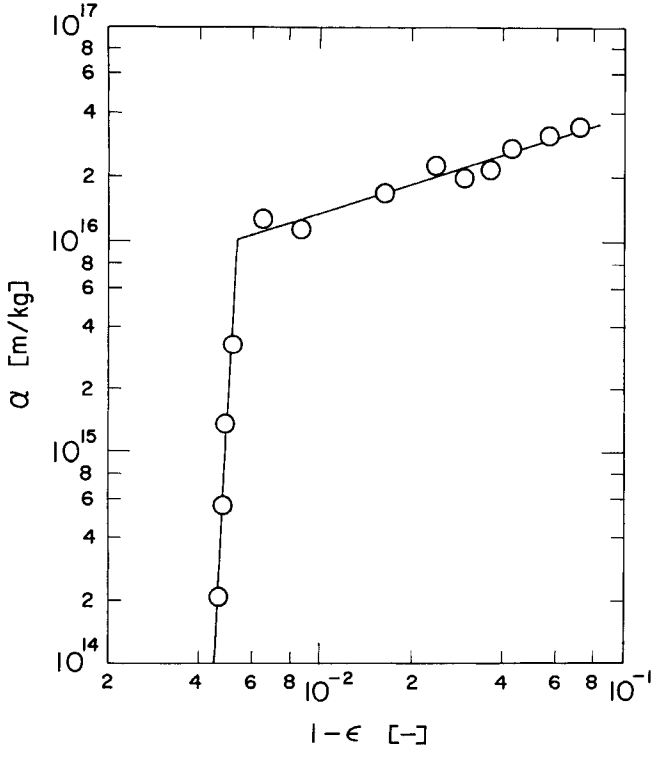

FIG. 10 Relation between flow resistance and porosity.

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the different slopes. Under relatively low load conditions, which result in large porosity, the slope is steep. In this first stage, it is mainly the water among the gel particles that is allowed to drain. All the water among the gel particles may be removed at the transition point at which the slope of the line changes. In the second stage under higher load conditions, the water inside gel particles may be removed, resulting in shrinking of the gel particles; the slope of the line becomes relatively gentle. As a fair approximation of compression–permeability data, the functional relation between α and ϵ in each part of the compression process can be represented by

$$\alpha = \alpha_0(1 - \epsilon)^{\beta} \tag{11}$$

where α_0 and β are the empirical constants. According to the classic Kozeny–Carman equation describing the flow of fluids through a randomly packed, porous bed of granular particles, the relation between specific flow resistance α and porosity ϵ may be written as

$$\alpha = \frac{kS_0^2(1 - \epsilon)}{\rho_s\epsilon^3} \tag{12}$$

where S_0 is the effective specific surface of the gel particles comprising the bed. The quantity k is Kozeny's constant, and the value of 5 appears to be a fair average for beds of granular material. The diameter d_m of the actual flow path of the bed of compressed gel particles is related to both the specific surface S_0 and the porosity ϵ by

$$d_m = \frac{4\epsilon}{S_0(1 - \epsilon)} \tag{13}$$

The pore size d_m in the above equation represents the average value since there is a distribution of crosslinks in the gel. Substituting Eqs. (11) and (12) into Eq. (13) results in

$$d_m = 4\sqrt{\frac{k}{\rho_s\epsilon\alpha_0(1 - \epsilon)^{1+\beta}}} \tag{14}$$

Equation (14) can be used to predict the pore size d_m of the network of the gel at any time during swelling provided that the values of α_0 and β and the value of the porosity ϵ of the gel at that time are known. In Fig. 11, the pore size d_m of the network of the gel is plotted against the swelling ratio R. In this paper, the swelling ratio is defined as the ratio of swollen mass to the original unswollen mass of the gel. The pore size d_m increases as the gel swells. The pore sizes are 3.1 and 37.4 nm at swelling ratios of 5 and 160 (equilibrium condition), respectively. Highly swollen gels are

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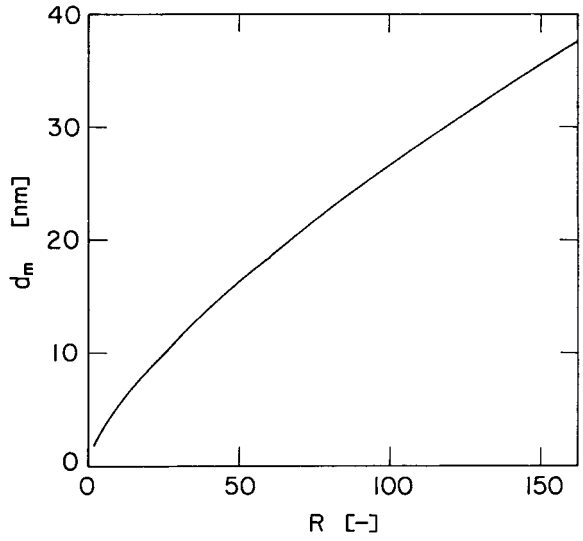

FIG. 11 Relation between pore size of network of gel and swelling ratio.

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expected to exhibit decreased exclusion of macromolecules. The BSA molecule is regarded as the prolate (rod-shaped) ellipsoid of revolution with a major axis of 14 nm and a minor axis of 4 nm (19). When the swelling ratio is small, the BSA molecules are excluded by the mesh of the gel. Since the gel swells water, the filter cake of the BSA molecules builds up on the gel surface. This filter cake alters the solute rejection characteristics of the gel (20, 21), and the gel excludes any macrosolute smaller than the pore size of the network of the gel. Therefore, the BSA molecules are excluded by the cake overlying the gel surface when the swelling ratio becomes large. Moreover, the negatively charged BSA molecules in the solution environment encountered in this study are excluded because of the fixed negative charges on the polymer backbone of the gel.

EFFECT OF STIRRING

In order to examine the effect of stirring on the concentration efficiency, the concentration experiments were conducted for various impeller rotational speeds. In Fig. 12, both the concentration ratio s/s_0 and the mass W_w of water absorbed by the gel particles are plotted against the rotational speed N. As can be seen, the value of W_w was little influenced by the rotational speed. Of particular importance is the surprising observation

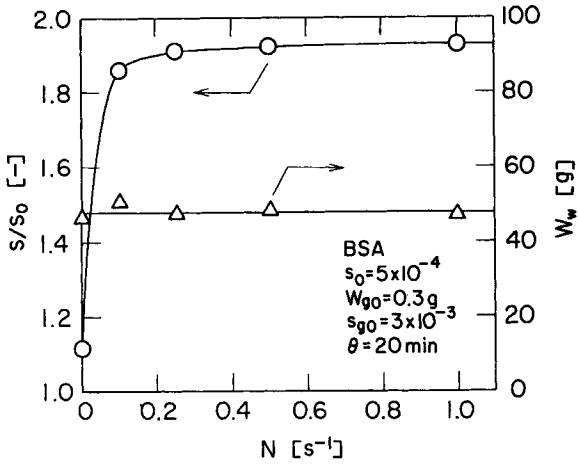

FIG. 12 Effect of stirring on concentration ratio and mass of water absorbed by gel.

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that the concentration ratio shows an extremely low value when the solution is not stirred. The concentration process of proteinaceous solutions with the hydrogel is regarded as a kind of ultrafiltration. It is expected that a filter cake of BSA molecules accumulates on the surface of the gel in the absence of stirring as the gel particles swell in proteinaceous solutions (22). As a result, the retentate is not extensively concentrated. As previously described (23, 24), we have been developing an upward ultrafiltration technique in which the filtrate flow is in the direction opposite to gravity. In this technique the filter cake formed on the membrane is easily exfoliated by the gravitational force acting on the protein molecules without employing the crossflow or stirred technique or any other energy-consuming techniques (25, 26). This finding leads to the surprising conclusion that, in general, much of the filter cake in ultrafiltration is quite easily displaced by a slight shear, despite evidence that such a cake is essentially nondiffusible under stagnant conditions. For this reason, the concentration ratio shows a high value when the solution is slightly agitated.

CONCLUSIONS

Concentration experiments of dilute BSA solutions were conducted with superabsorbent crosslinked hydrogels exhibiting a drastic change in volume by absorbing water only. Dilute proteinaceous solutions can be effectively concentrated with superabsorbent hydrogels. The degree of increase in the concentration ratio of the solution becomes marked as the

dosage of the gel is increased. Variations of the concentration ratio with time were successfully predicted on the basis of the theory of the kinetics of swelling of the spherical gel. The pore size of the network of the gel was simulated by employing the compression–permeability technique. It was found that a considerable amount of the filter cake formed on the surface of the gel was easily exfoliated by slight stirring.

NOMENCLATURE

a	final radius of gel sphere in equilibrium with surrounding solvent (m)
D	diffusion coefficient of gel (m^2/s)
d_m	diameter of actual flow path of bed of compressed gel particles (m)
f	friction coefficient between network and solvent ($kg/m^3 \cdot s$))
K	bulk modulus of polymer network (Pa)
k	Kozeny's constant
L	equilibrium thickness of compressed cake layer of gel particles (m)
M	value defined in Eq. (7)
N	rotational speed (s^{-1})
p	pressure (Pa)
R	ratio of swollen mass to original mass
r	radius (m)
S_0	effective specific surface of gel particles (m^{-1})
s	concentration of concentrated solution
s_0	concentration of feed
s_{g0}	mass fraction of dry gel added to solution
u	displacement vector (m)
u_1	permeation rate (m/s)
W	mass of feed (kg)
W_{g0}	mass of dry gel (kg)
W_w	mass of water absorbed by gel (kg)
W_0	mass of dry gel particles of entire cake per unit area (kg/m^2)

Greek

α_0	empirical constant in Eq. (11) (m/kg)
α	specific flow resistance (m/kg)
β	empirical constant in Eq. (11)
Δa_0	total increase in radius of sphere in entire process of swelling (m)

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$\Delta a(\theta)$	increase in radius of gel from time θ to equilibrium (m)
ΔW_0	total increase in mass of gel in entire process of concentration (kg)
$\Delta W(\theta)$	increase in mass of gel from time θ to equilibrium (kg)
ϵ	porosity
θ	time (s)
θ_s	characteristic time of swelling (s)
μ	viscosity of water (Pa·s)
ρ	density of solvent (kg/m^3)
ρ_g	apparent density of dry gel (kg/m^3)
σ_{rr}	stress in radial direction (Pa)
ω_0	mass of dry gel particles of entire cake per unit area (m)

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