Diffusive Permeability of Ionic Solutes in Charged Chitosan Membrane

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ABSTRACT: The permeabilities of three kinds of solutes with similar sizes such as anionic benzenesulfonic acid, neutral styrene glycol, and cationic theophylline in the chitosan membrane were investigated. The chitosan membrane becomes cationic below its pKa value. Benzenesulfonic acid showed the highest permeability, whereas theophylline showed the lowest, although these solutes have almost the same size. This can be explained by the electrostatic attraction or repulsion between the solute and the membrane instead of the size exclusion effect. The permeabilities of benzenesulfonic acid and theophylline increased and decreased, respectively, with the decrease of pH from 7.4 to 4.0 because of the increase of the charge density of the membrane. Thus, the selectivity of benzenesulfonic acid to theophylline increased and reached about 30 at pH 4.0, in contrast to the selectivity of nearly unity in poly(vinyl alcohol) (PVA) membrane. The partition coefficients for three solutes showed the similar tendencies to those in the permeabilities. Contrary to the results of the permeabilities, the partition coefficients increased with the increase of the degree of the crosslinking in the membrane. This is probably due to the increase of the electrostatic interaction between the solute and the membrane brought about by the smaller mesh size and also due to the increase of the bound water fraction in the membrane. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 72: 397-404, 1999

Key words: chitosan membrane; electrostatic interaction; partition coefficient; benzenesulfonic acid; styrene glycol; theophylline

INTRODUCTION

Hydrogel membranes with highly hydrophilic nature have high biocompatibility and low fouling potential. The hydrogel membranes have been applied to biochemical and biomedical separations such as plasmapheresis and drug release. For example, poly(vinyl alcohol) (PVA) gel membrane developed by Kuraray Co., Ltd. (Kurashiki, Japan) for plasmapheresis showed high hydraulic permeability and plasma flux.¹

Numerous studies have been reported on diffusive permeabilities of solutes in PVA gel membrane.^{2–11} The targeted solutes ranged in sizes from a small molecule to proteins. The permselectivity of the PVA gel membrane is generally attributable to only the size exclusion of solutes. Therefore, the sizes of the network meshes of PVA membrane and solute molecular size and shape are important factors in determining selectivity and permeability. The effective separation of two solutes with the similar molecular size is essentially impossible in PVA gel membrane.

On the other hand, ionized hydrogel membrane offers the valuable solute separation based on the electrostatic interaction between the gel membrane and the solute as well as the size exclusion effect when ionic solute is used. This enable solutes with similar sizes to be separated from each

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other. Miya et al. prepared charged PVA membrane and measured permeabilities of amino acids.¹² The experimental results suggested that amino acids might be separated owing to the difference in charges as well as the differences in the molecular sizes. Gudeman and Peppas prepared charged gel membranes with varying degrees of crosslinking and ionic content from interpenetrating networks of PVA and poly(acrylic acid).¹³ Permeation studies demonstrated that for ionic solutes such as tryptophan and urea, the diffusion coefficient depended on pH and was smaller at a pH of 3 than at a pH of 6. Uragami et al. investigated the transport of adenine and uracil through charged gel membrane prepared from PVA and poly(styrenesulfonic acid).¹⁴ These two solutes were transported against their concentration gradients. It was found that adenine and uracil molecules can be transported in the opposite direction and separated from each other.

Chitosan, derived from chitin by deacetylation, is a unique basic polysaccharide. Chitosan hydrogel has some advantages of nontoxicity, biocompatibility, and biodegradability.⁶ The value of pKa of chitosan was reported to be 6.3.¹⁵ Therefore, chitosan hydrogel membrane can become a cationic membrane in the low pH condition. In this work, the transport of three kinds of solutes with the similar molecular sizes through the charged chitosan membrane was investigated. The solute used was an anionic solute (benzenesulfonic acid), a cationic solute (theophylline), and a neutral solute (styrene glycol). Solute permeabilities and partition coefficients were measured in various pH conditions and the results are discussed based on the charged conditions of the chitosan membrane and the solutes.

EXPERIMENTAL

Materials

Chitosan was kindly supplied by Katokichi Co., Ltd. (Japan) and its deacetylation degree was about 100 mol %. Glutaraldehyde (50% aqueous solution, Aldrich Chemical Co.) was used as a chemical crosslinking agent.

The solutes used were the ophylline (M_w : 180), benzenesulfonic acid (M_w : 158), and styrene glycol (M_w : 138). These were purchased from Nacalai Tesque, Inc (Japan). The chemical structures of the solutes are shown in Figure 1. The values of pKa of benzenesulfonic acid and the oph-



Figure 1 Chemical structures of three kinds of solutes.

ylline are reported as -2.7 and 8.77, respectively.¹⁶ Thus, benzenesulfonic acid and theophylline are fully anionic and cationic in the pH range of 4.0-7.4 used in this work. On the other hand, styrene glycol is a neutral species. These three solutes have similar molecular weights, whereas the charged conditions are different from each other.

Preparation of Chitosan Membrane

Chitosan solution was prepared by dissolving chitosan in 10 wt % aqueous acetic acid solution at ambient temperature with stirring overnight. The mass fraction of chitosan was 0.04. The solution was allowed to stand for about half a day to remove the air bubbles. The solution was then cast onto a glass plate of desired thickness (1000 μ m) and placed in a drying oven at 60°C for 20 h. The dry film was immersed in a gelating agent such as 1N aqueous sodium hydroxide for 3 h. The chitosan membrane was washed repeatedly with water to remove all traces of alkali.

The resultant membrane was placed in various aqueous glutaraldehyde solutions (0.01, 0.1, and 0.2 wt %) at 25°C for 20 h for chemical crosslinking. The reaction leading to the crosslinked form in chitosan membrane was described in the literature.⁶

DSC Measurement

A Seiko 220 thermal analyzer was used for the melting measurement. A membrane sample equilibrated at 25°C in water with a pH of 6.0 was sealed in an aluminum pan and cooled down with liquid nitrogen to -70°C in the DSC cell. The cell was slowly heated at a program rate of 5°C/min up to 20°C.

Partitioning of Solutes Between Chitosan Membrane and Bulk Solution

The chitosan membrane was soaked in solute buffer solution at 25°C for one day. The solute concentrations in bulk solutions were 0.2 g/dm³ for benzenesulfonic acid, 5.0 g/dm³ for theophylline, and 1.0 g/dm³ for styrene glycol. Disodium hydrogen phosphate (0.01 mol/dm³)/potassium dihydrogen phosphate (0.01 mol/dm³) buffer solution was used for a pH of 7.4 and 6.0, whereas disodium hydrogen phosphate (0.01 mol/dm³)/citric acid (0.005 mol/dm³) buffer solution was used for a pH of 4.0. The chitosan membrane taken from the solute solutions was wiped carefully to remove the excess aqueous solution on the membrane surface and resoaked in buffer solution (pH 7.4) containing no solute at 25°C for one day. The concentrations of solutes released into the buffer solution were measured using an ultraviolet (UV) spectrophotometer (Hitachi, U-2000; wavelength: 262 nm for benzenesulfonic acid, 271 nm for theophylline, 256 nm for styrene glycol).

Partition coefficient can be defined in two ways. One is the ratio of solute concentration in gel membrane standardized by the total membrane volume to that in bulk solution, and the other is the ratio of solute concentration in membrane standardized by the water volume in the membrane to that in bulk solution.¹⁷ If we express the former as K and the latter as K', the two partition coefficients are related as follows, where H is the water volume fraction in the membrane:

$$K = HK' \tag{1}$$

Permeation Experiments

The procedure and the apparatus used in the permeation experiments were the same as described in the previous work.¹⁷ The diffusion cell consisted of two cylindrical half cells of a volume of 20 cm³ made of Pyrex glass. The chitosan membrane was sandwiched between two cells. The membrane area was 5.3 cm^2 . The solutions in the two cells were stirred by magnetic stirring bars at 250 rpm. The diffusion cell was placed in a water bath maintained at 25° C.

The feed solution was prepared by dissolving the solute in buffer solution. Solute concentrations and kinds of buffer solutions were the same as described in the partition experiment section. The receiving solution was the buffer solution alone. Samples (1 cm^3) of the receiving solution were taken at various time intervals and solute concentrations were analyzed by spectrophotometer at the wavelengths described above. After taking out a sample of 1 cm^3 , 1 cm^3 of the fresh buffer solution was always added to the receiving solution. The change in concentration due to the addition of fresh buffer solution was taken into account in the calculation of the solute amounts transported into the receiving solution.

The flux N is given by

$$N = (V/A) \ (dC_{b1}/dt) = K_0(C_{b2} - C_{b1})$$
(2)

where V is the receiving solution volume, A is the membrane area, K_0 is the overall mass transfer coefficient, and C_{b2} and C_{b1} are the bulk concentrations in feed and receiving solutions, respectively. In our previous study,¹⁷ it was already confirmed that even in a membrane showing the higher permeability than the highest permeability obtained in this work, the resistance of the membrane phase was predominant. Therefore, in this chitosan membrane, the liquid-phase mass transfer resistance can be ignored. Furthermore, C_{b1} is negligibly small compared with C_{b2} . Thus, the flux is expressed by eq. (3), as follows:

$$N = (P/L)C_{b2} \tag{3}$$

Here, P is the membrane permeability and L is the membrane thickness. The membrane permeability P was calculated from eq. (3) by using the membrane thickness measured by a micrometer. The solute diffusivity in the chitosan gel membrane D_{gel} was estimated from the following equation:

$$D_{\rm gel} = P/K \tag{4}$$

RESULTS AND DISCUSSION

The water volume fraction H in the chitosan membranes crosslinked by the glutaraldehyde solutions with various concentrations are listed in Table I. Membranes crosslinked with 0, 0.01, 0.1, and 0.2 wt % glutaraldehyde solutions are abbreviated as membrane 0, membrane 1, membrane 2, and membrane 3, respectively. The values of Hwere calculated from the water weight fraction obtained by the swelling experiment at 25°C using densities of both water and the chitosan membrane. The chitosan membrane density was determined as 1.445 g/cm³ by buoyant method. In the case of pH of 7.4, H of membrane 0 (no chemical crosslinking) showed the highest value and H decreased in the order of membrane 1, membrane 2, and membrane 3. This result suggests that the

Membrane	Water Volume Fraction			Membrane Thickness (μm)			
	pH 4.0	pH 6.0	pH 7.4	pH 4.0	pH 6.0	pH 7.4	Weight Fraction of Freezable Water
0	_	_	0.711	_	_	102	0.444
1	0.702	0.675	0.648	98	86	85	0.300
2	0.601	0.581	0.572	90	83	83	0.114
3	0.532	0.542	0.532	75	78	75	0.088

 Table I
 Water Volume Fractions H, Membrane Thickness, and Weight Fraction of Freezable Water W

 in the Chitosan Membranes

crosslinking density ρ increases with the increase of the concentration of the crosslinking agent. Since the mesh size is proportional to $\rho^{-1/2}$, ^{3,17} we can speculate that the mesh size decreases in the order of membrane 0, membrane 1, membrane 2, and membrane 3. In membrane 1, the decrease in pH of the solution led to the monotonous increase in H. The pKa value of chitosan was reported as 6.3.¹⁵ Therefore, the decrease in pH results in the increase in the charge density of the membrane, which makes the membrane more swollen due to the electrostatic repulsion. This is the reason that *H* became larger at the lower pH solution. However, in the membrane with higher crosslinking, that is, membrane 3, H was hardly influenced by pH of the solution. This may be because the highly crosslinked membrane cannot be swollen well and the effect of the charge density of the membrane is not significant. The data of the measured membrane thickness are included in Table I. The tendency is similar to that of the water content.

The differential scanning calorimetry (DSC) melting thermograms for the chitosan membrane with various crosslinking are shown in Figure 2. All the data were normalized as those per the same amount of water in the membranes. Membrane 0 showed the large endothermic peak at about 0°C. The endothermic peaks decreased in the order of membranes 1, 2, and 3; that is, with the increase of the crosslinking degree. The water in the gel membrane is roughly divided into two types such as bound nonfreezable water and free freezable water.^{18–20} The amount of the freezable water in the membrane was approximately estimated from the equation

$$W = Q_{\rm end}/Q_f \tag{5}$$

where W is the weight fraction of the freezable water, $Q_{\rm end}$ is the observed endothermic heat

(cal/g water) and Q_f is the heat fusion of ice (79.9 cal/g).¹⁸ The values of W for various chitosan membranes are listed in Table I. Even in the case of membrane 0 with no chemical crosslinking, W was found to be only 0.444. Membrane 3 had <10% freezable water. Furthermore, in this membrane, the structure of the freezable water must be different from that of the free-bulk water because the endothermic peak position was shifted to about -10° C.

As a reference, permeations of three kinds of solutes through PVA membrane without charge were examined. PVA membrane was prepared by



Figure 2 The DSC melting thermograms for chitosan membranes.

	Permeability (cm ² /s)	Diffusivity (cm ² /s)	Solute Radius ^a (Å)
Benzenesulfonic acid	$1.23 imes 10^{-6}$	$8.50 imes 10^{-6}$ b	3.0
Styrene glycol Theophylline	$1.03 imes 10^{-6} \ 1.27 imes 10^{-6}$	$7.93 imes 10^{-6}{ m c}$ $6.54 imes 10^{-6}{ m c}$	3.2 3.8

Table IIPermeabilities Through PVA Membrane, Diffusivities in Water,and Solute Radii

^a Calculated by Stokes-Einstein equation.

^b Estimated by Wilke–Chang equation.

^c Ref. 17.

casting the 10 wt % PVA dope solution onto the glass plate with $1000-\mu m$ thickness and drying it for 20 h at 60°C. The obtained permeabilities were summarized in Table II together with the solute diffusivities in bulk water $D_{\rm water}.$ In addition, the solute radii calculated by the Stokes-Einstein equation with these D_{water} values are included in this table. Almost the same permeabilities were obtained for three solutes. In the noncharged gel membrane such as PVA, the permselectivity is attributable to the size exclusion of solute. Benzenesulfonic acid, theophylline. and styrene glycol have the similar molecular weights, and therefore, the solute sizes are almost the same, which leads to similar permeabilities, as shown in Table II. Thus, the mutual separation of these three solutes cannot be achieved by the usual noncharged gel membranes.

Figure 3 shows the relations between the permeabilities and pH of the solutions in the case of chitosan membranes. In membrane 1, the permeability of benzenesulfonic acid increased with the decrease of pH of the solution. As described above,



Figure 3 Relations between permeabilities and pH. Benzensulfonic acid (\bigcirc) , styrene glycol $(\textcircled{\bullet})$, theophylline (\Box) .

benzenesulfonic acid is anionic in this pH range. The decrease of pH leads to the increase of the cation charge density of the chitosan membrane, and the electrostatic attraction between benzenesulfonic acid and the membrane is enhanced. This is the reason for the increase in the permeability. Furthermore, it is speculated that the decrease of pH brings about the increase in the amount of the freezable water because of the increase of water content. This may be another reason for the increase of the permeability because the solute is more likely to diffuse in the freezable water than in the nonfreezable water. On the other hand, in the case of cationic theophylline, the electrostatic repulsion between the solute and the membrane caused the decrease of the permeability with the decrease of pH. Thus, the selectivity of benzenesulfonic acid over theophylline, which is defined as the permeability ratio, increased with the decrease of pH. The neutral styrene glycol showed the slight increase of the permeability with the decrease of pH. In this case, there exists no electrostatic interaction between the solute and the membrane, and thus, only the size exclusion effect must be considered. As shown in Table I, the water volume fraction H increased with the decrease of pH in membrane 1. This highly swollen structure at the lower pH probably made the permeability of styrene glycol higher. In the order of membrane 1, membrane 2, and membrane 3, permeabilities of each solute decreased due to the decrease of the mesh size of chitosan membrane. The degree of the increase of benzenesulfonic acid permeability and that of the decrease of theophylline permeability with the decrease of pH were enhanced in the order of membrane 1, membrane 2, and membrane 3. This is probably because the smaller mesh size in the highly crosslinked membrane leads to the higher fraction of solutes that exist near the charged sites of the membrane and



Figure 4 Relations between partition coefficients and pH. Benzensulfonic acid (\bigcirc), styrene glycol (\bigcirc), theophylline (\Box).

are significantly influenced by the charged sites. Thus, the selectivity of benzenesulfonic acid over theophylline reached about 30 at pH of 4.0 in membrane 3. If we remember that this separation cannot be achieved by PVA membrane, the result by chitosan membrane clearly shows that the charged gel membrane enables the separation of solute with the similar size based on the electrostatic interaction.

Figure 4 shows the relations between the partition coefficient K' and pH in membranes 1, 2, and 3. The tendencies obtained are similar to those shown in Figure 3. That is, K' of benzensulfonic acid increased with the decrease of pH. whereas that of theophylline decreased. Such changes of two solutes were enhanced in the order of membranes 1, 2, and 3. K' of styrene glycol was nearly constant in this pH range. These tendencies can be explained by the electrostatic interaction between the solutes and the membrane, as described above. K' values of benzenesulfonic acid were fairly larger than unity, which shows that larger amounts of the solute exist in the membrane than in the ambient bulk solution. This is due to the electrostatic attraction in the membrane. Even in the case of the neutral styrene glycol, the obtained K' values were larger than unity. Kojima et al. reported that the partition coefficient of Congo Red in PVA membrane was as large as the order of $10^{3.2}$ In this case, there is no electrostatic interaction between the solute and the membrane. They mentioned that this is because the bound water in PVA membrane interconnects Congo red to the membrane. Also, in the chitosan membrane, the larger partition coefficient for styrene glycol is considered to be attributable to the existence of the bound water. The fraction of bound water in the membrane increases in the order of membranes 1, 2, and 3, as shown in Table II. This may be the cause of the increase in K' of styrene glycol in the highly crosslinked membrane in Figure 4. The difference between the results in Figure 4 and those in Figure 3 is that K' values for each solute increase as the degrees of the crosslinking increase; that is, the mesh sizes in the gel membrane decrease. whereas the permeabilities decrease. The increase of K' is due to the increase of the electrostatic interaction between the solute and the membrane brought about by the smaller mesh size and also due to the increase of the bound water fraction. On the other hand, the decrease of the permeability is attributable to the remarkable decrease in the diffusion coefficient due to the decrease of the mesh size.

Yasuda et al.^{21–23} and Peppas and Reinhart²⁴ developed theory for solute diffusion through hydrated polymer membrane or gel membrane based on the free volume theory. According to the theory, the normalized solute diffusion coefficient in the swollen gel membrane D_{gel} to that in pure water D_{water} is expressed as follows:

$$\frac{D_{\text{gel}}}{D_{\text{water}}} = B(v_d) \exp\left\{\frac{-\pi r_s^2 l(1/H - 1)}{V_1}\right\}$$
(6)

Here, r_s is the Stokes hydrodynamic radius of solute, l is the characteristic length, and V_1 is the average free volume of water. The term $B(v_d)$ denotes the probability of a diffusing species of volume v_d finding a mesh formed by the crosslinked chains of the polymeric network having a volume of at least v_d . The ratios of D_{gel} obtained from eq. (4) to D_{water} are plotted against pH of the solution in Figure 5. At a pH of 7.4, the ratios $D_{\rm gel}/D_{\rm water}$ were the highest for benzenesulfonic acid and the lowest for theophylline. Since three solutes have similar molecular weight, values of r_s are considered to be comparable for these solutes. Therefore, the difference in $D_{\rm gel}\!/\!D_{\rm water}$ is attributable to the difference in $B(v_d)$. In the case of benzenesulfonic acid, there exists the electrostatic attraction between the solute and the membrane. This attraction makes it difficult for the solute to leave the present mesh position and to move the next position, whereas it offers more chances to find the next position. The former effect decreases $B(v_d)$ and the latter effect increases $B(v_d)$. The experimental result shown



Figure 5 Relations between D_{gel}/D_{water} and pH of membrane 3. Benzensulfonic acid (\bigcirc), styrene glycol (\bigcirc), theophylline (\square).

in Figure 5 suggests that the latter effect is predominant in this system. On the other hand, the repulsion between theophylline and the chitosan membrane offers less chances to find the next mesh position for the diffusion, which leads to the lower $D_{\text{gel}}/D_{\text{water}}$, as shown in Figure 5. As the pH of the solution decreased, $D_{\text{gel}}/D_{\text{water}}$ for benzensulfonic acid increased, whereas that for theophylline decreased. This is because the attraction or repulsion between the solute and the membrane are enhanced due to the larger charge density of the membrane. However, these tendencies were not so remarkable compared with those in permeabilities shown in Figure 3. In membrane 3, Hwas almost independent of pH as shown in Table I. Therefore, the exponential term in eq. (5) is constant regardless of pH. Since $B(v_d)$ is hardly influenced by pH in the diffusion of the neutral styrene glycol, $D_{\text{gel}}/D_{\text{water}}$ for styrene glycol showed the nearly constant values in various pH ranges in Figure 5.

CONCLUSION

The permeation of ionic solutes through the charged chitosan membrane was investigated. The obtained conclusions are as follows.

- 1. The water volume fraction H in the membrane decreased with the increase of the crosslinking degree. In the membrane with the lower crosslinking degree, H decreased with the increase of pH of the solution, which is due to the increase of the charge density of the chitosan membrane. The DSC measurement indicated that the fraction of the free freezable water decreased remarkably in the highly crosslinked membrane. The fraction was found to be only 0.08 in the chitosan membrane crosslinked with 0.2 wt % glutaraldehyde solution.
- 2. The anionic benzensulfonic acid showed the highest permeability, whereas the cationic theophylline showed the lowest, although these solutes have almost the same sizes. This can be explained by the electrostatic attraction or repulsion between the solutes and the membrane instead of the size exclusion effect. The permeabilities of benzensulfonic acid and theophylline increased and decreased, respectively, with the decrease of pH because of the increase of the charge density of the membrane. Thus, the selectivity of benzensulfonic acid to theophylline increased and reached about 30 at a pH of 4.0. This result clearly showed the charged gel membrane enables the separation of solutes with the similar sizes based on the electrostatic interaction.
- 3. The partition coefficients K' for three kinds of solutes showed the similar tendencies to those in the permeabilities. Even in the case of the neutral styrene glycol, K' was larger than unity. This is probably attributable to the existence of the bound water in the membrane. Contrary to the results of the permeabilities, K' values increased as the degree of the crosslinking in the membrane increased. The increase of the electrostatic interaction between the solute and the membrane brought about by the smaller mesh size and also the increase of the bond water fraction are probably the cause of this increase in K'.
- 4. The ratios D_{gel}/D_{water} were the highest for benzensulfonic acid and lowest for theophylline. This result was explained by the difference in $B(v_d)$ based on the free volume theory.

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