Effect of Membrane Preparation Conditions on Solute Permeability in Chitosan Membrane

HIDETO MATSUYAMA, HIDETOSHI SHIRAISHI, YOSHIRO KITAMURA

Department of Environmental Chemistry and Materials, Okayama University, 2-1-1 Tsushima-naka, Okayama 700-8530, Japan

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ABSTRACT: Chitosan membrane was prepared in various conditions and diffusive permeabilities of theophylline and vitamin B_{12} were investigated. The membrane preparation procedure consists of dissolving chitosan in acid solution, cast on the glass plate, drying the dope solution, and immersion of the plate in the gelating agent. Effects of the kinds of acids to dissolve chitosan, chitosan concentration, drying time of the dope solution, and the kinds of the gelating agent on the membrane structure and performance were studied in detail. With increasing the chitosan concentration, the solute permeability decreased, while the selectivity of theophylline to vitamin B_{12} increased. The membrane changed from the wholly porous structure to the asymmetric structure by the increase of the chitosan concentration. Furthermore, the use of ethanol as the gelating agent brought about the wholly porous structure with the high permeability and the low selectivity. The asymmetric structure and the wholly dense structure were obtained in the cases of the gelating agents, such as aqueous NaOH solution and dimethyl sulfoxide (DMSO), respectively. Thus, the membrane structure can be controlled by the kinds of gelating agent. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 73: 2715–2725, 1999

Key words: chitosan membrane; membrane preparation condition; asymmetric structure; theophylline; vitamin B_{12}

INTRODUCTION

Chitosan (β-(1-4)-2-amino-2-deoxy-D-glucose), derived from chitin by deacetylation, is a natural polycationic polymer, that possesses valuable properties as a biomaterial. This unique basic polysaccharide is attractive as biopolymer for many biomedical applications. In addition, the biocompatibility and biodegradability of chitosan have been well established.[1,2]

The film-forming property of chitosan offers many applications in various membrane separation fields. A number of studies have been carried out on pervaporation separation of water–alcohol mixtures.[3–8] Mochizuki et al. reported that the permselectivity of chitosan membrane increased and reached 450 in the separation of water and ethanol with increasing the degree of the neutralization of chitosan with H_2SO_4.[5] Lee and Shin investigated the pervaporation performance of novel phosphorylated chitosan membranes to separate water from aqueous ethanol solution.[7] Chemical modification of the chitosan membrane contributed to an improved pervaporation performance.

In addition, application to ultrafiltration[9,10] and diffusive permeation[11,12] have been reported. Beppu et al. prepared the chitosan membrane for ultrafiltration by the phase inversion method.[9] The effects on various organic solvents used as

Correspondence to: H. Matsuyama.

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the gelating agents on the membrane performance were investigated in detail. Nakatsuka and Andrady studied the diffusive permeation of vitamin B_{12} in the crosslinked chitosan membrane.[11] The equilibrium swelling ratios, diffusion coefficients, and partition coefficients were measured; and they concluded that the transport mechanism in chitosan–vitamin B_{12} system was predominantly of the pore mechanism.

In the previous works on separations by these chitosan membranes, the membranes were usually prepared in the fixed procedure except for changing the degree of crosslinking. Thus, the relations between the membrane preparation conditions, and the membrane structure and performance have not yet been investigated systematically. The purpose of this work is to clarify how each step in the membrane preparation procedure influences the membrane characteristics. As the membrane performance, the diffusive permeabilities of theophylline and vitamin B_{12} and the selectivity of theophylline to vitamin B_{12} were studied. The permeabilities and selectivities obtained will be discussed with relation to the membrane preparation conditions, such as kinds of acid in the dope solution, chitosan concentration, drying time after casting, and kinds of gelating agents.

EXPERIMENTAL

Materials

Chitosan was kindly supplied by Katokichi Co., Ltd. (Japan). Its deacetylation degree was about 100 mol %. The solutes used for the transport experiment were theophylline (MW, 180; Stokes radius, 3.7 Å[13]) and vitamin B_{12} (MW, 1355; Stokes radius, 8.4 Å[13]). These were purchased from Nakalai Tesque Inc.

Membrane Preparation

Chitosan solution was prepared by dissolving chitosan in aqueous acid solution at ambient temperature with stirring overnight. The solution was allowed to stand for about half a day to remove the air bubbles. Several types of acids, such as acetic acid, nitric acid, formic acid, and hydrochloric acid, were used. The concentration of chitosan in acid solution was changed as variable. The dope solution was then cast onto a glass plate with desired thickness (1000 μm) and placed in a drying air oven (usually 60°C). The drying time was also changed as variable. The dry film was immersed in several gelating agents, such as 1*N* aqueous sodium hydroxide, ethanol, and dimethyl sulfoxide (DMSO) for 3 h. The chitosan membrane was repeatedly washed with water and stored in water until use.

Characterization of Chitosan Membrane

Infrared (IR) spectra of freeze-dried chitosan membranes were measured with a Shimadzu IR-460 spectrometer. For scanning electron microscopy (SEM) analysis, the freeze-dried membrane was sputtered with Au–Pd in vacuum. The structures of both air-facing and glass-facing surfaces were observed by a scanning electron microscope (Hitachi Co., Ltd., S-2150) under an accelerating voltage of 15 kV.

The water content of the membrane was calculated from the following equation by using the weight of membrane swollen and equilibrated in the buffer solution (W_s) and the dry membrane weight (W_d):

$$\text{Water content} = (W_s - W_d)/W_s \qquad (1)$$

The water content was evaluated as the weight fraction in this work instead of the volume fraction.

Permeation Experiments

The procedure and the apparatus used in the permeation experiments were the same as described in previous work.[14] The diffusion cell consisted of two cylindrical half cells of volume of 20 cm^3 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 a phosphate buffered saline solution (0.0027*M* KCl; 0.137*M* NaCl; pH = 7.4). Solute concentration was 5 g/dm^3 for theophylline and 0.5 g/dm^3 for vitamin B_{12}. 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 (Hitachi Co., Ltd., U-2000; wavelength, 271 nm for theophylline; 361 nm for vitamin B_{12}). After taking out a sample of 1 cm^3, 1 cm^3 of the fresh buffer solution was

Table I Membrane Thickness, Water Content, and Theophilline Permeability for Various Acid Cases

Acid	Membrane Thickness (mm)	Water Content (wt %)	Theophylline Permeability (cm^2/s)
Acetic acid	0.0645	52	10.6×10^{-7}
Nitric acid	0.0175	45	3.79×10^{-7}
Formic acid	0.0155	49	3.59×10^{-7}
Hydrochloric acid	0.0208	46	4.31×10^{-7}

Chitosan concentration: 2 wt %. Acid concentration: 10 wt %. Drying time: 20 h. Gelating agent: 1*N* NaOH solution.

always added to the receiving solution to maintain the constant volume. 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.

Total overall resistance for the solute transport consists of mass transfer resistances in the stagnant layers of feed and receiving phases and the resistance of the membrane phase. In our previous study,[14] it was confirmed that even in a membrane with the higher permeability than the highest permeability obtained in this work, the resistance of the membrane phase was predominant. Therefore, the flux N is given by

$$N = (V/A)(dC_{b1}/dt)$$

$$= (P/L)(C_{b2} - C_{b1}) \quad (2)$$

where V is the receiving solution volume, A is the membrane area, and C_{b2} and C_{b1} are the bulk concentrations in feed and receiving solutions, respectively. The membrane permeability P was calculated from eq. (2) by using the membrane thickness L measured by a micrometer (Mitsutoyo Co., Ltd., MDC-25M)

RESULTS AND DISCUSSION

Effect of Kinds of Acids to Dissolve Chitosan

The membrane thickness, water content and theophylline permeability are summarized in Table I for various acid cases. In the case of acetic acid, remarkably higher membrane thickness and theophylline permeability were obtained, compared with those in other acid cases. The gel membrane with high water content can increase its thickness remarkably due to the increase of the membrane swelling brought about by the looser membrane structure. Therefore, it is not surprising that the thickness of the membrane prepared using chitosan solution dissolved with acetic acid was about three times higher than that dissolved with other acids. The water content is also the highest in the acetic acid case, although the difference is not so distinguished. These experimental results suggests that the looser membrane structure is formed in the case of acetic acid.

To confirm the above expectation, IR spectra were measured for all membranes. The result is shown in Figure 1. Bands at 3455 and 665 cm^{-1} are reported to be related to crystallization of

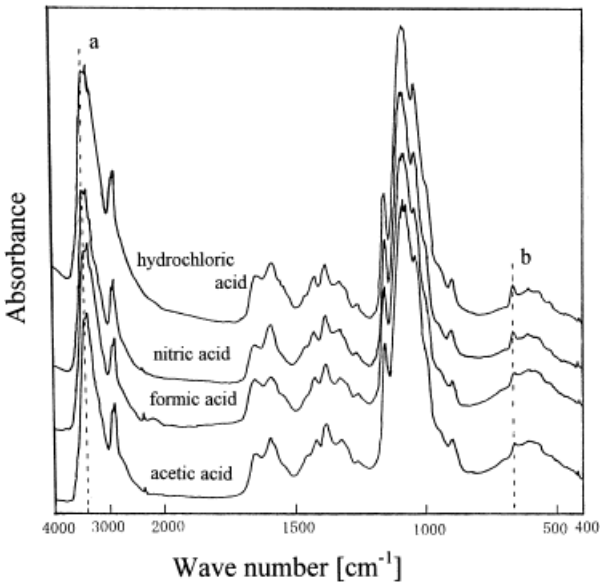

Figure 1 IR spectra of chitosan membranes. Bands of (a) and (b) are related to the crystallization of chitosan.

chitosan.[15,16] The band intensities at about 3455 and 665 cm^{-1} were clearly smaller in the case of acetic acid than those in the cases of hydrochloric acid and nitric acid. This lower crystallinity probably leads to the higher permeability and water content shown in Table I. Because acetic acid is bulky compared with other acids, the aggregation of chitosan in the gelating process may be inhibited by the existence of acetic acid near chitosan and may result in the lower crystallinity. The chitosan membrane prepared with formic acid has the low band intensities at about 3455 and 665 cm^{-1}. However, the resultant membrane permeability is not high, as shown in Table I. The reason for this result is not yet clear.

Effect of Acetic Acid Concentration

Figure 2 shows the effect of acetic acid concentration on theophylline permeability and the membrane thickness. The dotted lines in this figure show the critical amount of acetic acid necessary for carboxyl groups of acetic acid to correspond to amino groups of chitosan in the ratio of 1 : 1. When the acetic acid concentration was lower than this critical value, the dope solution became opaque and, therefore, had insoluble chitosan. In the high concentration region, the acetic acid concentration hardly influenced the permeability and the membrane thickness.

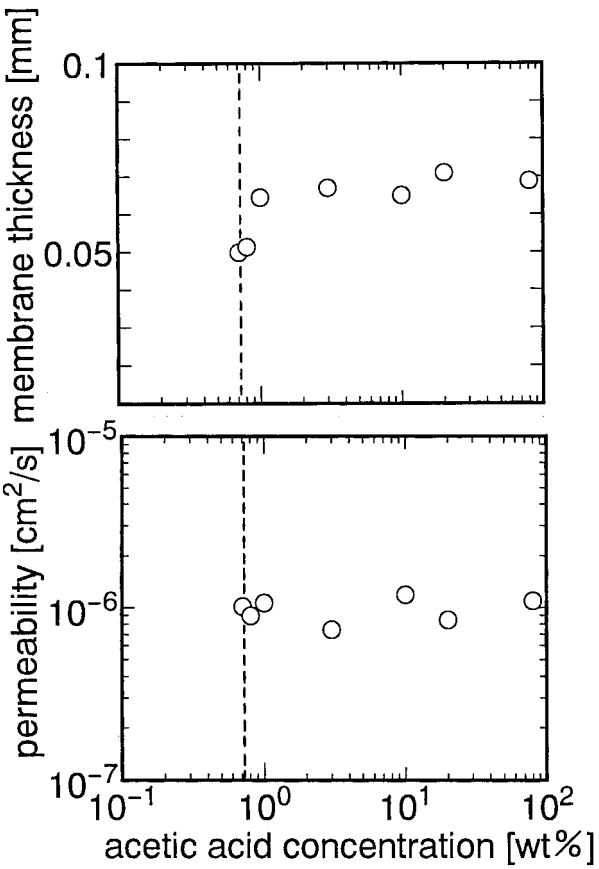

Figure 2 Effect of acetic acid concentration on theophylline permeability and the membrane thickness. Chitosan concentration: 2 wt %. Drying time: 20 h. Gelating agent: 1*N* NaOH solution. Dotted lines show the critical amount of acetic acid necessary for carboxyl groups of acetic acid to correspond to amino groups of chitosan in the ratio of 1 : 1.

Effect of Drying Time

Figures 3 and 4 shows the effects of the drying time on the permeability and the selectivity and the water content and the membrane thickness, respectively. The selectivity was defined as the ratio of theophylline permeability to vitamin B_{12} permeability. In all cases, theophylline with the smaller Stokes radius showed the higher permeability than vitamin B_{12}. Thus, all selectivities obtained were more than unity. In the shorter drying time, the permeabilities were higher with the lower selectivity. Between 1.5 and 2.0 h drying time, the membrane performances drastically changed. The obtained lower permeabilities and the higher selectivity means that the dense membrane structure was formed. For the drying time more than 2.0 h, it hardly influenced the membrane performances. As can be seen in Figure 4, both the water content and the membrane thickness decreased with the drying time, and after the drastic decrease between 1.5 and 2.0 h, these values became nearly constant. From these experimental results, the membrane formation is presumed as follows. In the shorter drying time, the membrane dope solution contains still large amount of water, which inhibits the aggregation of chitosan and formation of crystallization in the gelation process. This leads to the looser and porous membrane structure. Consequently, the higher permeabilities, the lower selectivity, and the higher water content and membrane thickness were obtained. Between 1.5 and 2.0 h drying time, evaporation of water is assumed to be nearly completed because the membrane performances changed drastically. Even for the longer drying time, the similar dense and nonporous structure will be formed. This is probably the reason that the nearly constant membrane performances were obtained after 2.0 h drying time.

Figure 5 shows the membrane structures of the air-facing and the glass-facing surfaces in various

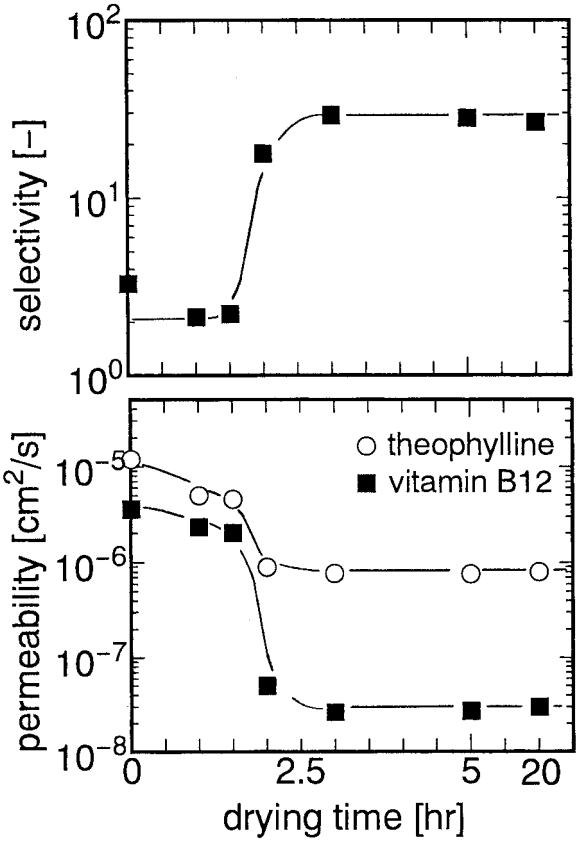

Figure 3 Effect of drying time on the permeability and the selectivity. Dope solution: 2 wt % chitosan in 10 wt % acetic acid solution. Gelating agent: 1*N* NaOH solution.

drying times. It should be noted that the magnification of SEM observation are different in each cases. When the drying times are 0 and 1.0 h, porous structures were formed at both surfaces. On the other hand, dense and nonporous structures were obtained in the cases of the drying times of 5.0 and 20 h. These observations are in agreement with our expectation in the membrane formation described above.

Effect of Chitosan Concentration

The effects of chitosan concentration on the permeability and the selectivity, and the water content and the membrane thickness are shown in Figures 6 and 7, respectively. Two kinds of experiments, such as the drying times of 1.5 and 20 h, were carried out. The experimental results were quite different in two cases. When the drying time is 20 h, water in the membrane dope solution is evaporated nearly completely, which probably leads to the similar dense structures regardless of the chitosan concentrations. Therefore, nearly constant permeability, selectivity, and water content were obtained, as shown in Figures 6 and 7, while the membrane thickness increased monotonously with the increase of the chitosan concentration because the membrane thickness is directly related to the chitosan concentration, even in the case of the similar membrane structure. On the other hand, when the drying time is 1.5 h, the permeability, the water content, and the membrane thickness decreased accompanying the increase of the selectivity as the chitosan concentration increased. It should be noted that the tendency of the change in the membrane thickness, in this case, is opposite to that in the drying time of 20 h. Because all of water in the dope solution cannot be evaporated in the drying of 1.5 h, the higher the initial chitosan concentration, the higher the chitosan concentration after the drying becomes. The higher chitosan concentration in

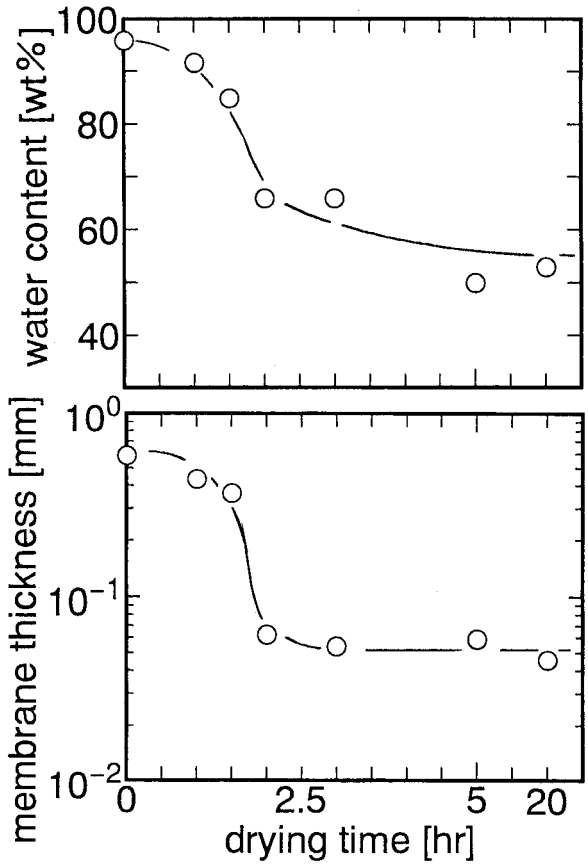

Figure 4 Effect of drying time on the water content and the membrane thickness. Membrane preparation conditions are the same as those described in the caption of Figure 3.

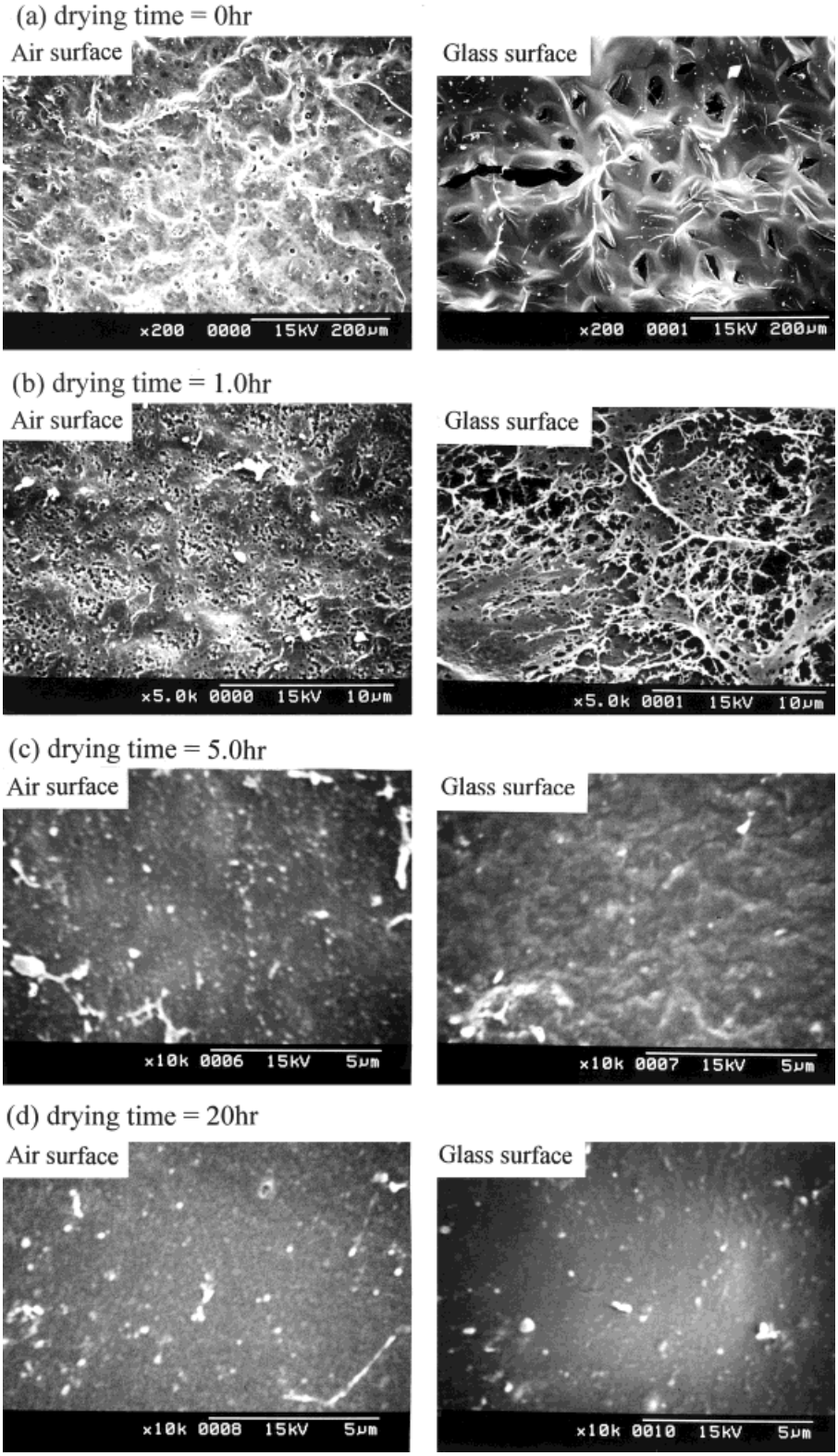

Figure 5 Membrane structures of the air-facing and the glass-facing surfaces in various drying times.

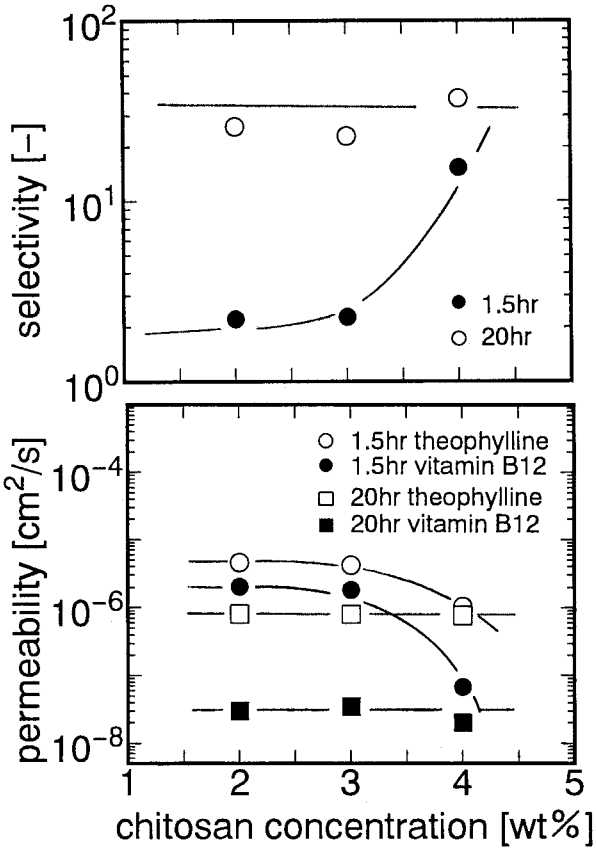

Figure 6 Effects of chitosan concentration on permeability and the selectivity. Dope solution: chitosan in 10 wt % acetic acid solution. Drying time: 20 h. Gelating agent: 1*N* NaOH solution.

dope solution leads to easy aggregation of each chitosan molecule in the gelation process and the resultant denser structure. This is the reason that the higher selectivity, lower permeability, and lower membrane thickness were obtained in the membrane prepared by the chitosan solution with the higher initial concentration.

Figure 8 shows the membrane surface structures in various cases with the different chitosan concentrations. When the concentration is 2.0 wt %, both surfaces had the porous structures. The structure at the air-facing surface approached to the dense structure, but some pores are still observed in the case of 3.0 wt % concentration. When the concentration increased to 4.0 wt %, the air-facing surface had the dense homogeneous structure, while the glass-facing surface had the porous structure. This means that the clear asymmetric structure was formed in this case. Since water evaporates only the air-facing surface of the dope solution in this membrane preparation condition, the chitosan concentration gradient, that is, the higher concentration at the air-facing surface and the lower concentration at the glass-facing surface, may be formed in certain special cases. The high and low chitosan concentration lead to the dense and porous structures, respectively. Thus, the asymmetric structure is probably attributable to such the chitosan concentration gradient in the membrane dope solution. The asymmetric structure is usually favorable because the higher permeability can be obtained with keeping the high selectivity. Although an attempt to produce the asymmetric structure for poly(vinyl alcohol) membrane had already been done,[13] the studies on the asymmetric structure of chitosan membrane were hardly reported. It should be noted that the asymmetric structure can be formed in chitosan membrane by the simple procedure in this work.

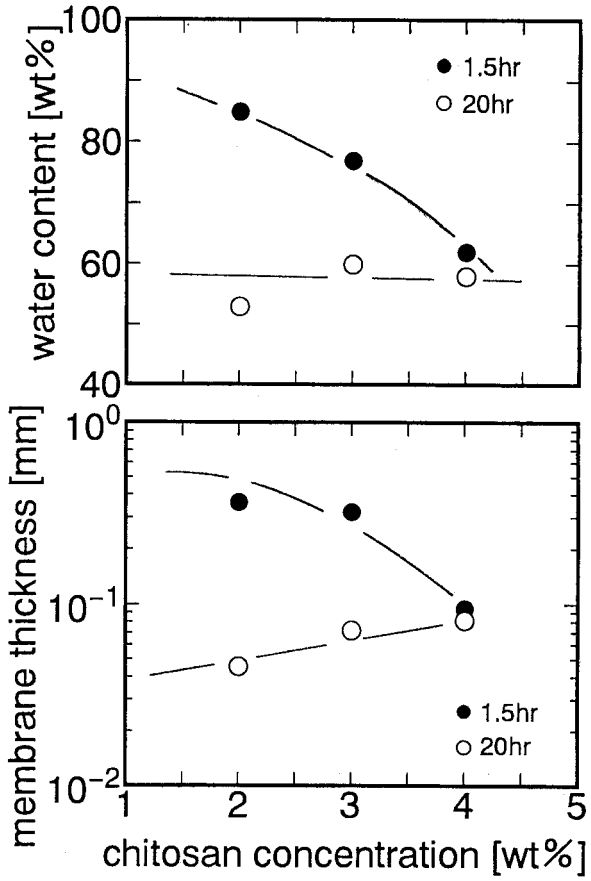

Figure 7 Effect of chitosan concentration on the water content and the membrane thickness. Membrane preparation conditions are the same as those described in the caption of Figure 6.

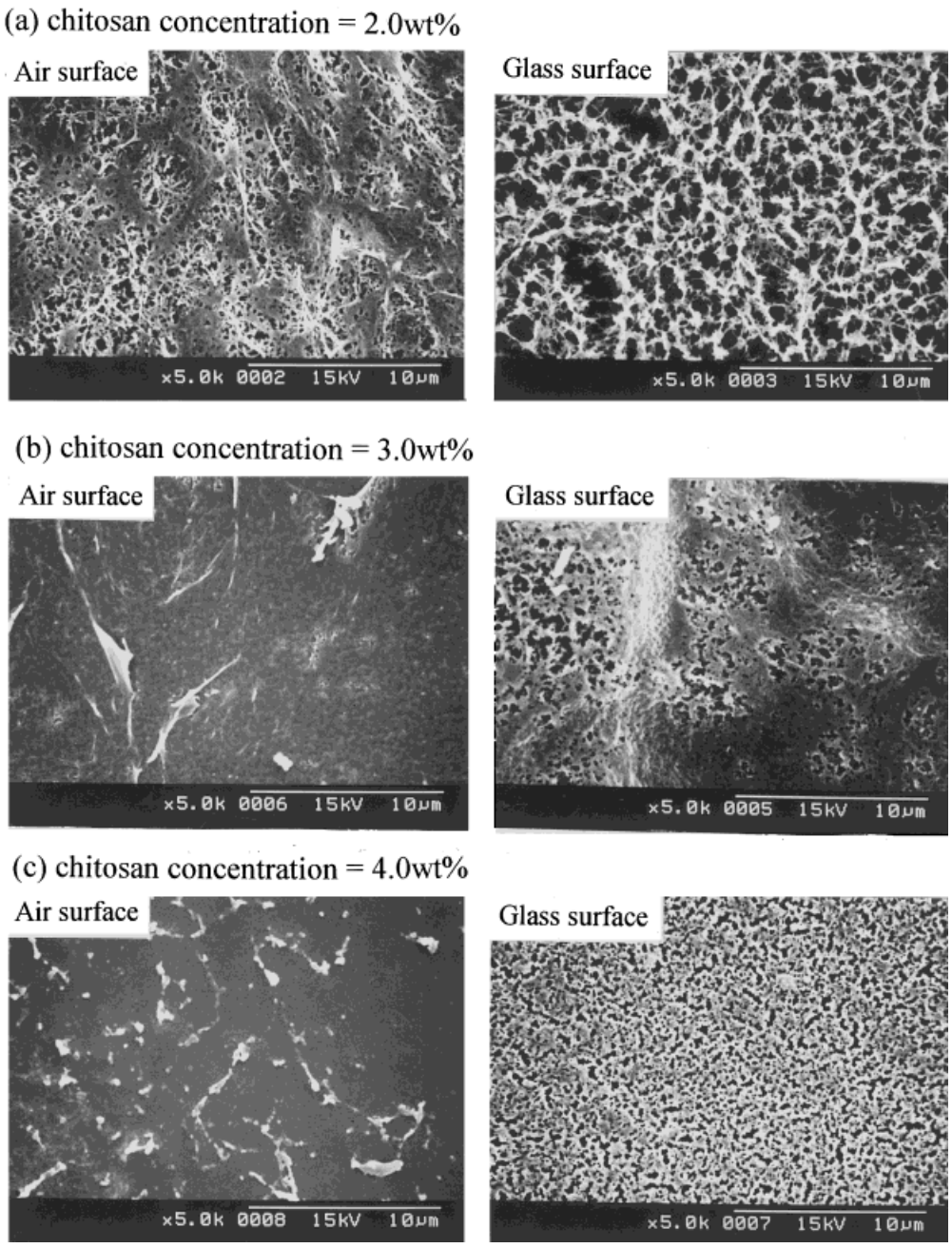

Figure 8 Membrane surface structures in various cases with the different chitosan concentrations. Dope solution: chitosan in 10 wt % acetic acid solution. Drying time: 1.5 h. Gelating agent: 1*N* NaOH solution.

Effect of Kinds of Gelating Agents

Effects of kinds of gelating agents were investigated, and the obtained results are summarized in Table II. Aqueous NaOH solution, ethanol, and DMSO were used as the gelating agents. The permeabilities, water contents, and membrane thicknesses decreased in the order of ethanol, NaOH solution, and DMSO. In the case of ethanol, it is expected based on the extremely low selectivity (2.3) that the porous structure are formed at both surfaces. When NaOH solution was used, the high selectivity was obtained as well as the relatively high permeabilities. Thus, the formation of the asymmetric structure is ex-

Table II Permeabilities, Selectivity, Water Content, and Membrane Thickness for Various Cases of Gelating Agents

	Permeability (Theophylline) (cm^2/s)	Permeability (Vitamin B_{12}) (cm^2/s)	Selectivity (−)	Water Content (wt %)	Membrane Thickness (mm)
Ethanol	3.9×10^{-6}	1.7×10^{-6}	2.3	86	0.330
1*N* NaOH	1.7×10^{-6}	9.1×10^{-8}	18.7	66	0.105
DMSO	7.2×10^{-7}	5.3×10^{-8}	13.6	50	0.046

Dope solution: 2 wt % chitosan in 10 wt % acetic acid solution. Drying time: 1 h at 80°C.

pected in this case. The lower permeabilities and the lower membrane thickness in the case of DMSO suggests that the dense nonporous structure forms at both surfaces.

Figure 9 shows the membrane surface structures when various solutions were used as the gelating agents. As it is expected above, wholly porous, asymmetric, and wholly dense structures were confirmed from these SEM observations when ethanol, NaOH solution, and DMSO were used, respectively. The asymmetric structure in the case of NaOH solution is probably due to the chitosan concentration gradient in the dope solution formed in the drying process. When other solvents were used, such concentration profiles are also formed because the drying process is the same regardless of the gelating agents. However, if the diffusion of ethanol into the dope solution is very fast, the chitosan concentrations at both surfaces similarly become very low. In this situation, the wholly porous structures are formed, as shown in Figure 9(a). Certainly, in the preparation of reverse osmosis membrane from cellulose acetate, it was reported that when the diffusion of the gelating agent into the membrane dope solution is faster than that of solvent in the dope solution into the gelating solution, the loose structure is formed; whereas the dense structure is formed in the opposite situation.[10,17] Contrary to the above, if the diffusion of DMSO into the dope solution is very slow, the chitosan concentrations at both surfaces become high because of the fast leakage of water in the dope solution. This leads to the wholly dense structure, as shown in Figure 9(c). The diffusion rate is related to the thermodynamic properties, such as the chemical potential difference, as well as the kinetic properties, such as the diffusion coefficient. Further investigation is necessary to estimate the diffusion rates of water and gelating agents in this phase inversion process.

CONCLUSION

1. The kinds of acids to dissolve chitosan in the membrane dope solution influenced the membrane performance. The membrane prepared with acetic acid showed the highest theophylline permeability. From IR analysis, the membrane was found to have the low crystallinity.
2. With an increase in the drying time of the dope solution, the permeability, the water content, and the membrane thickness decreased, while the selectivity increased. In a drying time of more than 2.0 h, it hardly influenced the membrane structure and performance. The SEM observation revealed the wholly porous structure in the shorter drying time and the wholly dense structure in the longer drying time.
3. The effect of chitosan concentration was investigated in two drying times, such as 20 and 1.5 h. In the case of the drying time of 20 h, the chitosan concentration did not influence the membrane performance. On the other hand, the increase of the concentration in the drying time of 1.5 h brought about the decrease of the permeability and the increase of the selectivity. The membrane structure changed from the wholly porous to the asymmetric with the increase of the chitosan concentration.
4. The kinds of the gelating agents greatly influenced the resultant membrane structure and performance. The use of ethanol leaded to the wholly porous structure with the high permeability and low selectivity. Asymmetric and wholly dense structure were obtained in the cases of NaOH solution and DMSO, respectively. This indicates that the membrane structure can be controlled by the gelating agent.

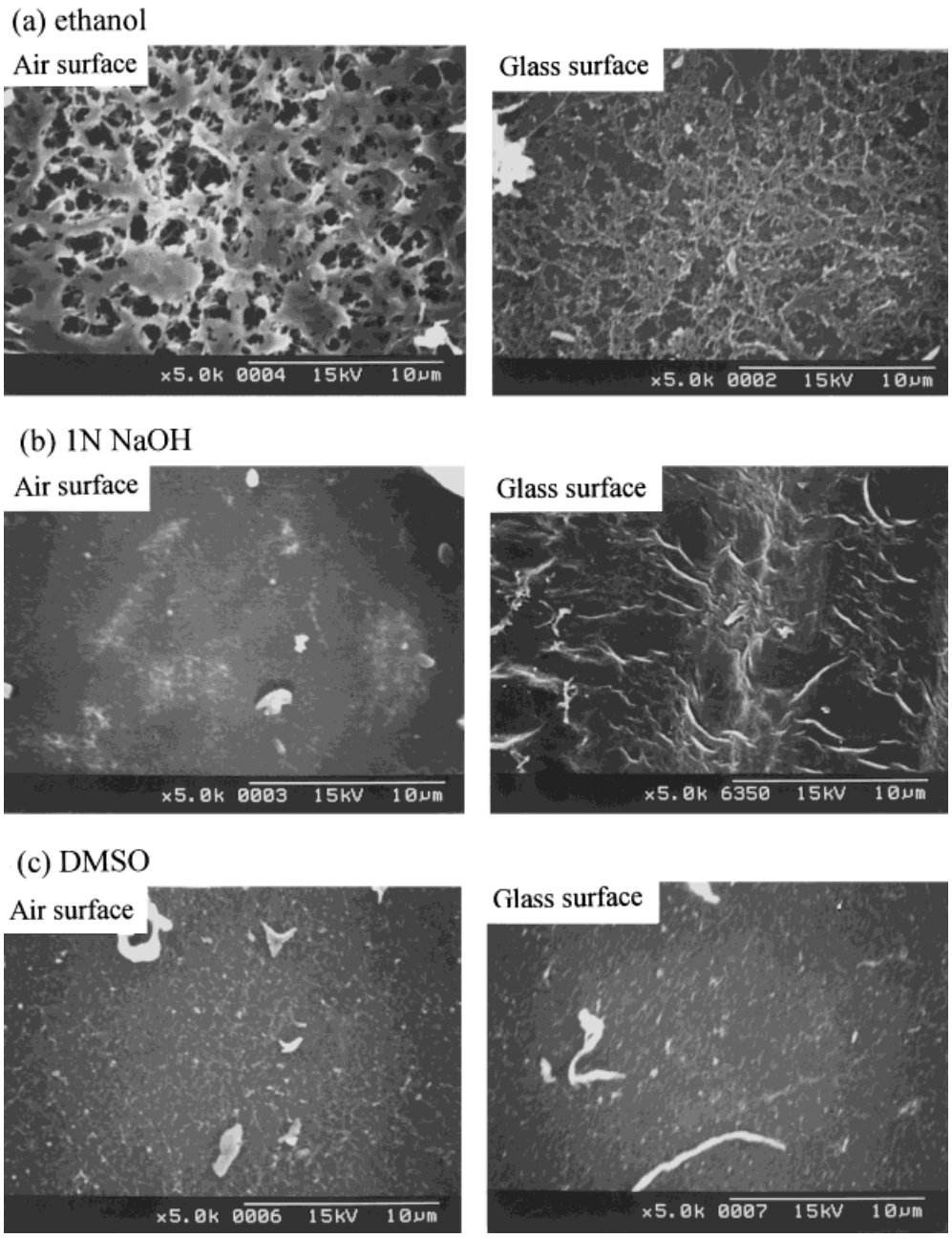

Figure 9 Membrane surface structures when various solutions were used as the gelating agents. Dope solution: 2 wt % chitosan in 10 wt % acetic acid solution. Drying time: 1 h at 80°C.

REFERENCES

1. Hirano, S.; Ohe, Y. Agric Biol Chem 1975, 39, 1337.
2. Chandy, T.; Sharma, C. P. Biomat Art Cells Art Org 1990, 18, 1.
3. Miya, M.; Iwamoto, R.; Mima, S.; Yamashita, S.; Mochizuki, A.; Tanaka, Y. Kobunshi Ronbunshu 1985, 42, 139.
4. Ishida, H.; Watanabe, M.; Kamagami, S. Konbunshi Ronbunshu 1986, 43, 449.
5. Mochizuki, A.; Amiya, S.; Sato, Y.; Ogawara, H.; Yamashita, S. J Appl Polym Sci 1989, 37, 3385.
6. Mochizuki, A.; Amiya, S.; Sato, Y.; Ogawara, H.; Yamashita, S. J Appl Polym Sci 1990, 40, 633.
7. Lee, Y. M.; Shin, E. M. J Membr Sci 1991, 64, 145.

8. Qunhui, G.; Ohya, H.; Negishi, Y. J Membrane Sci 1995, 98, 223.
9. Miya, M.; Iwamoto, R.; Yoshikawa, S.; Mima, S. Kobunshi Ronbunshu 1982, 39, 649.
10. Beppu, M.; Baba, T.; Kamizawa, C. Kobunshi Ronbunshu 1993, 50, 35.
11. Nakatsuka, S.; Andrady, A. L. J Appl Polym Sci 1992, 44, 17.
12. Thacharodi, D.; Rao, K. P. Int J Pharm 1993, 96, 33.
13. Brannon, M. L.; Peppas, N. A. J Membrane Sci 1987, 32, 125.
14. Matsuyama, H.; Teramoto, M.; Urano, H. J Membrane Sci 1997, 126, 151.
15. Mima, S.; Miya, M.; Iwamoto, R.; Yoshikawa, S. J Appl Polym Sci 1983, 28, 1909.
16. Miya, M.; Iwamoto, R.; Mima, S. J Polym Sci, Polym Phys Ed 1984, 22, 1149.
17. Strathman, H.; Scheible, P.; Baker, R. W. J Appl Polym Sci 1971, 15, 811.