Solid-Phase Modification of Chitosan Hydrogel Membranes and Permeability Properties of Modified Chitosan Membranes

NAOJI KUBOTA,[1,*] YASUO KIKUCHI,[1] YUKAKO MIZUHARA,[2] TATSUMI ISHIHARA,[2] and YUSAKU TAKITA[2]

[1]Marine Science Laboratory, Faculty of Engineering, Oita University, Dannoharu, Oita 870-11, Japan; [2]Department of Environmental Chemistry and Engineering, Faculty of Engineering, Oita University, Dannoharu, Oita 870-11, Japan

SYNOPSIS

A novel preparation method for modified chitosan membranes was developed. Chitosan hydrogel membranes were prepared by immersing an aqueous acetic acid solution of chitosan in KOH solution and modified with 3,3′-dithiodipropionic acid (DTPA), which has a functional group that causes the thiol ↔ disulfide transition through a redox reaction. It was smoothly modified with DTPA even in a solid-phase modification when carbodiimides were used as the condensing reagents. The chitosan membrane modified with DTPA was reduced with tri-*n*-butyl phosphine (M_{SH} membrane) and then oxidized with iodine (M_{SS} membrane). Permeabilities of KCl, sucrose, and urea through these modified chitosan membranes were investigated. The permeability of urea was quite high and followed by KCl and sucrose in that order. The permeabilities of KCl and sucrose through the M_{SS} membrane showed a decrease compared to those through the M_{SH} membrane. The thiol ↔ disulfide transition was responsible for changes in the permeabilities of KCl and sucrose. The permeability of urea was almost the same for both membrane systems, i.e., permeation of urea was not affected by the thiol ↔ disulfide transition, probably due to the break of hydrogen bonding in the membranes. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

Chitin [poly-β(1→4)-*N*-acetyl-D-glucosamine] has been regarded as a potential marine resource, because it is a useful aminopolysaccharide analogous to cellulose structurally and naturally abundant, especially in the cuticle of the marine crustacean. Deacetylation of chitin by alkaline hydrolysis yields chitosan, which is one of the few natural cationic polyelectrolytes. Chitosan appears to be more useful than is chitin, since it has both the hydroxyl and amino groups that can be chemically modified easily.[1] Consequently, chitin, chitosan, and their derivatives have recently found wide applications in the industrial and medical fields.[2,3] We have previously prepared several novel membranes from chitosan derivatives and reported on the transport of alkali metal ions through these membranes.[4-6] Moreover, membranes that change the permeability of ions and low molecular weight molecules according to the stimulation such as pH and redox reagents were also reported.[7-11]

In the present article, chitosan was used as a membrane matrix because of its excellent membrane-forming abilities. The chitosan hydrogel membrane regenerated from chitosan solution was modified in solid phase with 3,3′-dithiodipropionic acid (DTPA), which brings about the thiol ↔ disulfide transition with redox reagents. On the basis of the difference in the permeabilities of thiol-containing and disulfide-containing membranes, the feasibility of the permeation control for ions and low molecular weight molecules is discussed. These membranes may give us useful information about the chemical valve and dialysis or hemodialysis membranes.

* To whom correspondence should be addressed.

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EXPERIMENTAL

Materials

Chitosan was of reagent grade from Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan, and used after purification (M_η 1.7×10^6; degree of acetylation 14%). DTPA was purchased from Aldrich Chemical Co., Milwaukee, WI. *N,N*-Dimethylformamide (DMF), ethanol, methanol, and acetone were distilled just before use.

Preparation of Chitosan Hydrogel Membrane

A 2% solution of chitosan was made by dissolution in a 5% acetic acid solution under agitation. The resulting solution was very viscous and allowed to filter. We used vacuum filtration with a sintered glass filter. This filtered chitosan solution was poured onto a clean, flat PTFE plate. A well was created on the plate and a given volume of chitosan solution was poured with a syringe to control the thickness of the casting solution. The poured chitosan solution and plate were gently immersed in 2 mol/dm^3 KOH solution and left overnight. The hydrogel membrane obtained was washed several times with deionized water to remove KOH completely and immersed in acetone until the membrane floated off the plate. The membrane was stored in acetone for later use. The fine structure of the chitosan hydrogel membrane was observed with a Hitachi S-2400 scanning electron microscope at 10 kV in the usual way, after replacing acetone in the membrane with water and freeze-drying.

Modification of Chitosan Hydrogel Membranes

N,N-*Dicyclohexylcarbodiimide (DCC)–DMF System*

A mixture of DTPA (0.31 g) and DCC (0.61 g) was stirred in DMF (20 cm^3) at room temperature until the powder completely dissolved. To the resulting clear solution was added a chitosan hydrogel membrane (0.08 g by dry weight), in which acetone had been previously displaced by DMF, and the reaction solution was stirred slowly. After stirring overnight at room temperature, the membrane was washed well with ethanol. The modified chitosan membrane was immersed in a 20 cm^3 of a methanol–water (9:1) mixture, 0.4 cm^3 of tri-*n*-butyl phosphine (Bu_3P) was added, and the reaction solution was stirred overnight at room temperature. After the reaction, the membrane was washed with methanol and with acetone and finally immersed in water. It was then held between silicone rubber (50 × 50 × 0.5 mm), pressed in PMMA plates (50 × 50 × 5 mm), and dried. Oxidation of the membrane was achieved by immersing it in a 10 mmol/dm^3 solution of iodine.

IR spectra of these products and original chitosan were taken with a Hitachi 270-50 IR spectrophotometer. Elemental analysis was performed at the Institute of Physical and Chemical Research, Japan.

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide Hydrochloride (EDC)–DMF System

Chitosan hydrogel membrane was modified with DTPA using EDC (0.57 g) in DMF, reduced, and oxidized in the same manner as described above.

EDC–DMF–H_2O System

Chitosan hydrogel membrane was modified with DTPA using EDC (0.57 g) in the mixture of DMF and water (9 : 1), reduced, and oxidized in the same manner as described above.

Solute Permeation

The measurement of the permeability was carried out with a diaphragm-type cell that is made of PMMA and has 4.0 cm^2 of permeation area. The modified chitosan membrane was mounted vertically between the two chambers. Into the left-hand chamber of the cell, 25 cm^3 of deionized water was introduced, and 25 cm^3 of 0.05 mol/dm^3 KCl, 0.02 mol/dm^3 sucrose, or 0.01 mol/dm^3 urea was introduced into the right-hand chamber. The cell was placed in a thermostat controlled at 30°C, and 0.1 cm^3 of sample was withdrawn from both sides of the membrane every 2 h. The concentration of KCl was determined by measuring the K^+ ion with a Hitachi Z-8100 atomic absorption spectrophotometer. The sucrose and urea concentrations were determined with a Hitachi U-2000 spectrophotometer using the phenol–sulfuric acid method and the biacetylmonoxime method, respectively. The thickness was measured on the wet membrane.

RESULTS AND DISCUSSION

We adopted chitosan as a membrane matrix considering its excellent membrane-forming abilities and introduced the functional group into the side chain. Because the raw chitosan that we had obtained contained impurities, it was purified three times and characterized. As a result, the viscosity-average molecular weight[12] was 1.7×10^6, and the

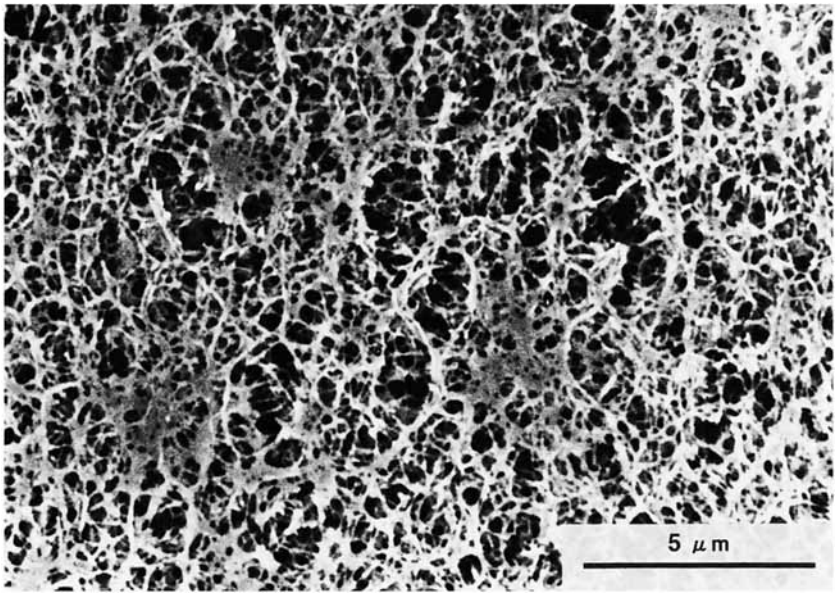

Figure 1 Scanning electron micrograph of freeze-dried chitosan hydrogel membrane.

degree of acetylation[13] was 14%. The fine structure of chitosan hydrogel membrane was estimated by scanning electron microscopic observation. As shown in Figure 1, chitosan hydrogel has highly porous structure. The pore size ranges from 0.1 to 1 μm. Accordingly, it is possible to introduce the new functional groups to this chitosan hydrogel membrane.

DCC and EDC were used as the condensing reagents for chitosan and DTPA. Although chitosan is soluble in acidic media, it became insoluble after introduction of these functional groups. Figure 2 shows IR spectra of chitosan, chitosan modified with DTPA, and *N*-(3-mercaptopropionyl)chitosan obtained in each reaction condition. As can be seen, chitosan has the absorption band assigned to the free amino group at 1600 cm^{-1}, whereas chitosan modified with DTPA does not have this absorption, but, rather, the absorptions assigned to the amide group at 3090, 1660, and 1550 cm^{-1}. A discernible shoulder at 1730 cm^{-1} for chitosan modified with DTPA suggests the presence of ester groups resulting from condensation of the hydroxyl groups of chitosan with the carboxyl group of DTPA. This shoulder seems to include also the absorption of the carboxyl group of DTPA that condensed with chitosan on one side, since the intensity of it lowers in the next reduction step. The decrease in absorbance at 1730 cm^{-1} probably results from the elimination of 3-mercaptopropionic acid produced in reduction of DTPA that condensed with chitosan on one side. As to *N*-(3-mercaptopropionyl)chitosan, the absorption at 1730 cm^{-1} in the case of the EDC–DMF system is lower than that in the case of the DCC–DMF system. The amino group is thought to be, somewhat specifically, modified with DTPA when EDC was used, if the absorption at 1730 cm^{-1} is all due to the ester carbonyl. Further, the amino group appears to be modified more specifically in the case of the EDC–DMF–H_2O system. This result is analogous to the fact that only *N*-acetylated chitosan is formed when acetylated in the presence of water.[14]

The absorption at 2560 cm^{-1} assigned to the mercapto group is observed in the spectra of *N*-(3-mercaptopropionyl)chitosan in each reaction condition. It was reported that mercapto groups were generally unstable in the monomer form, but appreciably stable in the polymer matrix.[15] Then, the stability of the mercapto group in chitosan matrix was studied by examining the change of absorbance at 2560 cm^{-1}. This absorption remains even after standing for 15 days in the atmosphere, as shown in Figure 3. In water saturated with oxygen, the mercapto group in the chitosan matrix was oxidized to sulfonic acid within 1 h, which is identified by the absorption around 1200 cm^{-1}. However, iodine oxidation of the mercapto group to the disulfide bond was achieved without formation of sulfonic acid. *N*-(3-Mercaptopropionyl)chitosan prepared in this way is useful as a new adsorbent of heavy metal ions or support for immobilized enzymes. The sulfur contents of disulfide-containing chitosans, which were obtained in oxidation of *N*-(3-mercaptopropionyl)chitosans in the DCC–DMF, EDC–DMF, and EDC–DMF–

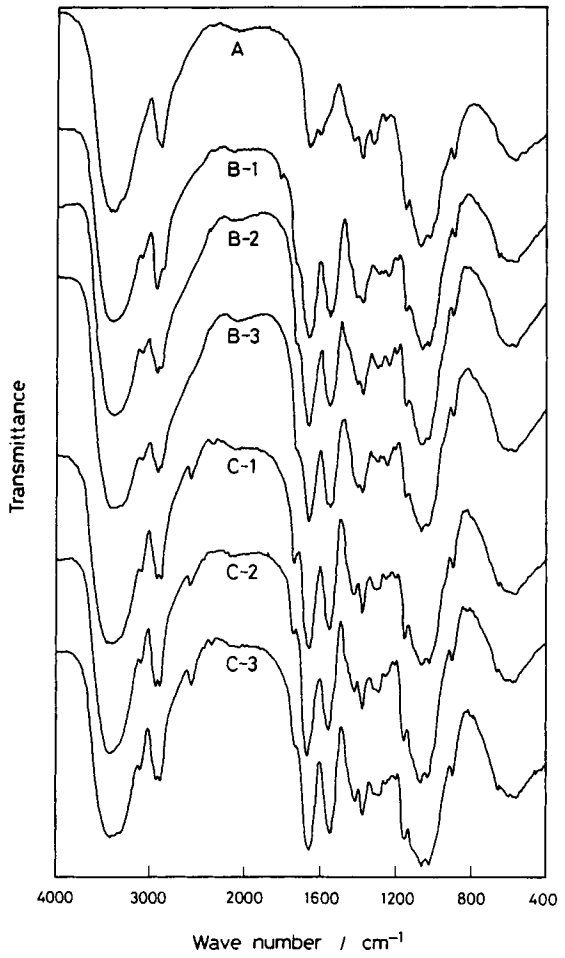

Figure 2 IR spectra of chitosan and modified products in each reaction condition: (A) chitosan; (B) chitosan modified with DTPA; (C) *N*-(3-mercaptopropionyl)chitosan. Series 1, 2, and 3 denote DCC–DMF, EDC–DMF, and EDC–DMF–H_2O systems, respectively.

H_2O systems, were 9.60, 10.09, and 9.50 wt %, respectively. Accordingly, the degrees of modification are estimated as 68, 72, and 67%, respectively. Because the degree of modification was the highest when EDC was used as a condensing reagent in DMF, the membrane that was modified with DTPA using EDC in DMF was employed in the next membrane permeation experiment.

In consideration of the fact that chitin derivatives reproduced hydrogen bonding to yield tough films when dried after being washed with water,[16] we tried to prepare the membrane as follows: The chitosan hydrogel membrane that was modified with DTPA and reduced with Bu_3P was washed well with methanol and with acetone and, finally, immersed in water. It was subsequently held between silicone rubber, pressed into PMMA plates, and dried as it was, since it shrinks significantly with drying. By this procedure, a translucent *N*-(3-mercaptopropionyl)chitosan membrane about 70 μm in thickness was obtained. Figure 4 shows the cross-sectional view of this modified chitosan membrane. The membrane apparently becomes nonporous. The sulfur distribution based on the mercapto group was uniform from one surface to the other, according to the quantitative analysis with an electron-probe microanalyzer. The remaining 3-mercaptopropionyl groups in this membrane are supposed to cause the reversible thiol $\leftrightarrow$ disulfide transition through a redox reaction. The thiol $\leftrightarrow$ disulfide transition in the membrane matrix is confirmed by the fact that IR absorption at 2560 cm^{-1} disappeared upon iodine oxidation (M_{SS} membrane) and reappeared upon reduction with Bu_3P (M_{SH} membrane).

The membrane that was obtained by the aforementioned technique was mounted between the two chambers of the permeation cell, and the permeabilities of KCl, sucrose, and urea were measured. The slope of the curve in Figure 5 exhibits the per-

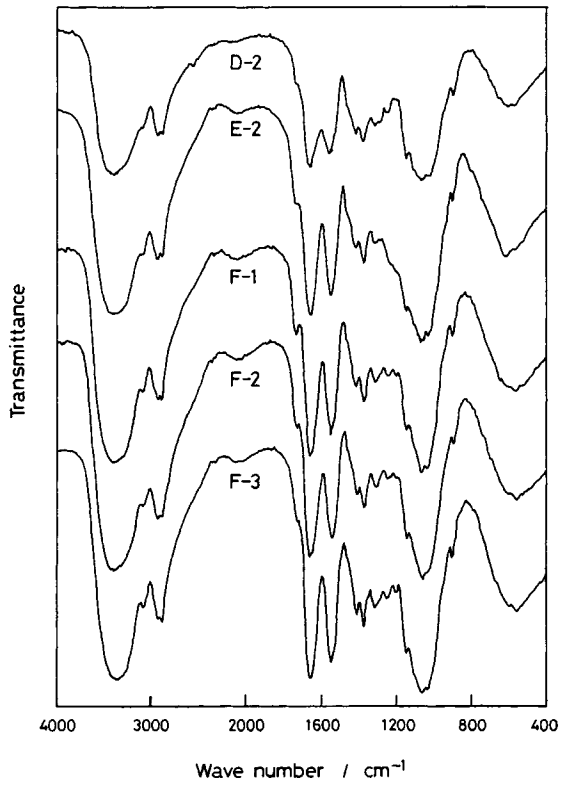

Figure 3 IR spectra of oxidized products in each reaction condition: (D) *N*-(3-mercaptopropionyl)chitosan after standing for 15 days; (E) *N*-(3-mercaptopropionyl)chitosan oxidized with O_2 saturated in water; (F) *N*-(3-mercaptopropionyl)chitosan oxidized with I_2. Series 1, 2, and 3 denote DCC–DMF, EDC–DMF, and EDC–DMF–H_2O systems, respectively.

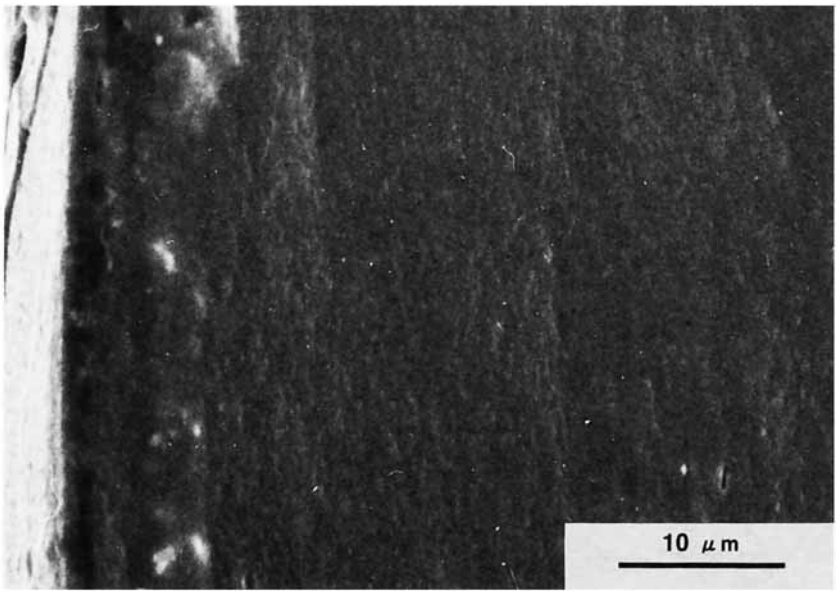

Figure 4 Scanning electron micrograph of cross-sectional view of the modified chitosan membrane.

meability evaluated by the following equation derived from Fick's law of diffusion[11]:

$$\text{Permeability in cm/s} = -\frac{V}{2At}\ln\frac{\Delta C}{C_0}$$

where V is the volume of each chamber of the cell; A, the permeation area of membrane; t, the time; C_0, the initial concentration of solute in the right-hand chamber; and ΔC, the concentration difference between the right- and left-hand chambers. The permeability of urea is quite high; it is followed by KCl and sucrose in that order for both the M_{SH} membrane and the M_{SS} membrane. This result is similar to that reported on chitin film.[17] In addition, the permeability of KCl through the M_{SS} membrane is less than that through the M_{SH} membrane. It was reported that the polypeptide membrane containing mercapto groups showed a 90% decrease in the permeability of KCl by the oxidative reaction,[9] whereas the decrease in the permeability of our system was about 50%. The permeability of sucrose has the same tendency as that of KCl, but the degree of decrease is small. There is a possibility that the mercapto group was oxidized in part to the sulfonic acid, since the change in the permeability of ion is larger than that of neutral molecule. On the other hand, the permeability of urea is almost the same for both membrane systems. This observation is interpreted in terms of the fact that urea breaks hydrogen bonding in the membranes.

The permeabilities of KCl and sucrose were also measured in the presence of 0.01 mol/dm^3 urea. The permeability properties of KCl and sucrose are the same both in the absence and presence of urea as shown in Figure 6. Therefore, the high permeability of urea seems to be based on the nature of urea itself.

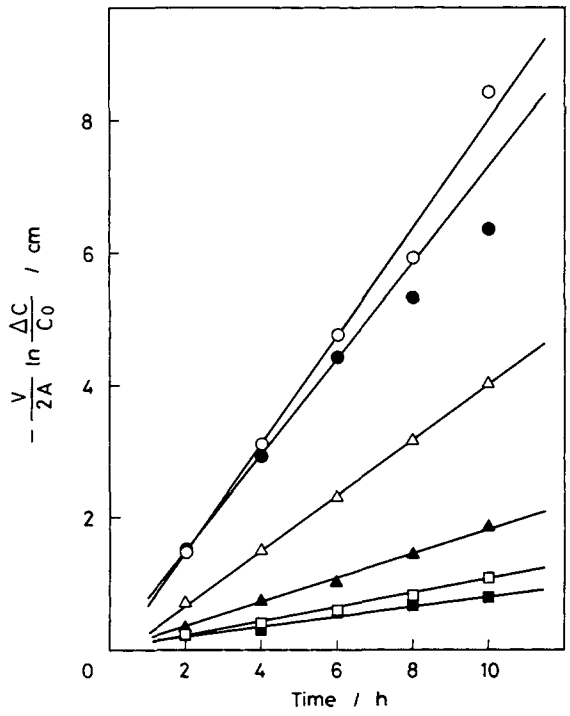

Figure 5 Permeabilities of KCl, sucrose, and urea through the modified chitosan membranes: (△, ▲) KCl; (□, ■) sucrose; (○, ●) urea; (○, △, □) M_{SH} membrane; (●, ▲, ■) M_{SS} membrane. Permeability is represented by the slope.

The permeability can be correlated to the permeability coefficient, P, by the following equation[11]:

$$P \text{ in cm}^2/\text{s} = (\text{permeability}) \times \delta$$

where δ is the membrane thickness. The permeability coefficients are summarized in Table I. Evidently, the change of the permeability coefficient of KCl is larger than that of sucrose, and there is no change in the permeability coefficient of urea. This indicates that the permeation of KCl can be controlled more easily than that of the other solutes studied. The change of the permeability coefficient with the thiol ↔ disulfide transition appears to be based on the degree of cross-linking by the disulfide bond and the compactness of the membrane.

In conclusion, it was revealed that chitosan hydrogel membrane was regenerated by immersing the aqueous acetic acid solution of chitosan in alkaline solution and modified easily. Chitosan membranes modified with the mercapto group, whose functional group changed reversibly by redox reaction, were capable of controlling the permeation of KCl and sucrose to some extent. The permeation of urea was not controlled at all.

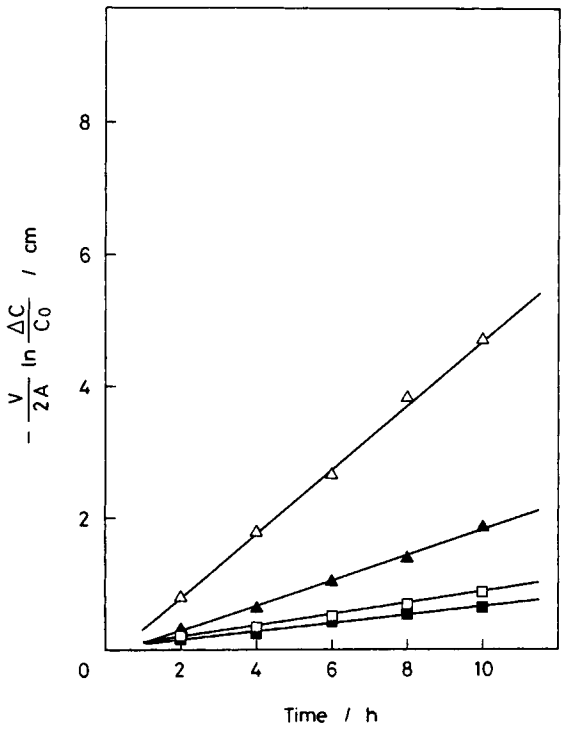

Figure 6 Permeabilities of KCl and sucrose through the modified chitosan membranes in the presence of 0.01 mol/dm^3 urea: (△, ▲) KCl; (□, ■) sucrose; (△, □) M_{SH} membrane; (▲, ■) M_{SS} membrane. Permeability is represented by the slope.

Table I Permeability Coefficients of Modified Chitosan Membranes

Membrane	$P_{KCl} \times 10^7$ (cm^2/s)	$P_{sucrose} \times 10^7$ (cm^2/s)	$P_{urea} \times 10^7$ (cm^2/s)
M_{SH}	13.0	3.0	26
M_{SS}	7.3	2.2	26
M_{SH}[a]	11.0	1.8	—
M_{SS}[a]	5.1	1.6	—

[a] Membrane in the presence of 0.01 mol/dm^3 urea.

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