Permeability Properties of Glycol Chitosan Membrane Modified with Thiol Groups

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SYNOPSIS

Water-soluble glycol chitosan (GC), having the ability to form a membrane, was modified with 3,3′-dithiodipropionic acid (DTPA), a functional group that causes the thiol ↔ disulfide transition through redox reaction. The membrane derived from this modified GC was water-insoluble. The permeabilities of KCl and sucrose through the GC membrane modified with DTPA (M_{DTPA}), the reduced membrane (M_{SH}), and the oxidized membrane (M_{SS}) were investigated. The permeability through the M_{SH} membrane increased twofold relative to that through the M_{DTPA} membrane, and the M_{SS} membrane showed a 60% decrease in the permeability, which had increased in the case of the M_{SH} membrane. The permeability of sucrose was lower and more changeable than that of KCl. These permeation phenomena were discussed from the viewpoint that the thiol ↔ disulfide transition is responsible for the water content of each membrane.

INTRODUCTION

Chitin, which is obtained mainly from the cuticle of the marine crustacean, has recently aroused great interest in the industrial and medical communities[1] because it is a mucopolysaccharide that resembles cellulose structurally and has been produced in nature as much as cellulose.[2] Deacetylation of the acetamide group at the 2-position in the acetylglucosamine unit of chitin by alkaline hydrolysis yields chitosan, which is a cationic polyelectrolyte. Chitosan appears to be more useful than chitin, since it has both hydroxyl and amino groups that can be modified easily.[3]

Therefore, these chitin and chitosan derivatives have increasingly developed many-sided applications. We have previously prepared many polymer membranes from chitosan derivatives and reported on the transport of alkali metal ions and low molecular weight solutes through these membranes.[4-7] Additionally, membranes that change the permeability according to stimulations such as pH, redox reagent, or light were also reported recently.[8-11]

In the present study, glycol chitosan (GC), which is a water-soluble chitosan derivative, was used as a raw material for the membrane matrix. The membrane was derived from the GC modified with 3,3′-dithiodipropionic acid (DTPA), which gives rise to the thiol ↔ disulfide transition with redox reagents. The difference in the permeabilities of the thiol-containing and disulfide-containing membranes and the feasibility of the permeation control for ions and low molecular weight molecules are discussed.

EXPERIMENTAL

Materials

GC (D.P. 400; nitrogen content 5.53 wt %) was the reagent for the colloidal titration of Wako Pure Chemical Industry, Ltd., Osaka, Japan. DTPA was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. The other chemicals were of reagent grade and used without further purification. Dimethylformamide (DMF) and the other solvents were distilled just before use. For the potentiometric titration, deionized water (0.3 μScm^{-1}), which was degassed by boiling, was used.

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Potentiometric Titration

The deionization of the GC solution (0.02 base mol/L) was performed by using a mixture of cation and anion exchangers. After adding NaCl up to 0.1 mol/L, 50 mL of GC solution was neutralized with HCl, followed by back titration with 0.5 mol/L NaOH at 25°C in a nitrogen atmosphere. In order to estimate the degree of dissociation, α, the hydrogen ion concentration was corrected by back titration of a GC-free blank solution. Both titrations were carried out with a Hiranuma UCB-7 autoburet and a Horiba M-8 pH meter.

Modification of GC

A mixture of DTPA (0.32 g) and 1-ethyl-3(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (0.58 g) was stirred in DMF (10 mL) at room temperature until the powder completely dissolved. The resulting clear solution was added drop by drop to a vigorously stirred aqueous solution of GC (0.21 g/10 mL) that was cooled with ice-cold water, and then the reaction mixture gelled. After standing over night at room temperature, the gel was precipitated with acetone, washed well with ethanol, and centrifuged. Several aliquots of the precipitate were dried in vacuo for analyses; the rest were suspended in a methanol–water mixture (9 : 1, 10 mL) before drying, and 0.4 mL of tri-*n*-butyl phosphine (Bu_3P) was added. The mixture was stirred over night at room temperature, centrifuged, washed with methanol, and dried in vacuo. IR spectra of these products and the original GC were taken with a Hitachi 270-50 IR spectrophotometer. Elemental analyses were performed at the Institute of Physical and Chemical Research, Japan. The disulfide content of the GC modified with DTPA was determined by titration with $KBrO_3$ solution.

Membrane Application

The GC gel modified with DTPA, obtained by the aforementioned technique in the reaction mixture, was washed with an acetone–water mixture (1 : 1), then washed again with water, and centrifuged. It was held between silicone rubber sheets (50 × 50 × 0.5 mm), pressed with PMMA plates (50 × 50 × 5 mm) and dried at 30°C. The reduction and the oxidation of the membrane were achieved by immersing it in a solution (methanol–water) of Bu_3P and a 10-mmol/L solution of iodine, respectively, over night. The measurement of the permeabilities of KCl and sucrose was carried out with a diaphragm-type cell that had 4.0 cm^2 of effective area. Into the left-hand chamber of the cell 25 mL of pure water was introduced, and 25 mL of 0.05 mol/L KCl or 0.02 mol/L sucrose was introduced into the right-hand chamber. The cell was then placed in a thermostat controlled at 30°C, and 0.1 mL of sample was withdrawn from both sides of the membrane every 2 h. The concentration of KCl was determined by measuring K^+ ion with a Shimadzu AA-640 atomic absorption spectrophotometer. The sucrose concentration was determined with a Hitachi U-2000 spectrophotometer by means of the phenol–sulfuric acid method. The degree of swelling and the water content of each membrane were calculated from the difference in the weights of the water-swollen and dried membranes.

RESULTS AND DISCUSSION

GC is a chitosan derivative whose primary hydroxyl group at the 6-position was 2-hydroxyethylated. It is water-soluble at all pH, in marked contrast to chitosan, which is soluble in acidic medium but insoluble in basic medium. On account of this property, GC is quite usable as a reagent for the colloidal titration.[12] In addition, GC seems to be more suitable for studying the solution properties of chitosan derivatives. For example, potentiometric titration of glycol chitosan hydrochloride (GCH) in the presence of 0.1 mol/L NaCl gave the pK-α curve shown in Figure 1. This curve indicates the dependence of the dissociation constant, K, on the degree of dis-

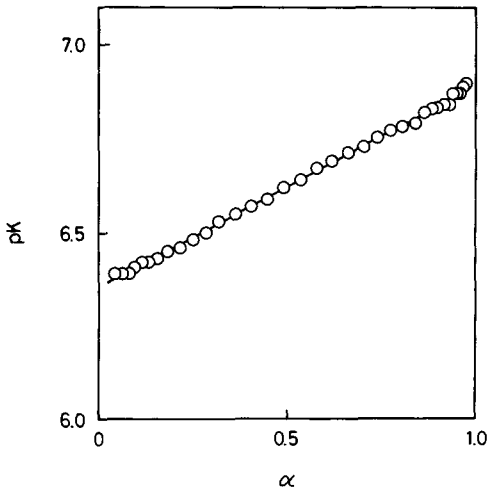
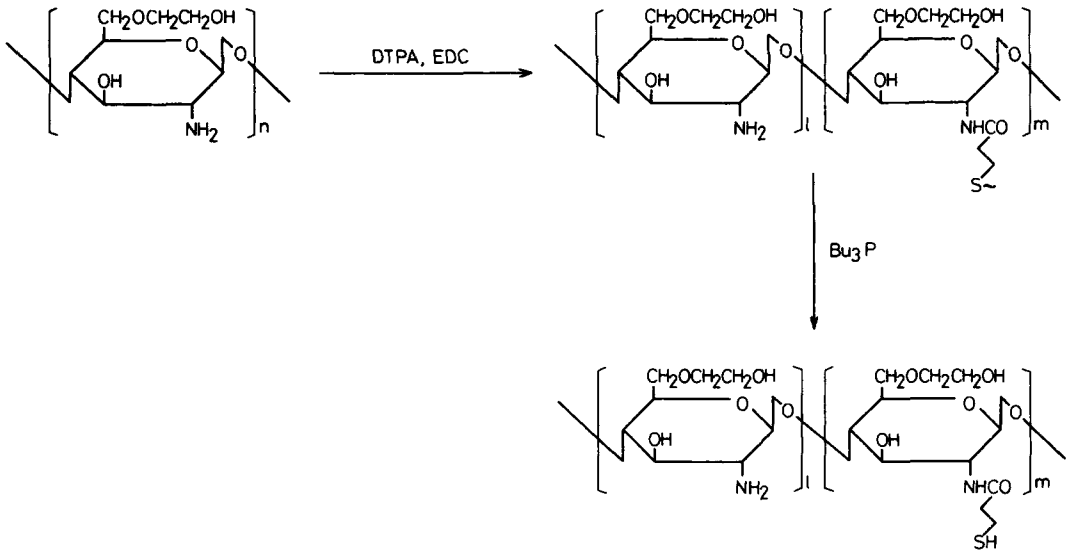


Figure 1 The dependence of the dissociation constant on the degree of dissociation of GCH in the presence of 0.1 mol/L NaCl at 25°C. $pK = pH - \log\{\alpha/(1-\alpha)\}$.

sociation, α, of GCH. It is due to the good solubility of GC that such a pK-α curve was obtained over a wide range of α. The slope of the curve demonstrates the intramolecular electrostatic interaction between the neighboring dissociable functional groups on GC chain. It is known that the break is observed in the pK-α curve when the polyelectrolyte brings about a reversible conformational change such as the helix $\leftrightarrow$ coil transition of poly(L-glutamate)[13] or poly(L-lysine).[14] However, the linear pK-α curve obtained for GC suggests that such a conformational change of the backbone of GC has not occurred. That is to say, GC is a rather rigid polyelectrolyte of which the β-(1,4) linkage is identical to that of cellulose. The flexibility of some polysaccharides was investigated in terms of "stiffness," the dependence of the intrinsic viscosity on the ionic strength. Terbojevich et al.[15] obtained values of stiffness from 0.043 to 0.091 for chitosan with degrees of acetylation ranging from 52.2 to 12.1%. These values were nearly equal to those for carboxymethylcellulose (0.065) and hyaluronate (0.080), and greater than DNA (0.0055), but less than poly(acrylate) (0.23). GC also seems to have the same stiffness as these polysaccharides.

GC with the preceding properties was used as the membrane matrix, and functional groups were introduced into the side chains. Introduction of the functional groups was conducted according to Scheme I. Figure 2 shows the IR spectra of GC, GC modified with DTPA, and N-(3-mercaptopropionyl) GC, which was obtained in reduction of GC modified with DTPA. As can be seen, GC has the absorption band assigned to free amino groups at 1600 cm^{-1} whereas GC modified with DTPA does not have this absorption, but rather the absorptions assigned to amide groups at 3090, 1660, and 1550 cm^{-1}. The shoulder at 1730 cm^{-1} of GC modified with DTPA indicates the presence of the ester carbonyl resulting from the combination of hydroxyl groups of GC with carboxyl groups of DTPA. Additionally, this shoulder seems to include the absorption of carboxyl groups of DTPA, which condensed with GC on one side because the intensity of it lowered in the next reduction step. This point will be discussed later. The absorption at 2560 cm^{-1} assigned to thiol groups was observed in the spectrum of N-(3-mercaptopropionyl)GC. It was reported that thiol groups were generally unstable in the monomer form, but appreciably stable in the polymer matrix.[16] The stability of thiol groups in GC was thus studied by examining the change of the absorption at 2560 cm^{-1}. The absorption was reduced to about half the initial value after standing for 30 days in the atmosphere, as shown in Figure 2.

An attempt was made to cast a polymer membrane from GC modified with DTPA, but it was impossible because the polymer was completely insoluble in common organic solvents and water. In consideration of the fact that chitin derivatives reproduced the hydrogen bonding to yield films when dried after being washed with water,[17] we tried to prepare the membrane as follows: The GC gel modified with DTPA, which was obtained in the reaction mixture, was washed well with an acetone–water mixture to keep the precipitate highly swollen,



Scheme 1

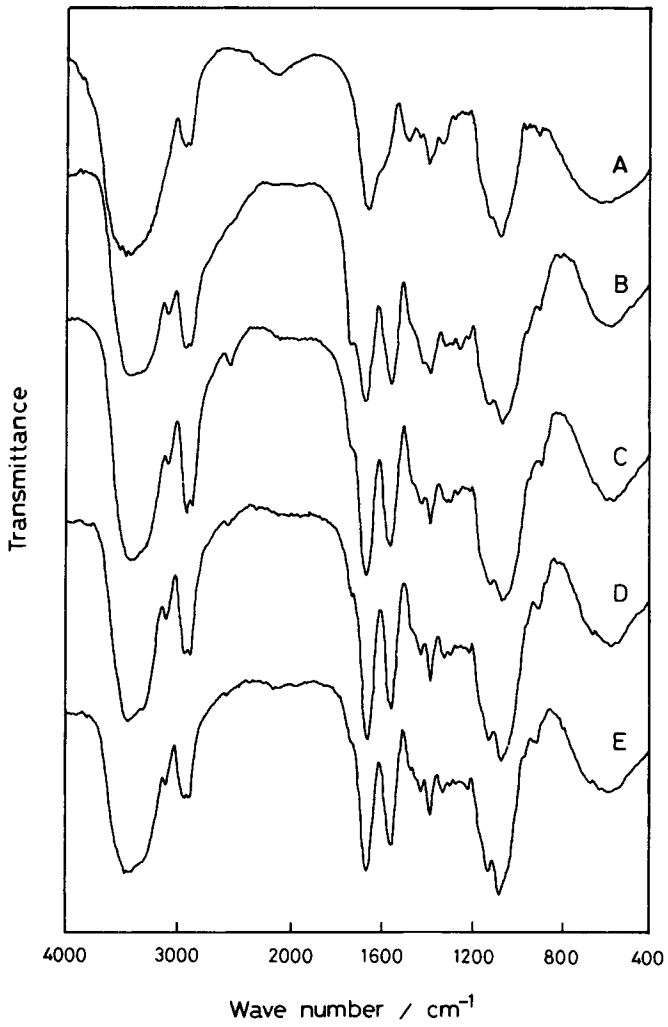


Figure 2 IR spectra of GC and each product: (A) GC, (B) GC modified with DTPA, (C) *N*-(3-mercaptopropionyl) GC, (D) *N*-(3-mercaptopropionyl) GC after standing for 30 days, (E) *N*-(3-mercaptopropionyl) GC after standing for 90 days.

washed finally with water, and dried while being held between silicone rubber sheets and PMMA plates. By this procedure, GC membrane modified with DTPA about 90 μm in thickness was obtained. The thiol $\leftrightarrow$ disulfide transition was found to occur in the membrane matrix because the IR absorption at 2560 cm^{-1} disappeared upon iodine oxidation and reappeared upon reduction with Bu_3P, as shown in Figure 3.

The modified GC membrane was then placed in a diaphragm-type cell, and the permeabilities of KCl and sucrose were measured. The slopes of the curves in Figure 4 exhibit the permeabilities evaluated by the following equation derived from Fick's law of diffusion (see Appendix):

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

where V is the volume of each chamber of the cell, A is the effective area of membrane, t is the time, C_0 is the initial concentration of solutes in the right-hand chamber, and ΔC is the concentration difference between the right- and left-hand chambers. The permeability of KCl through the GC membrane modified with DTPA (M_{DTPA}) increased by reduction (M_{SH}), and that through the M_{SH} membrane decreased by oxidation (M_{SS}). Takizawa et al. reported that the polypeptide membrane containing thiol groups showed a 90% decrease in the perme-

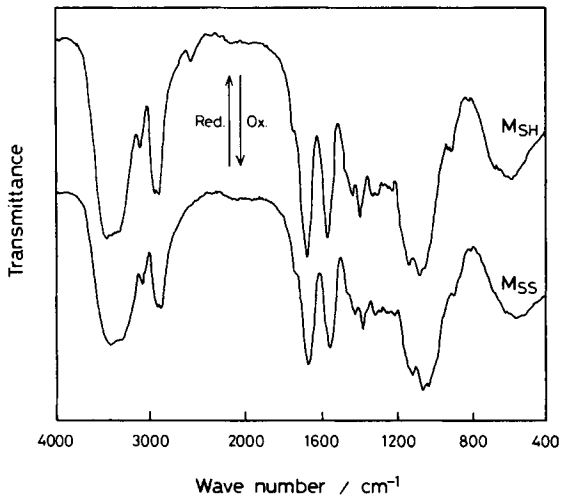


Figure 3 IR spectra of the modified GC membranes. M_{SH} membrane was obtained in reduction with Bu_3P, and M_{SS} membrane was obtained in oxidation with I_2.

ability of KCl by the oxidative reaction,[10] while the decrease in the permeability of our system was about 60% the permeability that had increased by the previous reduction. The permeability of sucrose had the same tendency as shown in Figure 4. In the case of sucrose, all of the permeabilities were lower than those of KCl. This result may be attributed to the molecular and ionic size of each solute.

The permeability coefficients, P, are summarized in Table I along with the sulfur content, the degree of swelling, and the water content of each membrane. The sulfur contents of the M_{DTPA} and M_{SH} membranes were 9.32 and 5.99 wt %, respectively. The decrease in the sulfur content may have resulted from the elimination of 3-mercaptopropionic acid produced in reduction of DTPA, which had condensed with GC on one side. On the basis of the difference in the sulfur contents of the M_{DTPA} and M_{SH} membranes, the amount of 3-mercaptopropionic acid eliminated was estimated as 44%. The remaining 3-mercaptopropionyl groups in the M_{SH} membrane are supposed to cause the reversible thiol ↔ disulfide transition through redox reaction.

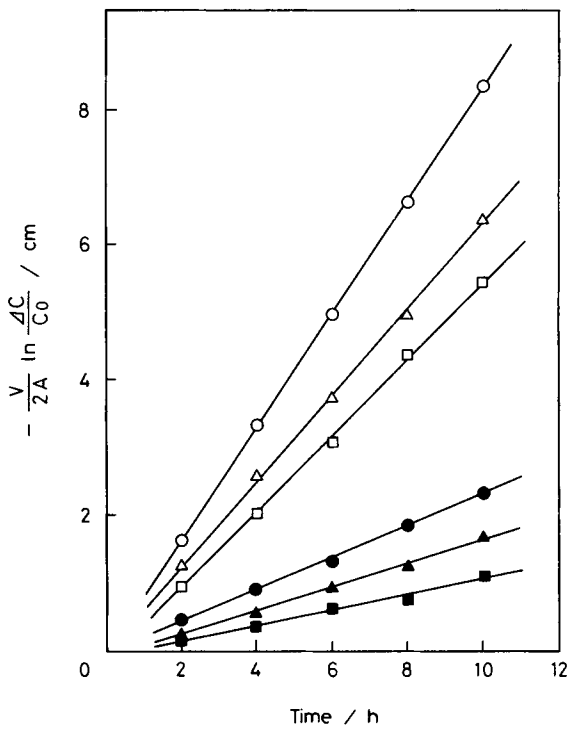


Figure 4 Permeabilities of KCl and sucrose through the modified GC membranes: (○, △, □) KCl, (●, ▲, ■) sucrose, (○, ●) M_{SH} membrane, (△, ▲) M_{SS} membrane, (□, ■) M_{DTPA} membrane. Permeabilities are represented by slopes.

Figure 5 illustrates the schematic structure of each membrane. In the case of the M_{DTPA} membrane, 1.45 mmol/g of disulfide groups, as measured by elemental analysis and titration with $KBrO_3$, were introduced into the side chains. In the case of the M_{SH} membrane, 44% of 3-mercaptopropionic acids were eliminated by reduction. And in the M_{SS} membrane, the remaining thiol groups, 1.87 mmol/g, combined again to form disulfide crosslinkings by the oxidative reaction. Actually, the M_{SH} membrane shrank immediately when it was immersed in a solution of iodine. The backbone of GC, however, is so stiff that the vacancy due to the elimination of 3-mercaptopropionic acid cannot be filled. Consequently, the

Table I Properties and Permeability Coefficients of the Modified GC Membranes

Membrane	Sulfur Content (wt %)	Degree of Swelling	Water Content (wt %)	$P_{KCl} \times 10^6$ (cm^2/s)	$P_{sucrose} \times 10^6$ (cm^2/s)
M_{DTPA}	9.32	2.24	55.4	1.6	0.38
M_{SH}	5.99	5.90	83.1	3.2	1.0
M_{SS}	—	3.76	73.4	2.3	0.69

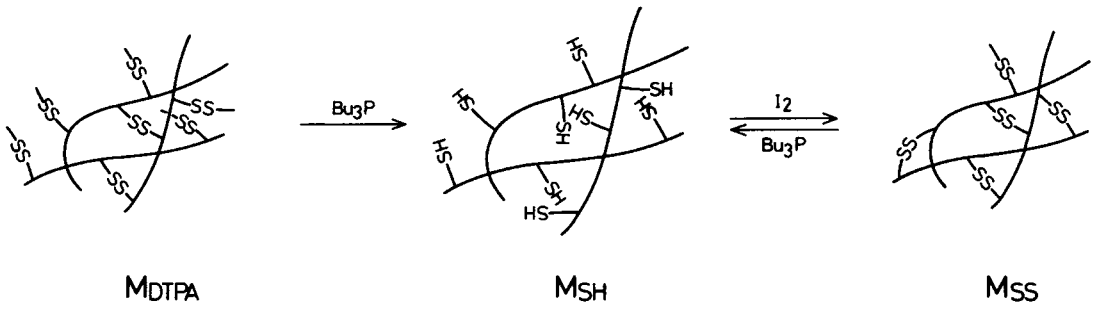


Figure 5 Schematic representation of the structure of each membrane: (—SS—) disulfide group; (—SH) thiol group.

structure of the M_{SS} membrane is thought to be rather dilate in comparison with that of the M_{DTPA} membrane. In order to control the permeability by introducing thiol groups into the side chains, it may be better to use a more flexible macromolecule for the membrane matrix than GC, although the ability to form a membrane should be examined.

The degree of swelling and the water content, which reflect the degree of crosslinking and compactness of the membrane, support the hypotheses explained by the schematic models in Figure 5. The degree of swelling and the water content were in the order of $M_{DTPA} < M_{SS} < M_{SH}$. Similarly, the permeability coefficients of KCl and sucrose through each membrane were in the same order, $M_{DTPA} < M_{SS} < M_{SH}$. The relation between the permeability coefficient and the water content has been studied previously, and the permeability through the hydrophilic membrane is known to depend on the water content.[18] The relation between the permeability coefficients of KCl and sucrose and the water content is shown in Figure 6. Although the water content of our membranes was only in the high region, it is evident that the logarithmic permeability coefficients and the water contents are in a linear relationship and that the slope in the case of sucrose is larger than that in the case of KCl. This suggests that the permeability of sucrose can be controlled more easily than that of KCl in our system.

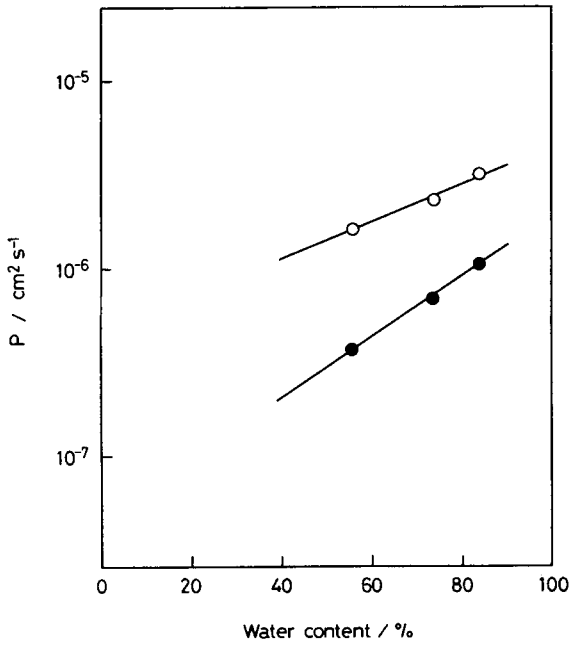


Figure 6 Relationship between the permeability coefficient and the water content: (○) KCl, (●) sucrose.

As with the aforementioned, it was revealed that the GC membrane modified with thiol groups, whose functional groups changed reversibly by redox reaction, was capable of controlling the permeabilities of KCl and sucrose to some extent. Changes in the permeability could be explained in terms of the extent of disulfide crosslinking and the water content of the membrane. In order to accomplish the ON/OFF permeation control, it seems necessary to extend the change of the water content by increasing the number of thiol groups.

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Appendix

For the steady-state analysis of the permeation, Fick's law of diffusion is expressed as follows:

$$\frac{dm}{dt} = -AD\frac{dC}{dx} \tag{1}$$

Here dm/dt is the amount of solute that permeates the membrane in a unit time, A is the area of the membrane, and dC/dx is the concentration gradient in the membrane. The term on left-hand side of Eq. (1) is represented by

$$\frac{dm}{dt} = V\frac{dC_t}{dt} \tag{2}$$

where V is the solution volume of each chamber and C_t is the concentration of solute in the left-hand chamber at a time t. In the term on right-hand side of Eq. (1)

$$\frac{dC}{dx} = S\frac{\Delta C}{\delta} \tag{3}$$

where S is the partition coefficient of solute between the membrane and solution, ΔC is the concentration difference between the left- and right-hand chamber, and δ is the membrane thickness. Assuming that the amount of solute in the membrane and the change of the solution volume are negligible, ΔC is approximated as

$$\Delta C = C_0 - 2C_t \tag{4}$$

where C_0 is the initial concentration of solute in the right-hand chamber. Substituting Eqs. (2), (3), and (4) into Eq. (1), we have

$$V\frac{dC_t}{dt} = -ADS\frac{(C_0 - 2C_t)}{\delta} \tag{5}$$

Integrating Eq. (5) under the initial condition, $C_t = 0$ ($t = 0$), we obtain

$$\frac{1}{2}\ln\frac{(C_0 - 2C_t)}{C_0} = -\frac{At}{V}\frac{DS}{\delta} \tag{6}$$

Therefore,

$$\text{Permeability}\ \frac{DS}{\delta} = -\frac{V}{2At}\ln\frac{\Delta C}{C_0} \tag{7}$$

$$\text{Permeability coefficient}\ DS = -\frac{V\delta}{2At}\ln\frac{\Delta C}{C_0} \tag{8}$$