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Effects of Charge Density on Drug Permeability through Alginate Gel Membranes

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Preparation methods for the alginate (Alg) gel membranes were developed and characterized. Three kinds of Alg gel membrane (mannuronate/guluronate ratio (M/G ratio)=1.00, 0.43, and 0.19) were prepared by a casting method, and the effects of M/G on characteristics such as transmittancy, viscoelasticity, effective charge densities, and drug permeability were investigated. The water content and membrane thickness of the Alg gel membranes were not affected by the M/G ratio, however, transmittancy and viscoelasticity were found to be significantly affected by them. Charge densities (θ) were obtained using a membrane potential method, and these θ values increased with M/G ratio, and dissociation of the carboxylic groups of uronates was found to be suppressed significantly in the network. The permeability coefficients (*P*) of sodium benzoate through the membranes decreased with increasing M/G ratio. The *P* values are discussed in terms of electrostatic interaction, structure of the gel network, and bound and restricted water.

Keywords alginate; hydrogel; membrane; charge density; permeability

Hydrogels are formed by three-dimensional hydrophilic polymer networks and can store molecules such as drugs. They swell or shrink corresponding to changes in the bulk conditions. Using these characteristics, hydrogels have been applied widely in the pharmaceutical field, for example as controlled delivery devices for various drugs.

Alginate (Alg) is a major structural polysaccharide found in brown algae as intercellular material, and is a linear block copolymer composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues, *i.e.*, the chain is composed of G-blocks including only G residues, M-blocks including only M residues, and MG-heteroblocks including both M and G residues. Alg forms hydrogels in the presence of divalent metal ions such as Ca^{2+}, and the crosslinks are formed by chelate complexing between divalent ions and the carboxylate anions of G–G blocks.[1,2] The important characteristics of Alg gel are summarized as follows. 1) The sol–gel transition occurs without any alteration in the temperature and/or any chemical crosslinking agents. 2) Alg gel can be easily converted into the sol by adding Na^+, Mg^{2+}, and EDTA. 3) Alg gel has charge densities due to the carboxyl groups. 4) Alg is non-toxic and biodegradable. Taking advantage of these characteristics of lyotropic gels, Alg is a very useful polysaccharide for applications in the pharmaceutical field, and can be used for basic studies of gelling effects on immobilized enzymes[3—5].

The release from Alg gels has been studied using beads and microgels, readily prepared by dropping sodium alginate (NaAlg) solution into calcium chloride solution. A few basic studies of permeability through Alg gel membranes have been reported so far. Parisa and Ross reported[6] the permeabilities of Alg gel membranes prepared by a drying method. Since Alg gels are charged due to the carboxylic acid groups of the M–M and M–G blocks, elucidation of the characteristics of charged Alg gel membranes is very important. In this report, to elucidate the effects of composition on the characteristics of Alg gels, three kinds of Alg gel membranes were prepared, and the effective charge densities and permeability were studied. Alg gel membranes prepared by the drying method had relatively low water content resulting from the effects of entanglement of the Alg molecules during the drying process. In this study, Alg gel membranes were prepared without drying and had much higher water content than those by the drying method.

Experimental

Three kinds of NaAlg sample (45G, 45 and 45M) with different M/G ratio were purchased from Kibun Food Chemifa Co. (bioreactor grade duck algine), and used without purification. The M/G ratios of 45G, 45 and 45M were 0.19, 0.43, and 1.00, respectively, and the degree of polymerization was 660. Sodium benzoate (NaBA) (Katayama Chemical Co.) and all other reagents were of special grades. Distilled and deionized water was used for the preparation of aqueous solutions.

Preparation of Alg Gel Membranes Alg gel membranes were prepared by casting NaAlg aqueous solutions (7 w/v%, 3 cm^3) on a glass plate (diameter: 8.5 cm) as shown in Fig. 1 and allowed to stand at room temperature for 1 h to make their thickness homogeneous. The NaAlg solutions did not effuse from the edge of the glass plate due to high viscosity. Next, the samples were immersed in 0.1 $mol \cdot dm^{-3}$ $CaCl_2$ solution at room temperature for 1 d, and the NaAlg solutions on the glass plate were gelled in the form of a flat membrane. They were then left to stand in 0.1 $mol \cdot dm^{-3}$ $CaCl_2$ solution at 4 °C for at least 1 week, and before measuring permeability, were preconditioned at 25 °C in 0.1 $mol \cdot dm^{-3}$ $CaCl_2$ and then in water.

Water contents of Alg gel membranes were obtained from the changes in the weight after drying at 90 °C for 6 h. The transmittance was measured

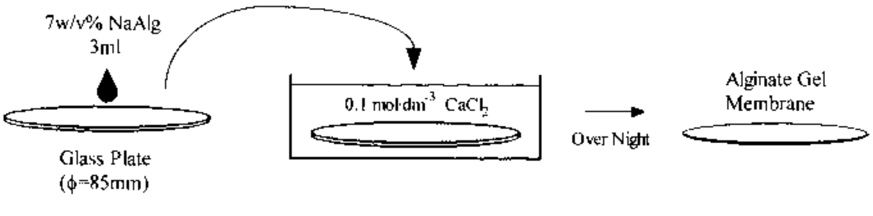

Fig. 1. Schematic Diagram of Preparation Method for Alg Gel Membrane

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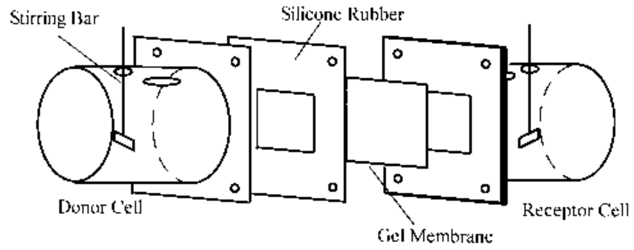

Fig. 2. Schematic Diagram of Diffusion Cell

using a spectro multi channel photo detector (MCPD-1000: Otsuka Electronics Co.) at $\lambda=600$ nm, a wavelength at which Alg do not have characteristic absorbance.

Measurement and Analysis of Stress Relaxation Stress relaxation of the Alg gel membranes was measured using Creep Meter (Yamaden Co. RE-33005). Strips (length: 3 cm, width: 1 cm, thickness: 0.905—0.922 cm) were fixed by clamps and suspended in $CaCl_2$ solution (0.1 mol·dm^{-3}). Strains γ were imposed and stress relaxations $\sigma(t)$ were measured for 24 h at 25 °C.

The stress relaxation was analyzed by the Maxwell model which is expressed by Eq. 1.

$$\sigma/\gamma=G_0+\sum G_i\exp(-t/\tau_i) \tag{1}$$

Where G_0 is equilibrium elastic modulus, G_i and τ_i are ith component of elastic modulus and relaxation times. These parameters were obtained using a curve fitting method.

Measurement and Determination of Permeability of Alg Gel Membranes Membrane permeability coefficients (P) of NaBA were measured at 25 °C using a diffusion cell made of an acrylate resin, as shown in Fig. 2. The Alg gel membrane was fixed between compartments I (donor cell) and II (receptor cell), which were filled with 50 cm^3 NaBA solution and water, respectively. The surface area of membranes was 4.0 cm^2. Both solutions were stirred continuously at 600 rpm by an external drive unit. From the receptor cell, sample solutions were removed at intervals of 20 min and replaced with equal volumes of water. The absorbency of NaBA in the sample solutions was determined using a spectro multi channel photo detector (MCPD-1000: Otsuka Electronics Co.) at 228 nm.

By approximating the movement of water due to osmotic pressure as negligible, total amount of NaBA permeating from the donor to the receptor compartment until ith sampling time Q_i is obtained by Eq. 2

$$Q_i=C_i^{\mathrm{II}}V+\sum_{j=1}^{i-1}C_j^{\mathrm{II}}V_{\mathrm{s}} \tag{2}$$

where C_i^{II} is the concentration of NaBA in the receptor compartment at ith sampling, and V_{s} is the volume of the sample solution. The flux J was obtained from the linear slope between Q and t, and the membrane permeability coefficient P was obtained by Eq. 3,[7)]

$$J=-P(C^{\mathrm{II}}-C^{\mathrm{I}})/l \tag{3}$$

where l is the thickness of the membrane.

Measurement of Membrane Potential In order to estimate the effective charge densities of the Alg gel membranes, the membrane potentials between compartments I and II were studied by inserting an Ag–AgCl electrode in each compartment using the same cell as shown in Fig. 2. Different concentrations of $CaCl_2$ solution were poured into both compartments and the concentration ratio R ($=C^{\mathrm{I}}/C^{\mathrm{II}}$) was kept at 2. Both solutions were well stirred by magnetic stirrers, so the effect of stagnant layers could be neglected. The membrane potentials E between solutions I and II were measured at 25 °C using Ag–AgCl electrodes, whose electrode potentials had been obtained by using Kieland's individual activity coefficients.

The membrane potential E is the summation of the diffusion potential in the membrane phase E_{D} and the Donnan potentials (boundary potentials) E_{B} at both interfaces; they are expressed by the following equation[8)]

$$E=\frac{RT}{F}\left\{(1/z_{\mathrm{M}}-t_{\mathrm{A}}(1/z_{\mathrm{M}}-1/z_{\mathrm{A}}))\ln\frac{\bar{C}_{\mathrm{A}}^{\mathrm{II}}+(1-t_{\mathrm{A}})\theta/z_{\mathrm{M}}}{\bar{C}_{\mathrm{A}}^{\mathrm{I}}+(1-t_{\mathrm{A}})\theta/z_{\mathrm{M}}}\right.$$
$$\left.+1/z_{\mathrm{M}}\ln\left(\frac{C_{\mathrm{A}}^{\mathrm{I}}}{C_{\mathrm{A}}^{\mathrm{II}}}\right)\left(\frac{\bar{C}_{\mathrm{A}}^{\mathrm{II}}}{\bar{C}_{\mathrm{A}}^{\mathrm{I}}}\right)\right\} \tag{4}$$

where θ and t_{A} are the effective charge density of the membrane and the transference number of an anion in it. $\bar{C}_i^{\mathrm{I}}$ and $\bar{C}_i^{\mathrm{II}}$ are the surface concentrations of the anion and can be obtained by using the Donnan equilibrium between ions in the membrane phase and in the bulk solutions at each interface (Eq. 5), and the electroneutrality condition in the membrane phase (Eq. 6)

$$(\bar{C}_{\mathrm{A}}/C_{\mathrm{A}})^{1/z_{\mathrm{A}}}=(\bar{C}_{\mathrm{M}}/C_{\mathrm{M}})^{1/z_{\mathrm{M}}} \tag{5}$$

$$z_{\mathrm{M}}\bar{C}_{\mathrm{M}}+z_{\mathrm{A}}\bar{C}_{\mathrm{A}}+\theta=0 \tag{6}$$

where z_{A} and z_{M} are valencies of the anion and the cation, respectively.

Table 1. Characteristic Values of Alg Gel Membranes

M/G	Transmittancy (%)	W_{w} (%)	l (mm)
1.00	3.87	92	0.905
0.43	42.03	93	0.922
0.19	74.30	92	0.908

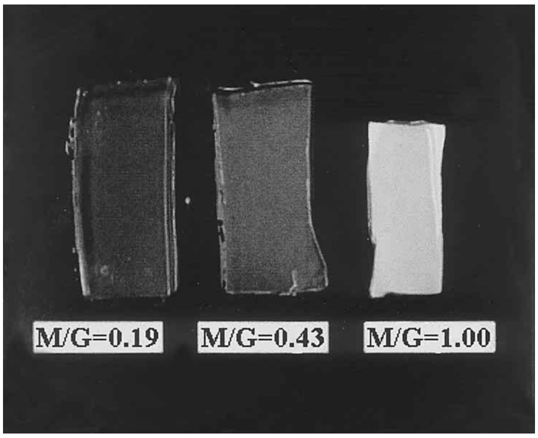

Fig. 3. Appearance Change of Alg Gel Membranes due to M/G ratio

Results

Characteristic of Alg Gel Membranes Three kinds of Alg gel membranes differing in M/G ratio were prepared by immersing a flat layer of NaAlg solution (7 w/v%) in $CaCl_2$ solution (0.1 mol·dm^{-3}). Thickness could be controlled by the amount of NaAlg solution cast on the glass plate (diameter: 8.5 cm). In these experiments, 3 cm^3 of the NaAlg solution was cast. Irrespective of the M/G values, the gel membranes had almost the same thickness l and water content W_{w}. The values of l were in the range 0.905—0.922 mm and W_{w} were in the range 92—93%, as shown in Table 1.

However, transmittancy and viscoelasticity of the Alg gel membranes were affected significantly by the M/G ratio. Figure 3 shows a photograph of the three kinds of Alg gel membranes. They become opaque with increasing M/G ratio. The transmittance was measured at λ=600 nm, at which the absorbency was 0. As shown in Table 1, the transmittance values decreased from 0.74 to 0.039 with increasing M/G ratio.

The tensile stresses of Alg gel membranes (M/G=0.19 and 1.0) were measured under the strain $\gamma=0.1$ at 25 °C. The results showed long relaxation and attained equilibrium states after more than 5 h as shown in Fig. 4. These results were analyzed by a curve fitting method using Maxwell's multicomponent model expressed by Eq. 1, and were found to be approximated by a model composed of long and short relaxation components. The solid curves of Fig. 4 are the re-

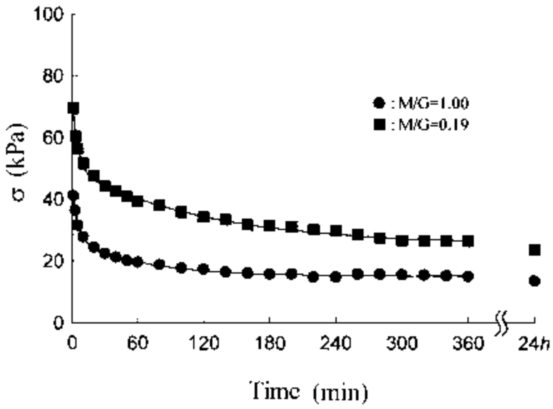

Fig. 4. Stress Relaxation Curves of Alg Gel Membranes under a Constant Tensile Strain ($\gamma=0.1$)

Table 2. Elastic Moduli and Relaxation Times of Alg Gel Membranes

M/G	G_0 (kPa)	G_1 (kPa)	τ_1 (min)	G_2 (kPa)	τ_2 (min)
1.00	151.9	175.6	4.71	118.7	61.1
0.19	253.4	241.4	4.49	242.5	121.8

sults calculated using the most suitable parameters, shown in Table 2. The equilibrium elastic moduli G_0 of Alg gel membranes of M/G=0.19 and 1.0 were 2.53×10^5 and 1.52×10^5 Pa. The elastic moduli G_1 and G_2 of M/G=0.19 were also greater than those of M/G=1.0.

From these results, it was found that the water content of the Alg gel membranes were not affected by the values of the M/G ratio, but transparency and viscoelastic properties were affected significantly by them.

Permeability of NaBA through Alg Gel Membranes Permeability of Alg gel membranes was studied using NaBA as a solute at 25 °C. The original concentration of NaBA in donor compartment I was $C^{\mathrm{I}}=0.01\ \mathrm{mol\cdot dm^{-3}}$ and diffusion into compartment II (water) was measured. Total amounts of NaBA Q_{NaBA} permeating through Alg gel membranes are shown in Fig. 5 as a function of time *t*. The values of Q_{NaBA} increased linearly over the experiment time (2 h), irrespective of the composition of Alg and the lag times were negligible. The values of Q_{NaBA} during 2 h were in about 3% of the original amount of NaBA in the donor compartment. The thicknesses of Alg gel membranes were almost the same before and after the experiments.

From the flux values $J_{\mathrm{s}}\ \mathrm{mol\cdot cm^{-2}\cdot s^{-1}}$, being the inclinations of the linear relations, permeability coefficients P $\mathrm{cm^2\cdot s^{-1}}$ were obtained by Eq. 3 using the averages of the initial and the final concentrations in each compartment. As shown in Fig. 6, the values of P were found to increase with decreasing M/G ratio although the difference between the membranes of M/G=0.43 and 1.0 was slight.

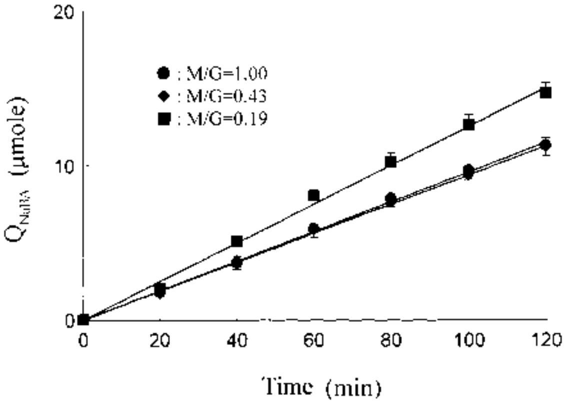

Fig. 5. Time Courses of NaBA through Alg Gel Membranes

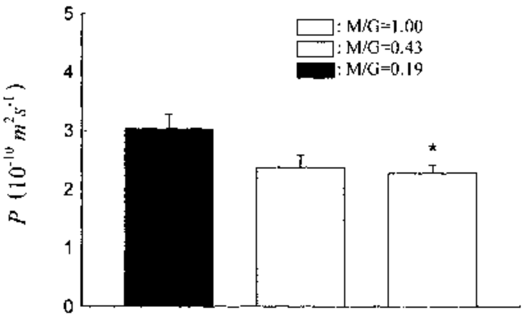

Fig. 6. Permeability Coefficients of NaBA through Alg Gel Membranes

Each bar represents the mean±S.E. Significantly different from M/G=0.19 value, $*p<0.05$.

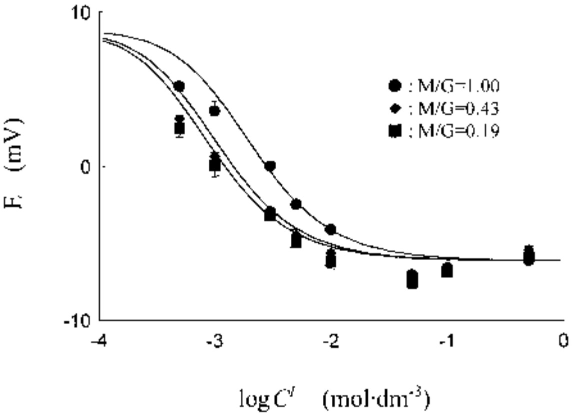

Fig. 7. Membrane Potentials of Alg Gel Membranes

Effective Charge Densities of Alg Gel Membranes The effective charge densities θ of the Alg gel membranes were estimated by the membrane potential method. The junctions of the Alg gel membranes formed by $\mathrm{Ca^{2+}}$ ions can be destroyed by counterion exchange with non-crosslinking counterions such as $\mathrm{Na^+}$ and $\mathrm{Mg^{2+}}$ ions. Thus, when electrolyte solutions in the compartments separated by the membranes contain non-crosslinking counterions, the charge densities should change. As shown in a previous paper,[9] $\mathrm{Ca^{2+}}$ ion binding of Alg gel prepared in $\mathrm{CaCl_2}$ solution ($0.1\ \mathrm{mol\cdot dm^{-3}}$) is in the saturated state. As the binding of $\mathrm{Ca^{2+}}$ ions with G–G block are strong, the junctions is considered to be maintained in more dilute $\mathrm{CaCl_2}$ solutions. In this experiment, the membrane potentials E between compartments I and II were measured using $\mathrm{CaCl_2}$ solutions with increasing concentrations and with a constant ratio ($C^{\mathrm{I}}/C^{\mathrm{II}}=2$) at 25 °C. The results E are shown in Fig. 7 as a function of $\log C^{\mathrm{I}}$. As expected from the theory of membrane potential of a charged membrane, the results showed sigmoidal curves decreasing with increasing C^{I} and attaining to constant values. Their reflection points decreased with decreasing values of M/G ratio. The concentrations C^{I} of the reflection points are a

Table 3. Charge Densities θ and Membrane Constants f of Alg Gel Membranes

M/G	θ (mol·dm^{-3})	θ/θ_0*	f
1.00	-9.00×10^{-3}	3.11×10^{-2}	0.234
0.43	-4.68×10^{-3}	—	0.236
0.19	-3.96×10^{-3}	3.74×10^{-2}	0.299

* θ_0: Concentration of the carboxylic groups in the Alg gel membrane.

rough indication of the effective charge densities. To obtain more precise values, effective charge densities θ were estimated using a curve fitting method. The solid curves in Fig. 7 show theoretical plots obtained with Eq. 4 by selecting the most suitable θ values so as to fit the experimented results in the region of $C^{\mathrm{I}}>1.0\times10^{-3}$ mol·dm^3 since experimental values at lower concentrations contain errors. For the transference number of Cl$^-$ ion in Eq. 4, the reference value in aqueous solution[10] was used. As shown in Table 3, the θ values of Alg gel membranes were negative and in the range of -4×10^{-3}—9×10^{-3} mol·dm^{-3}. Their absolute values were found to decrease with decreasing M/G ratios.

Discussion

Suppressing Effect on Alg Network of Dissociation of Carboxylic Groups Alg possesses one carboxylic group per one uronate unit. The concentration of the carboxylic group in the NaAlg solution (7 w/v%) was 0.353 mol·dm^{-3} and those of the Alg gel membranes θ_0 are shown in Table 3. The θ_0 values were obtained using approximate density values ($\rho=1.04$ g·cm^{-3}) for the Alg membranes, estimated from the water content as shown in Table 1. Crosslinking of Alg by divalent ions occurred between the G–G blocks and at the resultant junctions, the divalent ions bind to two carboxylic groups. The carboxylic groups of M–M and M–G blocks do not contribute to formation of the junction. Therefore, their free carboxylic groups should contribute to the values of θ. When the fraction of the junctions in a Alg molecule is F_j on the basis of the concentration of uronate units and the binding ratio of Ca^{2+} ions on the Alg chains between the junctions is $(\sigma_i)_{\mathrm{Ca}}$, the fraction of ionized carboxyl groups in CaAlg gels can be expressed by

$$\theta/\theta_0=(1-F_j)(1-(\sigma_i)_{\mathrm{Ca}}) \tag{7}$$

where Ca^{2+} ions entrapped in the junctions are assumed to bind completely. Assuming all G–G blocks contribute to form the junction, $\theta_0(1-F_j)$ is the concentration of free carboxylic groups in the Alg gel membranes θ_0. Table 3 shows θ and the ratio of θ/θ_0. The values of θ/θ_0 were in the range 0.037—0.031, from which the values of $(\sigma_i)_{\mathrm{Ca}}$ were determined to be 0.96—0.97. The binding ratio of Ca^{2+} ions was found to be significant. In our previous paper,[11] the effective charge densities of CaAlg gels were obtained by measuring the membrane potentials of multimembranes in which the concentration of Alg was very low (0.25—0.63 w/v%), compared with the Alg gels used in this work (7—8 w/w%). The values of $(\sigma_i)_{\mathrm{Ca}}$ were reported to be almost 0.88. By comparing the binding ratio of Mg^{2+} in the Alg sol state ($(\sigma_i)_{\mathrm{Mg}}=$ 0.72), Ca^{2+} ion binding was suggested to be enhanced by gellation in the regions between the junction, *i.e.*, in the network. From the results of this paper and the previous paper, the values of $(\sigma_i)_{\mathrm{Ca}}$ are considered to increase with decreasing water content of Alg gel.

Effects of Charge Density of Gel Membrane on Permeability of Ionic Solute When a nonelectrolyte solute permeates through a membrane, the permeability coefficient P can be expressed by Eq. 8 using the diffusion coefficient of the solute in aqueous solution D_0,

$$P=fbD_0 \tag{8}$$

where b is the partition coefficient of the solute at the membrane surface, and f is the membrane constant relating to the porosity ε and the tortuosity q ($f=\varepsilon/q^2$). When an electrolyte (valencies of cation; z_{M} and anion; z_{A}) permeates through a charged membrane possessing an effective charge density θ, the value of P can be expressed by Eqs. 9—11,[12]

$$P=fb(1+X)D_0 \tag{9}$$

$$b=\frac{\bar{C}_{\mathrm{A}}^{\mathrm{II}}-\bar{C}_{\mathrm{A}}^{\mathrm{I}}}{C_{\mathrm{A}}^{\mathrm{II}}-C_{\mathrm{A}}^{\mathrm{I}}} \tag{10}$$

$$X=\frac{(1/z_{\mathrm{M}}-z_{\mathrm{A}})+t_{\mathrm{A}}/z_{\mathrm{A}})\theta}{\bar{C}_{\mathrm{A}}^{\mathrm{II}}-\bar{C}_{\mathrm{A}}^{\mathrm{I}}} \times\ln\frac{((z_{\mathrm{M}}-z_{\mathrm{A}})/z_{\mathrm{M}})\bar{C}_{\mathrm{A}}^{\mathrm{II}}+((z_{\mathrm{M}}-z_{\mathrm{A}})(1-t_{\mathrm{A}})/z_{\mathrm{M}}z_{\mathrm{A}})\theta}{((z_{\mathrm{M}}-z_{\mathrm{A}})/z_{\mathrm{M}})\bar{C}_{\mathrm{A}}^{\mathrm{I}}+((z_{\mathrm{M}}-z_{\mathrm{A}})(1-t_{\mathrm{A}})/z_{\mathrm{M}}z_{\mathrm{A}})\theta} \tag{11}$$

where X is the electrostatic term. The term X depends on the concentration of the solute. Using the mean concentrations of NaBA in compartments I and II, the values of X and b were calculated. The values of X were negative and with decreasing M/G ratios approached to 0, as expected from the values of θ, and the values of b were almost equal to 1.

The membrane constants f of Alg gel membranes were obtained by Eq. 9 using the diffusion coefficients of NaBA in aqueous solution[13] D_0 at the mean concentrations. As shown in Table 3, the values of f were found to increase slightly with decreasing M/G ratios.

Effects of Water Content on Membrane Constant Assuming there is no interaction between a solute and a gel network, the value of P/D_0, *i.e.*, membrane constant f, is obtained using a lattice model by the following Mackie & Meares's equation,[14]

$$P/D_0=\left(\frac{\varepsilon}{2-\varepsilon}\right)^2 \tag{12}$$

where ε is porosity which is a volume fraction of free water in a network. Figure 8 shows the theoretical values of f as a function of ε.

Solutes can diffuse through the free water in networks according to their diffusion coefficients. Alg are reported[15] to possess much bound water around them because of the hydrophilicity. The bound water contents of NaAlg solutions estimated from differential scanning calorimetry (DSC) measurements were independent of M/G ratios and were 0.58 g per 1.0 g NaAlg. Approximating this bound water content to those in Alg gels, the bound water contents of the Alg gel membranes W_{b} were estimated to be 0.05. The porosities ε of Alg gel membranes were obtained using the densities of Alg gel ($\rho=1.03$ g·cm^{-3}) reported previously[16] and the density of water ρ_0 at 25 °C as that of the free water.

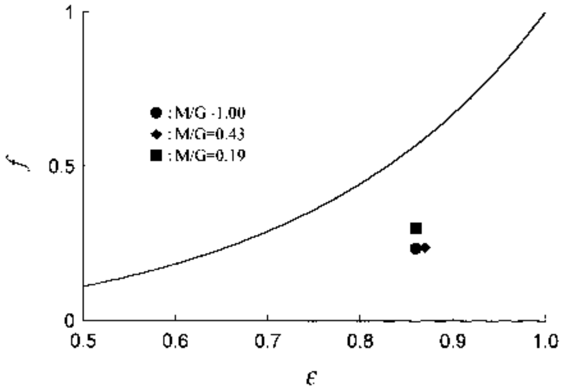

Fig. 8. The Relation between Membrane Constants *f* and Porosity ε

Solid line shows theoretical values of Mackie and Meares's equation.

$$\varepsilon=(\rho/\rho_0)(W_w-W_b) \tag{13}$$

The results of *f* for the Alg gel membranes shown in Table 3 are plotted in Fig. 8 as a function of ε. They were found to be much smaller than the theoretical values. Comparing the results *f* of Alg gel membranes with the same theoretical values, the discrepancies of ε were $\Delta\varepsilon\approx 0.15$. As shown in the previous paper,[17] the relation between the values of *f* obtained from the permeabilities of KCl through gelatin membranes and the values of ε were matched to Eq. 13. The discrepancies in the case of Alg gel membranes are considered to result from underestimation of the bound water. The bound water obtained by the DSC measurement is an unfrozen water. Furthermore, besides bound water, restricted water could present in the Alg gel networks which suppresses the diffusion of NaBA through Alg gel membranes.

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