Characteristics of Hyaluronate-hydroxyethyl Acrylate Blend Gel and Release of Cationic Amphiphilic Solutes

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Hyaluronate-hydroxyethyl acrylate blend gel (HA-PHEA) were prepared to modify the brittleness of hyaluronate gel (HA) and the characteristics of HA-PHEA gel were compared with those of HA and polyhydroxyethyl acrylate (PHEA) gels. These gels were high in water content and transparent. HA-PHEA gel was improved in viscoelastic properties due to the elasticity and the high affinity with water of PHEA, and the drying-swelling cycles became reversible. The effective charge densities θ of the gels estimated from membrane potentials were -0.002, -0.008 and 0 mol dm^{-3} for HA-PHEA, HA and PHEA gels. Effects of electro- static and nonelectrostatic interactions on absorptions and releases were studied using sodium benzoate (NaBA) as an anionic solute, and methylene blue (MB), chlorpromazine (CPHCl) and benzethonium chloride (BZTCl) as cationic solutes, in which CPHCl and BZTCl are cationic amphiphilic solutes. The releases of MB, CPHCl and BZTCl from HA-PHEA and HA gels were suppressed comparing with those of NaBA. By adding salts, the releases of MB and CPHCl were enhanced but those of BZTCl were suppressed due to enhancement of the intra- and intermicelle formation. In the releases of the cationic solutes from HA-PHEA gel, electrostatic and nonelectrostatic interactions with HA were found to play important roles. Behaviors of the releases from HA-PHEA gel were found to possess the features of HA gel.

Key words hyaluronate gel; hydroxyethyl acrylate; blend hydrogel; cationic solute; absorption; release control

Hydrogels are formed from hydrophilic synthetic polymers and many natural polymers such as proteins and polysaccharides. Natural polymer gels are useful in pharmaceutical fields for such uses as controlled delivery devices because of their biocompatibility and biodegradability. We are interested in hyaluronate (HA) from a pharmaceutically applicable point of view. HA is an acid polysaccharide and an important component of vitreous body, cartilages and connective tissues such as intercellular matrixes of skins. It is a linear block copolymer of repeating units composed of *N*-acetyl-D-glucosamine and D-glucuronic acid connected by alternate β (1-3) and β (1-4) glucosidic bonds. HA solutions are significantly viscous due to their very high molecular weight and possess counterion exchange abilities because of the presence of carboxylic groups. The solutions are transparent and are used as a surgical tool for the eye and a remedy for arthritis. HA is also a hydrophilic polymer and has high ability preserving water. However, it is reported to possess a hydrophobic plane on which axial H groups are extended, and to form single and double helical structures in solutions due to intra- and intermolecular interactions.[1] In our laboratory, the characteristics of HA have been studied for its interfacial adsorptions and the substance was found to form network structures on a bovine serum albumin (BSA) monolayer.[2,3]

As HA itself is unable to form a gel, it was gelled using a chemical crosslinking agent, and their useful characteristics have been reported for application in the pharmaceutical field.[4—9] In our laboratory, blended HA-PHEA gels composed of HA and polyhydroxyethyl acrylate (PHEA) have been studied and their characteristics reported.[10,11] Their water contents and release rates were shown to be controlled by the ratio of PHEA to HA. In this paper, the characteristics of HA-PHEA gel were compared with HA and PHEA gels and the validity of HA-PHEA gel was studied. These gels differ in crosslinking mechanisms. PHEA gel is formed by crosslinks due to hydrogen bonds between OH groups, but HA and HA-PHEA gels are formed by chemical crosslinks. Especially, the effects of electrostatic and nonelectrostatic interactions on adsorptions and releases were studied using sodium benzoate (NaBA) as an anionic solute, and methylene blue (MB), chlorpromazine hydrochloride (CPHCl), and benzethonium chloride (BZTCl) as cationic solutes. MB is a cationic dye, CPHCl is reported to form an association[12] and BZTCl is an cationic surfactant.

Experimental

Materials Sodium hyaluronate (NaHA) (M_w: 2.09×10^6 g mol^{-1}) from *Streptococcus zooepidemicus* was purchased from Kibun Food Chemifa. (Tokyo, Japan) and was used without any purification. Ethylene glycol diglycidyl ether (EGDE) (Aldrich Chemical Company, Inc.), Glycidyl methacrylate (GMA) and 2,2′-azobis [2-methyl-*N*-(2-hydroxyethyl)-propion amide] (VA086) as an initiator of polymerization were special grades (Wako Pure Chemical, Osaka), and 2-hydroxyethyl acrylate (HEA) (Wako Pure Chemical) was of first grade. Other chemicals were of analytical grades. Distilled and deionized water was used for the preparation of aqueous solution.

NaBA (Katayama Co., Ltd.), methylene blue trihydrate (MB) (Nakalai Tesque. Inc.), chlorpromazine hydrochloride (CPHCl) (Wako Pure Chemical), benzethonium chloride (BZTCl) (Wako Pure Chemical), triton X-100 (Nakalai Tesque. Inc.) were special grades. D_2O was 99.8% in D_2 (Wako Pure Chemical). Chemical structures of cationic solutes are shown in Fig. 1a.

Preparation of HA-PHEA, HA and PHEA Gels HA-PHEA blend gel was prepared by the method previously described.[10,11] Glycidyl methacryl hyaluronate (GMA-HA) was prepared by mixing NaHA (0.05 mol dm^{-3} in carbonate buffer (pH 11), 40 cm^3) and GMA (7.6 mmol dm^{-3}, 1.0 cm^3) at room temperature.[13—17] After adjusting to pH 7, the solution was purified by dialyzing. The molar mass of the GMA-HA was determined to be $M_w=2.06\times10^6$ g mol^{-1} using GPC-MALLS (DAWN, Wyatt Co., Ltd.) and was almost equal to that of the original NaHA. HEA was dissolved in GMA-HA solution (2 w/v%) at a weight ratio of 5/1 and a photo induced polymerization initiator VA086 was added at the ratio of 0.4 mg cm^{-3}. The degassed solutions (1 cm^3) were put into a disk cell for the gel preparation ($16\phi\times2$ mm) shown in Fig. 1b and irradiated by a 400 W Hg lamp (Type H400-P, Toshiba

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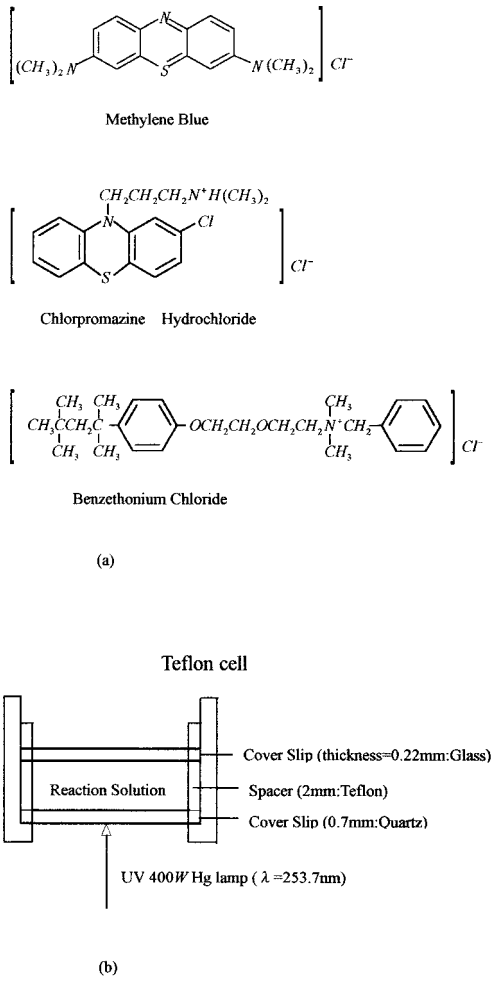

Fig. 1. Chemical Structures of Cationic Solutes and Cell for Preparation of Gels by Photoinduced Radical

(a) Chemical structures of cationic solutes. (b) Cell for preparation of gels by photoinduced radical.

Co., Ltd., Tokyo) for 20 min. The obtained HA-PHEA gels were dialyzed in distilled water. Schematic diagrams of the preparations of GMA-HA and HA-PHEA gel are shown in Figs. 2a and b.

PHEA gel was prepared by adding VA086 to HEA solution (7 w/v%) at the ratio 0.4 mg cm^{-3}. The degassed solution was put into the disk cell and was irradiated by a 400 W Hg lamp using the same methods as for HA-PHEA gel. Scheme of the preparation of HA gel is shown in Fig. 2C. HA gel was prepared by adding EGDE to NaHA solution (20 w/v% in 1 mol dm^{-3} NaOH) at the volume ratio 1/9 and by heating at 60 °C for 15 min.[5,6)]

Measurements of ^{1}H-NMR ^{1}H-NMR spectra of GMA, NaHA and GMA-HA were measured using a Lambda 400 MHz spectrometer (LAM BDA-NMR, JEOL) in D_2O. Sample solutions were prepared by dissolving GMA, NaHA and GMA-HA (10 mg) in D_2O (0.8 ml). D_2O was used as a reference (δ= 4.8 ppm) and accumulation was 64 times.

Measurement of Viscoelasticities Viscoelasticities of HA-PHEA, HA and PHEA gels were measured using a Creep meter (RE-3305, YAMADEN Co. Tokyo) at 25 °C. The front of the probe was a circular plate 0.8 cm in diameter and creeps can be measured with the accuracy 0.001 cm. Results were analyzed by Voigt's model and viscoelastic parameters were estimated by a curve fitting method. Using a 6 element model composed of springs and dashpots, a strain γ is expressed by Eq. 1,

$$\gamma=\frac{P_0}{E_0}+\frac{P_0}{E_1}(1-\exp(-tE_1/\eta_1))+\frac{P_0}{E_2}(1-\exp(-tE_2/\eta_2))+\frac{P_0}{\eta_N}t \quad (1)$$

where P_0 is constant stress, E_0 is instantaneous elastic modulus, E_1 and E_2 are moduli of elasticity of Voigt's model, τ_1 and τ_2 are retardation time, η_1, η_2 and η_N are viscosity coefficient of Voigt's model. A two element model is expressed by the summation of the first and fourth terms.

Measurement of Membrane Potential To estimate the effective charge densities of the gels, the membrane potentials E between compartments I and II separated by the gel disks were measured by a potentiometer (175 Autoranging Multimeter, KEITHLEY) using calomel electrodes connected by salt bridges. KCl solutions were used under the constant concentration ratio of C^{I}/C^{II}=2 at 25 °C. Both solutions were well stirred by magnetic stirrers, so that the effect of stagnant layers was negligible.[18,19)] 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 Eq. 2 when compartment I is a reference,[18,19)]

$$E=\frac{RT}{F}\left\{(1/z_M-t_A(1/z_M-1/z_A))\ln\frac{\bar{C}_A^{II}+(1-t_A)\theta/z_M}{\bar{C}_A^{I}+(1-t_A)\theta/z_M}+1/z_M\ln\left(\frac{C_A^{I}}{C_A^{II}}\right)\left(\frac{\bar{C}_A^{II}}{\bar{C}_A^{I}}\right)\right\} \quad (2)$$

where θ and t_A are the effective charge density of the membrane and the transference number of an anion in it. $\bar{C}_i^{I}$ and $\bar{C}_i^{II}$ are the surface concentrations of the ion i (A: anion; M: cation). They are obtained by using the Donnan equilibrium between the ions in the membrane phase and in the bulk solutions at each interface (Eq. 3) and the electroneutrality in the membrane phase (Eq. 4)

$$(\bar{C}_A/C_A)^{1/z_A}=(\bar{C}_M/C_M)^{1/z_M} \quad (3)$$

$$z_M\bar{C}_M+z_A\bar{C}_A+\theta=0 \quad (4)$$

where z_A and z_M are valencies of the anion and the cation.

Measurements of Adsorption Amounts and Equilibrium Partition Coefficients The equilibrium adsorption amounts n_e of NaBA, MB, CPHCl and BZTCl in the gels were measured by immersing them in the solutions of C_0=0.01 mol dm^{-3} for 72 h at 25 °C. The values of n_e were determined from the differences of the absorbance between the initial and the equilibrated solutions using a spectrophotometer (UV-2400PC, Shimadzu, Kyoto) at λ=225 nm for NaBA, at λ=292 nm for MB, λ=254 nm for CPHCl and at λ=269.5 nm for BZTCl. Equilibrium partition coefficient b was obtained by Eq. 5.

$$b=(n_e/V_g)/C_0 \quad (5)$$

where V_g is equilibrium volume of the gel.

Measurement of Releases of NaBA and Cationic Solutes through Gels NaBA, MB, CPHCl and BZTCl were used as solutes, and the gel disks were equilibrated with a solution of 0.01 mol dm^{-3} for 72 h at 25 °C. Release of solutes into bulk solutions (volume V: 50 cm^3) from the gels was measured under various ionic strengths at 25 °C. Bulk solutions were stirred at 800 rpm under which release rates were confirmed to be constant. Sample solutions (3 cm^3) were withdrawn at regular intervals of 30 min and replaced with equal volumes of the media. The absorbances of NaBA, MB, CPHCl and BZTCl in the sample solutions were determined using a spectrophotometer. Total released amounts up to ith sampling time Q_i are obtained by Eq. 6

$$Q_i=C_iV+\Sigma C_jV_s \quad (6)$$

where C_i is the concentration of the solute in ith sampling solution, V is the volume of the bulk solution and V_s is the volume of the sampling solution.

Measurements of Critical Micelle Concentration of CPHCl and BZTCl Specific conductivities κ of MB, CPHCl and BZTCl solutions were measured under various ionic strengths I adjusted by NaCl at 25 °C using a conductivity meter (DS-12, Horiba, Kyoto, Japan). Critical micelle concentrations (C.M.C.) were obtained from reflection points of the slopes of κ *versus* concentration.

Results and Discussion

Determinations of Binding Ratio of GMA to HA and Degree of Polymerization of PHEA in HA-PHEA Gels

^{1}H-NMR spectra of GMA, NaHA and GMA-HA dissolved in D_2O are shown in Figs. 3a—c. Peaks of B, C, D, E and F in (a) were assigned to H of each position of GMA.[15)] Peaks of B and C (δ=5.744, 6.163 ppm) of GMA-HA in (c) were assigned to H corresponding to those of GMA as these peaks

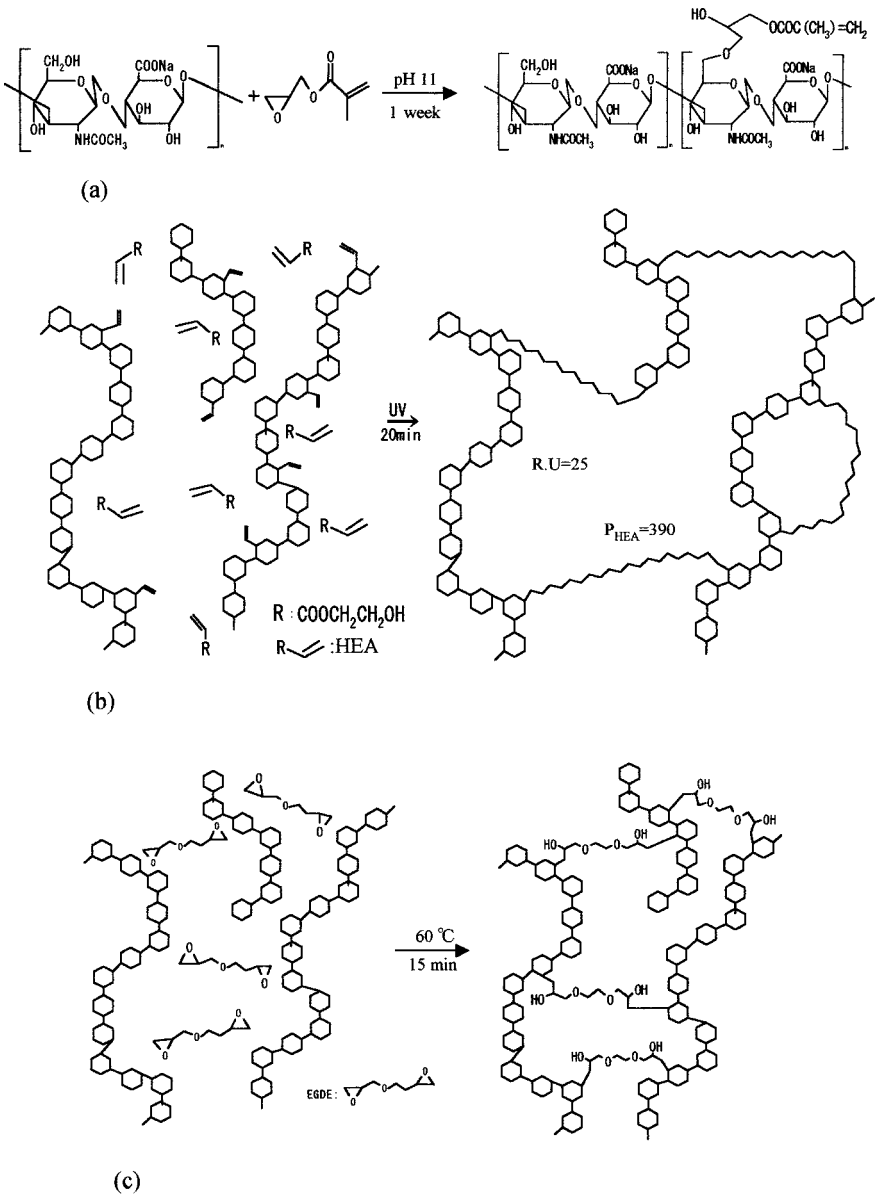

Fig. 2. Schematic Diagrams of Preparations of GMA-HA, HA-PHEA Gel and HA Gel

(a) Scheme of preparation of GMA-HA. (b) Scheme of preparation of HA-PHEA gel from GMA-HA and HEA: P_{PHEA}, mean degree of polymerization of HEA between crosslinks; R.U., mean numbers of repeating units of HA between crosslinks. (c) Scheme of preparation of HA gel.

could not be found in the spectrum of NaHA shown in Fig. 3b. GMA reacts primarily with $-CH_2OH$ groups of NaHA according to the report of Van Dijk-Wolthuis *et al.*[15] From their peak areas, the binding molar ratio of GMA to NaHA was found to be 0.056 on the basis of the repeating units. The binding molar ratio obtained using the more reliable dialysis method was 0.040, a little less than the result of the NMR method.

The mixtures of GMA-HA and HEA were polymerized by adding VA086 as an initiator and irradiating (400 W Hg lamp, λ=253.7 nm) for 20 min, and blend HA-PHEA gels were prepared. The concentrations of free HEA obtained by dialyzing the HA-PHEA gels were 0, *i.e.*, all HEA were polymerized in the gel. Assuming all HEA were polymerized between the GMA groups, the mean number of the repeating unit of HA and the mean degrees of the polymerization of HEA PHEA between the crosslinks were estimated to be 25 and 390. A model of the HA-PHEA gel is shown in Fig. 2b.

Characteristics of HA-PHEA, HA and PHEA Gels

HA-PHEA gel is composed of HA and a rather long chain of PHEA. Thus PHEA affects the properties of the gel. HA-PHEA, HA and PHEA gels swelled significantly in water and their water content W_w was 0.97, 0.97 and 0.93 respectively as shown in Table 1. They were transparent and their transmittances T were 0.98, 0.99 and 0.97, respectively. Viscoelastic properties of these gels were measured in water using the creep meter at 25 °C. The creeps under the constant stress of 3.99×10^4 N m^{-2} are shown in Fig. 4. The results of PHEA and HA gels could be analyzed by the 2 element model but the results of HA-PHEA gel could be analyzed by the 6 element model. Their viscoelastic parameters are shown in Table 1. The HA gel was rigid and brittle, but the PHEA gel was elastic. HA-PHEA gel was found to become elastic due to the presence of PHEA.

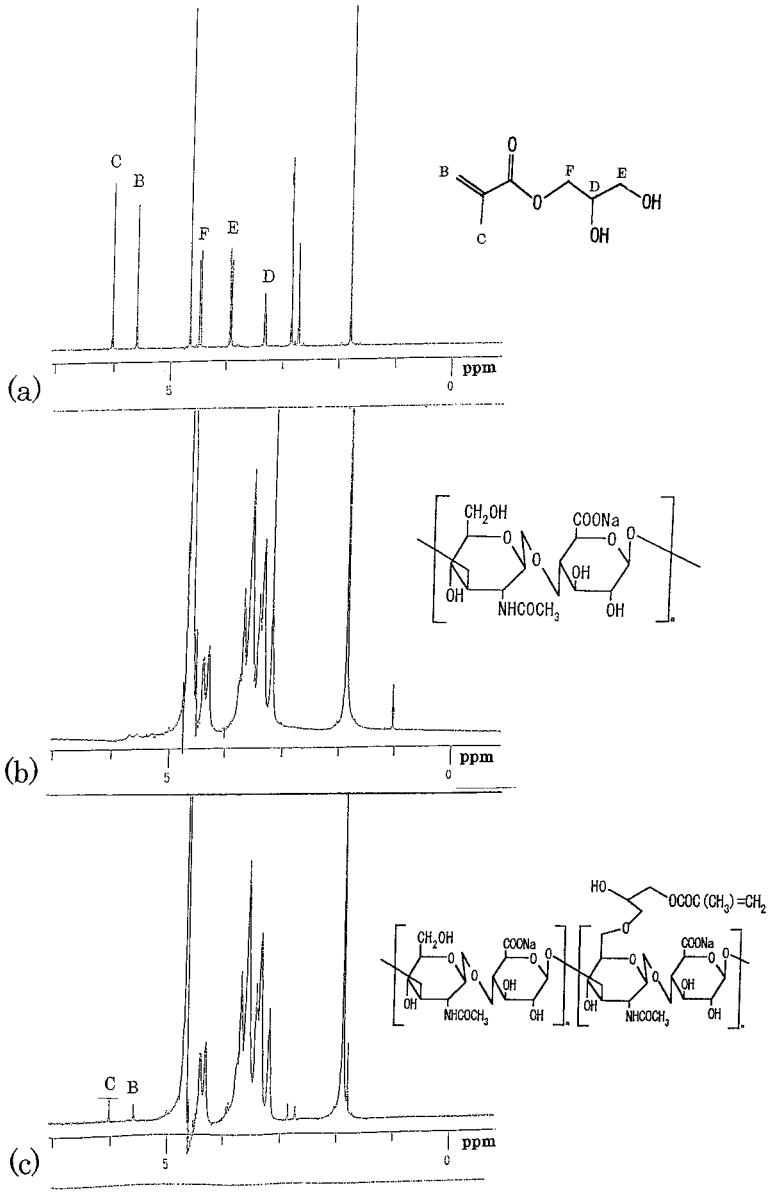

Fig. 3. ^{1}H-NMR Spectra and Molecular Formula of GMA, NaHA, GMA-HA in D_2O

(a) Glycidyl methacrylate (GMA). (b) Sodium hyaluronate (NaHA). (c) Glycidyl methacryl hyaluronate (GMA-HA).

Table 1. Characteristics of PHEA, HA and HA-PHEA Gel

Gel	W_w	T	θ/mol dm^{-3}	θ/θ_0	E_0/N m$^{-2}\times10^5$	η_N/Pa s$\times10^8$
PHEA	0.93	0.97	0	0	3.16	9.31
HA	0.99	1.00	−0.008	0.60	4.71	0.25
HA-PHEA	0.97	0.98	−0.002	0.45	1.35	3.10

W_w: water content. T: transmittance. θ: effective charge densities, θ_0: concentration of carboxylic groups. Viscoelastic parameters obtained under constant stress P_0=3.99×10^4 N m^{-2} constant stress: E_0; instantaneous elastic modulus, η_N; Newtonian viscosity coefficient of Voigt's model. Other viscoelastic parameters of HA-PHEA gels in Fig. 4 obtained by six-element model: E_1, 3.75×10^6 N m^{-2}; E_2, 5.23×10^6 N m^{-2}; τ_1, 59.6 s; τ_2, 7.22 s; η_1, 2.31×10^8 Pa s; η_2, 2.49×10^7 Pa s. τ_i ($=\eta_i/E_i$) is retardation time.

Swollen gels were dried and reswollen to an equilibrium state. Weight ratios to the original swollen gel W/W_0 in the drying-swelling processes are shown in Fig. 5. The dried HA-PHEA gel swelled to the original state. The drying-swelling cycles were continued many times and were found to be reversible processes. However, the cycles of HA and PHEA gels were irreversible. The dried HA gel was destroyed after 30 min in the first swelling step. The crosslinks of HA gel formed by EGDE are believed to be weak and to be destroyed in the swelling process. The dried PHEA gel swelled but did not recover to its original swollen state. As PHEA gel is crosslinked by hydrogen bonds between hydroxyethyl residues, the crosslinks were enhanced in the drying processes and this resulted in the irreversible swellings.

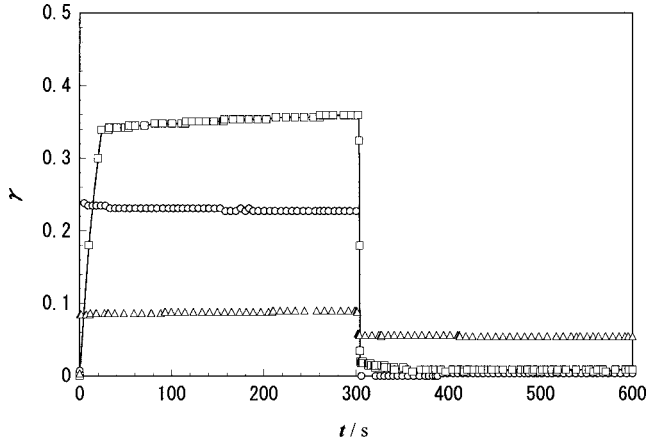

Fig. 4. Creep of HA-PHEA, HA and PHEA Gels
□, HA-PHEA gel; △, HA gel; ○, PHEA gel.

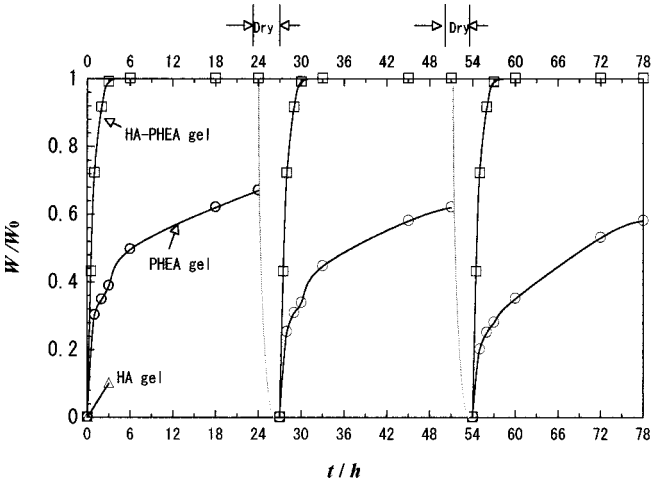

Fig. 5. Recycles of Swelling and Drying States of PHEA, HA and HA-PHEA Gels
□, HA-PHEA gel; △, HA gel; ○, PHEA gel.

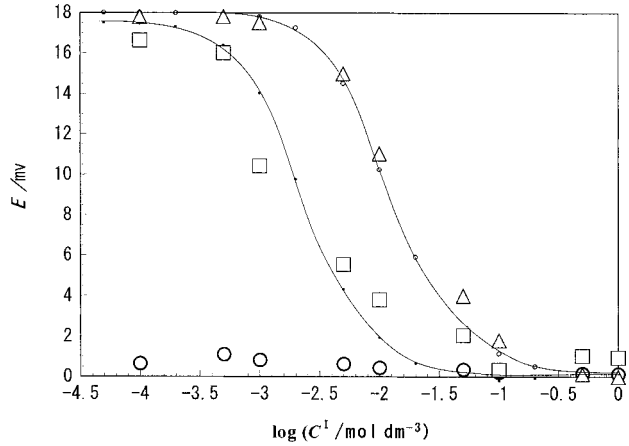

Fig. 6. Membrane Potential of PHEA, HA and HA-PHEA Gels
□, HA-PHEA gel; △, HA gel; ○, PHEA gel. Solid curves are calculated values from Eq. 2 under $C^{I}(KCl)/C^{II}(KCl)=2$.

Table 2. Characteristics of Gel

Characteristics of gel	Gels	Solutes			
		NaBA	MB	CPHCl	BZTCl
W_w	PHEA	0.95	0.96	0.98	0.98
	HA	0.99	0.79	0.65	0.66
	HA-PHEA	0.93	0.78	0.61	0.87
V/V_0	PHEA	1.0	1.42	2.12	4.4
	HA	0.92	0.06	0.05	0.07
	HA-PHEA	0.38	0.09	0.07	0.23
b	PHEA	0.76	0.57	0.9	0.54
	HA	0.33	65	81	119
	HA-PHEA	0.31	9.0	9.8	3.9
b_{cal}^+ or b_{cal}^-	PHEA	1.0	1.0	1.0	1.0
	HA	0.68	1.48	1.48	1.48
	HA-PHEA	0.9	1.10	1.10	1.10

W_w: water content in various solutes ($C=0.01\,mol\,dm^{-3}$). V/V_0: volume ratio to original ones. V_0: original volume of gels equilibrated in water. b: partition coefficient. b_{cal}^+ and b_{cal}^-: partition coefficients of cation and anion calculated from Donnan equilibrium ($\theta_{HA}=-0.008\,mol\,dm^{-3}$, $\theta_{HA\text{-}PHEA}=-0.002\,mol\,dm^{-3}$).

The reversible cycles of HA-PHEA gels are thought to result from the strength of the crosslinks, the elasticities of PHEA and the high affinities with water of HA and PHEA.

Determinations of Effective Charge Densities of HA-PHEA, HA and PHEA Gels Effective charge densities θ of HA-PHEA, HA and PHEA gels were estimated from the membrane potential method. The membrane potentials E between compartments I and II separated by the gel disks were measured using KCl solutions under the constant concentration ratio ($C^I/C^{II}=2$) at 25 °C. The results E are shown in Fig. 6 as a function of $\log C^I$. Results of PHEA gel were almost 0 in the experimental concentration region. Those of HA and HA-PHEA gels showed sigmoidal curves decreasing with increasing $\log C^I$, and attained to the diffusion potential of KCl (=0) as expected from the theory of the membrane potential of a charged membrane. Effective charge densities θ of the gels were estimated using a curve fitting method. Solid curves in Fig. 6 are the theoretical ones obtained from Eq. 2 when the most suitable θ values were selected to fit them the experimental results in the region of $C^I>1.0\times10^{-3}\,mol\cdot dm^3$, because experimental values in the lower concentration region had some errors. The values of θ were -0.002, -0.008 and $0\,mol\,dm^{-3}$ for HA-PHEA, HA and PHEA gels as shown in Table 1. The ratios of θ to the concentrations of carboxylic groups in the gels θ/θ_0 were 0.45 and 0.6 for HA-PHEA and HA gels. The values of θ_0 were obtained from the amounts of carboxylic groups n_c in the gels. Almost half of the carboxylic groups were found to work as dissociation groups in the HA and HA-PHEA gels.

Adsorption Amounts of Solutes and Volume Changes of HA-PHEA, HA and PHEA Gels Equilibrium adsorption amounts n_e in HA-PHEA, HA and PHEA gels were measured in NaBA, MB, CPHCl and BZTCl solutions ($C_0=0.01\,mol\,dm^{-3}$). Adsorbing solutes, the gels deformed. Their equilibrium water content W_w and the volume ratio to the original gel in water V/V_0 were also measured. As shown in Table 2, negatively charged HA-PHEA and HA gels decreased in W_w and V/V_0 which were affected significantly by cationic solutes. On the other hand, PHEA gels equilibrated with cationic solutes increased in W_w and V/V_0, which would be due to the electrostatic repulsions of adsorbed cationic solutes. HA-PHEA and HA gels would shrink due to the effects of electrostatic shielding and electrostatic bindings on

the carboxylic groups of HA. NaBA would shrink them primarily due to the electrostatic shielding effects and cationic solutes would shrink drastically due to the electrostatic bindings. BZTCl being a cationic surfactant forms intra- and intermicelles in gel matrixes in the experimental concentration (>C.M.C). The intramicelle formation would contribute to the swelling due to the electrostatic repulsion of the positive charges, but the intermicelle formation would shrink gel networks. The results of W_w and V/V_0 of HA-PHEA gel equilibrated with BZTCl were much higher than those of MB and CPHCl. This would result from electrostatic repulsion caused by the intramicelle formation. The results of HA gel however were almost equal to those of MB and CPHCl, as a result of the predominant intermicelle formation between close HA chains.

Partition Coefficients and Donnan Equilibrium Partition coefficients b of the solutes were obtained from the results of n_e and V. As shown in Table 2, in the case of neutral PHEA gel, the values of b were less than 1 for all solutes. In the cases of HA and HA-PHEA gels, the results showed features of negatively charged gels, *i.e.*, the results of NaBA were less than 1 and those of the cationic solutes were much more than 1.

Partition coefficients of ions in charged gels can be estimated from Donnan equilibrium using effective charge densities θ. As solutes used in our studies were 1–1 type electrolytes, partition coefficients of anion and cation b_{cal}^- and b_{cal}^+ can be expressed by Eqs. 7, 8,

$$b_{cal}^- = -\theta/2C_0 + ((-\theta/2C_0)^2+1)^{1/2} \quad (7)$$

$$b_{cal}^+ = \theta/2C_0 + ((-\theta/2C_0)^2+1)^{1/2} \quad (8)$$

Calculated vales of b_{cal}^- and b_{cal}^+ are shown in Table 2. The differences between experimental results b and calculated values were great in all systems, so that nonelectrostatic interactions were found to play important roles. The hydrophobic plane of HA and the hydrogen bonding ability of PHEA would contribute significantly to nonelectrostatic interactions. The results of HA-PHEA gel were less than those of HA gel, this is believed due to the lower value of $|\theta|$ and the unaffinity of PHEA for the cationic solutes. The great values of b of the cationic solutes showed the availability of HA and HA-PHEA gels as a storing matter.

Release of NaBA and Cationic Solutes from HA-PHEA, HA and PHEA Gels The release from HA-PHEA, HA and PHEA gels was measured in water at 25 °C. Time course of the releases of NaBA, CPHCl and BZTCl is shown in Figs. 7a—c, respectively. The ordinate is the relative release rate Q_t/Q_0 in which Q_0 is an initial equilibrium adsorption amount in the solutions of 0.01 mol dm^{-3}. As shown in Fig. 8a, release rates of NaBA were in the order of HA>HA-PHEA≅PHEA gels. The releases of NaBA from HA-PHEA and PHEA gels are believed to be suppressed by hydrogen bindings between NaBA and PHEA. As shown in Figs. 7b, c, release of CPHCl and BZTCl was in the order PHEA>HA-PHEA>HA. The release from anionic charged HA-PHEA and HA gels would be suppressed significantly due to electrostatic interactions. The suppression of HA gel was much greater than HA-PHEA gel, as expected from the more effective charge density $|\theta|$. Characteristics of releases from HA-PHEA gel were intermedate between HA and PHEA gels, as expected from the results of W_w and θ.

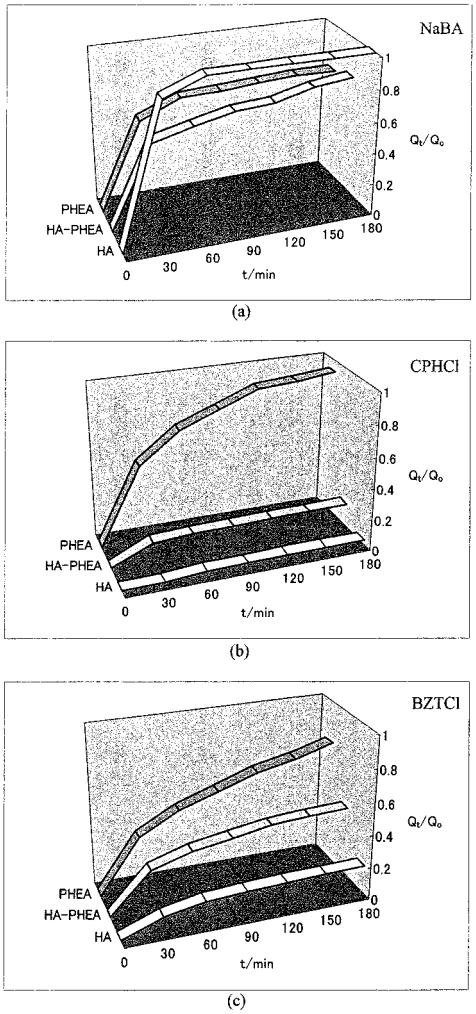

Fig. 7. Time Course of Relative Releases of NaBA, CPHCl and BZTCl from HA-PHEA, HA and PHEA Gels in Water at 25 °C

Solute: (a) NaBA, (b) CPHCl, (c) BZTCl. The original gels were equilibrated in 0.01 mol dm^{-3} solutions, respectively, and the concentration of solutes in each gel is denoted by Q_0.

Effects of Adding Salts on Releases of Cationic Solutes from HA-PHEA Gel Effects of adding salts and HCl on the release of cationic solutes from HA-PHEA gel were measured to elucidate the effects of electrostatic and nonelectrostatic interactions. The ionic strengths were adjusted to I=0.01 and 0.1 mol dm^{-3} by NaCl. The results of Q_t/Q_0 of MB, CPHCl and BZTCl are shown in Figs. 8a—c. The releases of MB and CPHCl were enhanced by increasing I but they were not completely released even in 0.1 mol dm^{-3} NaCl solutions. The effects of adding HCl (=0.1 mol dm^{-3}) were also examined. The concentration of HCl was 0.1 mol dm^{-3} and the value of pH was 1.1 at which carboxylic groups of HA change to H-type. The concentration of H^+ ion in HA-PHEA gel is more than 0.1 mol dm^{-3} as calculated from Donnan equilibrium. Then, cationic solutes binding electrostatically with carboxylic groups would become free due to counterion exchanges. However, the results of Q_t/Q_0 were almost the same level as 0.1 mol dm^{-3} NaCl and did not approach 1. These results indicated that considerable nonelectrostatic interactions were present.

Contrary to the results of MB and CPHCl, releases of

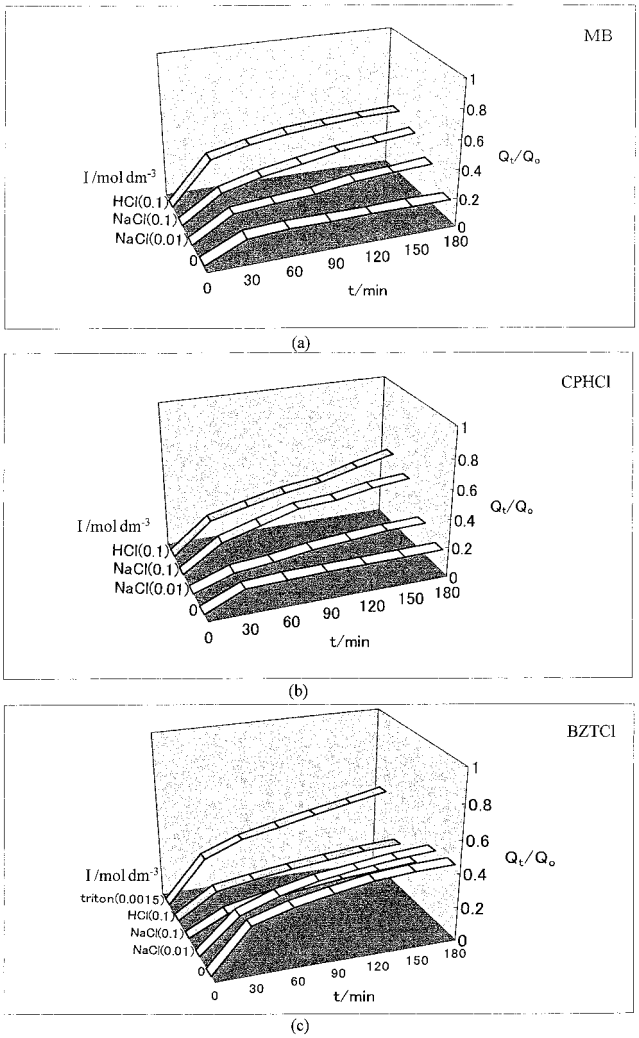

Fig. 8. Adding Effects of NaCl and HCl on Release of MB, CPHCl and BZTCl from HA-PHEA Gels at 25 °C

Solute: (a) MB, (b) CPHCl, (c) BZTCl.

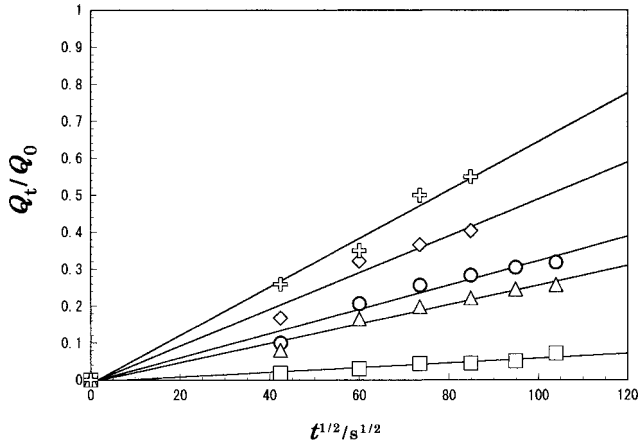

Fig. 9. Relative Release of BZTCl Q_t/Q_0 from HA-PHEA Gels Plotted as a Function of $t^{1/2}$

◇, Water; ○, I=0.01 mol dm^{-3} (NaCl); □, I=0.1 mol dm^{-3} (NaCl); △, I=0.1 mol dm^{-3} (HCl); ✠, C=0.0015 mol dm^{-3} (triton X-100).

Table 3. Effect of Ionic Strength on Kinetic Constant in PHEA, HA and HA-PHEA Gels

Solute	I/mol dm^{-3}	$k/10^{-3}$		
		PHEA	HA	HA-PHEA
NaBA	0	12.9	22.6	19.5
MB	0		0.23	1.50
	0.01 (NaCl)		0.84	2.63
	0.1 (NaCl)		2.63	3.25
	0.1 (HCl)		9.86	4.39
CPHCl	0	10.5	0.20	0.58
	0.01 (NaCl)		2.66	3.05
	0.1 (NaCl)	10.2	3.00	4.12
	0.1 (HCl)		5.86	4.62
BZTCl	0	7.20	2.17	4.98
	0.01 (NaCl)		3.01	3.29
	0.1 (NaCl)	2.75	0.51	0.64
	0.1 (HCl)		1.35	2.63
	0.0015 (Triton)		4.42	6.56

I: ionic strength.

BZTCl were suppressed by adding NaCl and HCl as shown in Fig. 8c. The characteristic releases of BZTCl would result from the intra- and intermicelle formations. The results of BZTCl were enhanced when triton X-100, a destructive agent of micelles, was added, as shown in Fig. 8c. However, BZTCl was not completely released and some electrostatically bound remained in the gel.

The critical micelle concentrations C.M.C. of MB, CPHCl and BZTCl were determined by a conductometric method under various ionic strengths adjusted by NaCl at 25 °C. The C.M.C. values of CPHCl and BZTCl were 34.1 and 3.5×10^{-3} mol dm^{-3} at I=0 and that of MB could not be determined. It should be noted that although CPHCl and BZTCl form an association or a micelle, MB does not. The C.M.C. value of CPHCl was 10 times greater than BZTCl. With increasing I, the C.M.C. of CPHCl and BZTCl decreased as did other ionic surfactants.[20,21] In the gel networks, BZTCl is believed to form micelles, but CPHCl is thought to form associations instead, as true in solutions.[12] By adding salts, the release of BZTCl was suppressed but that of CPHCl was enhanced in the same manner as MB. The former is due to the enhancement of intra- and intermicelle formations of BZTCl on the gel matrixes, and the latter is because the counterion exchanges occurred preferentially rather than enhancement of the intra- and inter-association formations.

Estimation of Kinetic Constants of Releases PHEA gel interacted nonelectrostatically with NaBA, MB, CPHCl and BZTCl. The adsorptions and the releases resulted in the swelling and shrink of the gel. In contrast, HA and HA-PHEA gels interacted electrostatically and nonelectrostatically with the solutes. The adsorptions and the releases resulted in the shrinking and swelling of the gels. The releases from these gels are thus processes of simultaneous diffusion and reactions accompanied by significant volume changes. Although theoretical analysis of these complex releases is very difficult, generally Q_t/Q_0 can be expressed by the following Eq. 9,

$$Q_t/Q_0 = kt^n \tag{9}$$

where k is the kinetic constant, and n is the exponent index. When releases from gels are controlled by diffusion, n should be 0.5.[22,23] As shown in Fig. 9, the results of Q_t/Q_0 of HA-

PHEA gels showed the linear function of $t^{1/2}$ as well as HA and PHEA gels. In the initial stages of the release, deformation of the gels is considered to be minimal and a slower process than diffusion. Then, the values of n are believed to be almost 0.5 in all systems. Further detailed analysis should be made. The values of k are shown in Table 3.

Conclusion

HA gel was brittle but HA-PHEA gel was stiff and elastic due to the elasticity of PHEA. Drying-swelling cycles of HA-PHEA gel were reversible. The partition coefficients of cationic solutes were much greater than calculated values obtained from the Donnan equilibrium. Nonelectrostatic interactions were found to play an important role. The release of CPHCl from HA-PHEA gel was enhanced by adding salts as well as MB, while the release of BZCl was suppressed by adding salts due to its enhancement of the intra- and intermicellar formation. Although the releases from these gels was processes of simultaneous diffusion and reactions accompanied by significant volume changes, Q_t/Q_0 were linear functions of $t^{1/2}$ in the initial stages.

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