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Effect of Interactions on Releases of Cationic Amphipathic Solutes from Hyaluronate Gels

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Abstract: Hyaluronate gel (HA) and Hyaluronate-hydroxyethyl acrylate blend gel (HA-PHEA) were prepared. Both HA and HA-PHEA gels were transparent, high water contents and negative charged gels. Characteristic releases of solutes were studied using an anionic solute, sodium benzoate (NaBA), cationic amphiphilic solutes, chlorpromazine (CPHCl) and benzethonium chloride (BZTCl). Effects of electrostatic and non-electrostatic interactions on releases were investigated. By adding salts, the releases of CPHCl were enhanced but those of BZTCl were found to be suppressed due to the enhancement of the intra- and intermicelle formation.

Key words: hyaluronate; hydroxyethyl acrylate; blend hydrogel; cationic amphipathic solutes; release control

1. INTRODUCTION

Hydrogels are formed from hydrophilic synthetic polymers and many natural polymers such as proteins and polysaccharides. Natural polymer gels are useful for pharmaceutical fields such as controlled delivery devices because of their biocompatibility and biodegradability. We are concerned with hyaluronate (HA) from pharmaceutical applicable point of views. HA is an acid polysaccharide that is an important component of vitreous body, cartilages and connective tissues such as intercellular matrixes of skin. 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. The important properties of HA solutions are high viscosity due to its very high molecular weights and counterion exchange abilities due to the presence of carboxylic groups. Furthermore, HA possess high preserving ability of water and their solutions are transparent, and are used as a surgical tool of eye and remedy for arthritis.

HA is reported to form single and double helical structures in solutions due to intra- and intermolecular interactions [1]. In our laboratory, the characteristics of HA in aqueous solutions have been studied concerning interactions with proteins [2]. HA was found to form network structures on BSA monolayer [3]. However, HA itself is unable to form gels, HA gels were prepared by using chemical cross-linking agents. The useful characteristics of HA gels in pharmaceutical fields have been reported so far [4−8]. Blend hydrogels composed of HA and polyhydroxyethyl acrylate (PHEA) have been studied and their characteristics were reported [9-10]. Their water contents and release rates could be controlled by the ratios of PHEA to HA. Furthermore, the blend gel of HA and PHEA is stable against cyclic change of swelling and drying processes.

In this paper, we study the release processes of substances from HA and HA-PHEA gels to clarify the effects of interactions between the polymer network of gels and the substances. The substances used here are sodium benzoate (NaBA; anionic substance), chlorpromazine hydrochloride (CPHCl; cationic substance), and benzethonium chloride (BZTCl; cationic substance). CPHCl form an aggregate [11] and BZTCl is an cationic surfactant.

2. EXPERIMENTALS

Materials and methods

Materials

Sodium hyaluronate (NaHA) (*Mw*: 2.09×10^6 g mol^{-1}) from Streptcoccus 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 *N*-(2-hydroxyethyl)-propion amide] (VA086) (Waco Pure Chemical (Osaka)) as an initiator of polymerization were special grade, and 2-hydroxyethyl acrylate (HEA) (Waco Pure Chemical (Osaka)) was the first grade. Other chemicals were of analytical grades. Distilled and deionized water was used for the preparation of aqueous solution.

Sodium benzoate (NaBA) (Katayama Co. Ltd), chlorpromazine hydrochloride (CPHCl) (Wako Pure Chemical (Osaka)) and benzethonium chloride (BZTCl) (Wako Pure Chemical (Osaka)) were of special grades.

Preparations of HA and HA-PHEA blend gels

HA gel was prepared by adding EGDE to NaHA solution (20 w/v% in 1 mol dm^{-3} NaOH) at the volume ratio of 1/9 and by heating 60℃ for 15 m. HA-PHEA blend gel was prepared by following ways. ① Glycidyl methacryl hyaluronate (GMA-HA) was synthesized 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 [10]. ② GMA-HA and HEA were mixed at weight ratio of 5/1 and then polymerized radically by adding VA086 as an initiator and irradiating ultraviolet rays (400 W Hg lamp, λ =253.7 nm) for 20 min. The chemical structure was determined by H-NMR. In GMA-HA, one molecule of GMA is bound within 25 repeating units of HA. The shape of sample gels is a disk of 0.80 and 0.2 cm in radius and thickness, respectively. They were dialyzed in water for 3 days.

Measurements of equilibrium adsorption amounts

The equilibrium adsorption amounts n_e of NaBA, CPHCl and BZTCl in HA and HA-PHEA gels were measured by immersing the gels in their solutions of

C=0.01 mol dm^{-3} for 72 *h* at 25℃. The values of n_e were determined from absorbance differences between the initial and the equilibrated solutions using a spectrophotometer (UV−2400PC, Shimadzu, Kyoto.) at λ=225 nm for NaBA, λ=254 nm for CPHCl and at λ =269.5 nm for BZTCl.

Measurement of destruction

Destruction stresses, destruction strains, destruction strengths and destruction energies of HA and HA-PHEA gels were measured using a creep meter (RE−3305, YAMADEN, Tokyo) at 25 ℃. The cylindrical probe was 0.8 cm in a diameter and the squeezing speed was 1 mm/s. The deformation were measured with an accuracy of 0.001 cm.

Measurement of releases from HA and HA-PHEA gels

NaBA, CPHCl and BZTCl were used as solutes. HA and HA-PHEA gels were equilibrated with the solutions of 0.01 mol dm^{-3} for 72 *h* at 25℃. Releases of the solutes into bulk solutions from them were measured under various ionic strengths at 25℃ using a diffusion cell (Volume: 50 cm^3). One disk of HA or HA-PHEA gel was put into the bulk solutions stirred by a magnetic stirrer continuously at 800 r.p.m under which the release rate was confirmed to be constant. Sample solutions (3 cm^3) were withdrawn at regular intervals and replaced with equal volumes of the media. The absorbance of the sample solutions was determined using the spectrophotometer. The total amount of the released solutes till *i*th sampling time Q_i are obtained by Eq. (1)

$$Q_i = C_i V + \sum C_j V_s \qquad (1)$$

where C_i is the concentration of the solute in *i*th sampling solution, V is the volume of the diffusion cell and V_s is the volume of the sampling solution.

3. RESULTS

Characteristics of HA and HA-PHEA gels

The concentrations of free HEA obtained by dialyzing HA-PHEA gels were found to be 0. Then, all HEA would be polymerized in the gel and react with GMA groups to form crosslinks and branches. The compositions of HA-PHEA gels were 5 in the weight ratios of PHEA to HA. Assuming all HEA were polymerized between the GMA groups, the mean degrees of the polymerization P_{PHEA} between the crosslinks were estimated to be 390. On the other hand, assuming all HEA are branches, the value of P_{PHEA} was estimated to be 195. Actually, both should exist in HA-PHEA gels.

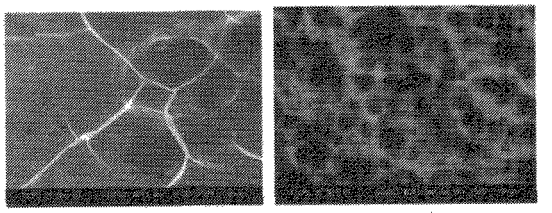
HA and HA-PHEA gels were transparent. These gels swelled significantly when soaked in water. As shown in Table 1, their water contents W_w were 0.993 and 0.980 respectively, and their transmittances were almost 1. The amounts of carboxylic groups n_c of HA and HA-PHEA gels were estimated to be 83×10^{-6} and 17×10^{-6} mol from the weights and the compositions of the gel matrixes. Their viscoelastic properties were measured by destruction modes. It was found from the mechanical measurements that the destruction energy, E, of HA-PHEA gel was much larger than that of HA gel as shown in Table 1. The results, thus, suggest that HA-PHEA gel is stiffer than HA gel.

Structures of HA and HA-PHEA gels were observed by a wet scanning electron microscope. As shown in Fig.1, mesh sizes of HA-PHEA gel were found to be smaller than HA gel.

Table. I Characteristics of HA and HA-PHEA gels and equilibrium adsorbed amounts.

Gels	*W*w	*T*	n_c $\times10^{-6}$	*P* $\times10^{3}$	*h*	*S* $\times10^{3}$	*E* $\times10^{2}$
HA	0.99	1.00	82.8	3.65	0.17	21.5	3.10
HA-PHEA	0.97	0.98	16.7	3.84	0.39	9.8	5.64

*W*w: water content, *T*: transmittance, n_c: concentration of carboxylic groups in HA and HA-PHEA gels (/mol dm^{-3}), *P*: destruction stress (N m^{-2}), *h*: strain, *S* (=*P*/*h*): destruction strength (N m^{-2}), *E*: destruction energy (J m^{-3}).



HA gel HA-PHEA gel

Fig.1 Structures of HA and HA-PHEA gels observed by Wet SEM. —: 200 μ m.

Recycles of swelling and drying states

HA and HA-PHEA gels equilibrated in water were dried and reswelled. As shown in Fig. 2, dried HA-PHEA gel was swelled to the original state and the cycles were continued many times. The HA-PHEA gels, which once dried, swelled until its original volume after 3hrs. These processes were found to be completely reversible. However, dried HA gel was destructed in the first swelling step after 30 min. In general, natural polymer gels such as alginate and gelatin gels do not recover the original swelling states after drying. Their relative swelling ratio decreased with increasing the numbers of the swelling-drying cycles. These swelling behaviors of HA-PHEA gels occur from two reasons; one is flexibility of PHEA and the other is the affinity of HA and PHEA with water.

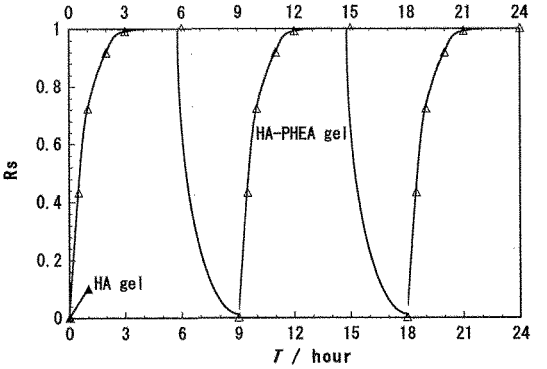


Fig.2 Recycles of swelling and drying states of HA-PHEA hydrogels. Relative swelling (Rs) = $(W_{SG}-W_{DG})$ / W_{SG}

Equilibrium adsorption amounts of NaBA and cationic solutes

Equilibrium adsorption amounts n_e of NaBA, CPHCl and BZTCl in HA and HA-PHEA gels were measured in their solutions of C_0=0.01 mol dm^{-3} and the water content W_w were also measured. The results are

shown in Table 2 together with the values of n_e/n_c. The results of negative solute NaBA were 0.16 and 0.26 for HA and HA-PHEA gels, and those of cationic solutes CPHCl and BZTCl were in the ranges of 1.2-3.1. These results strongly suggest that both the electrostatic interaction and the non-electrostatic interaction play essential roles in the binding behaviors of the cationic substance.

Both HA and HA-PHEA gels swelled significantly in water because of the affinity with water. The presence of carboxylic group in HA chains is also responsible for the swelling behavior of HA-PHEA gel. The HA-PHEA gel, however, collapsed into compact state when soaked in the solutions of NaBA, CPHCl, and BZTCl (C_0=0.01 mol dm^{-3}). The equilibrium swelling ratio of the gel in these solutions, V/V_0, where V_0 denotes the volume of the gel in water, are given in Table 2. In the case of NaBA solution, the swelling ratios of the gels were not much smaller than other solutions. These results suggest that collapse of the gels are caused mainly by the shielding of charges on the HA chains. In contrast, the gels collapsed completely in the solution of CPHCl. These results strongly suggest that the binding of charges on the carboxylic groups of HA chains are responsible for the collapse of gels. Since BZTCl is a cationic surfactant, the formation of micelles in the gel may affect the swelling behaviors of the gels. In fact, the swelling ratio of HA-PHEA gel in this solution was larger than that in CPHCl solution. These results suggest the structure effects of the positively charged micelles in the polymer network of gel.

Table. II Equilibrium adsorption amounts of NaBA and cationic solutes

Gels	*W*w			$n_e/10^{-6}$mol			n_e/n_c			V/V_0		
	NaBA	CPHCl	BZTCl	NaBA	CPHCl	BZTCl	NaBA	CPHCl	BZTCl	NaBA	CPHCl	BZTCl
HA	0.992	0.654	0.660	13	97	105	0.16	1.2	1.3	0.92	0.05	0.07
HA-PHEA	0.927	0.614	0.868	4.3	34	52	0.26	2.0	3.1	0.38	0.07	0.23

*W*w: water content,
n_c: concentration of carboxylic groups in HA and HA-PHEA gels, (HA=83×10^{-6}mol, HA-PHEA=17×10^{-6}mol)
n_e: equilibrium adsorption amounts in HA and HA-PHEA gels,
V_0: original volumes of HA and HA-PHEA gels equilibrated in water, (V_0(HA)=6.3cm^3, V_0(HA-PHEA)=3.8 cm^3),
V/V_0: volumes ratios to original ones.

Releases of NaBA and cationic solutes

The releases of NaBA, CPHCl and BZTCl from HA and HA-PHEA gels were measured in water at 25℃. Time courses of the releases are shown in Fig.3 and 4.

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}. The results of the anionic solute NaBA from HA and HA-PHEA gels approached to 1 after 0.5 and 8 *h*, and the adsorbed solutes were completely released from gels. However, in the cases of CPHCl and BZTCl, equilibrium values of Q_t/Q_0 were attained after 2 to 3 hrs. Besides, the equilibrium value was much less than unity. The releases of these substances were much affected by the interactions between the substance and polymer network.

Effects of adding salts on releases of cationic solutes

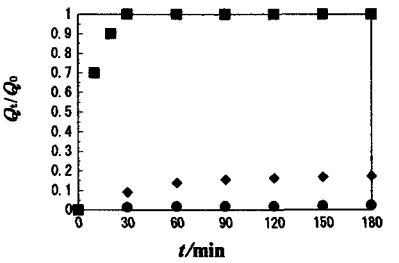


Fig.3 Time dependences of relative releases from HA gel in water. ■, NaBA; ●, CPHCl; ◆, BZTCl.

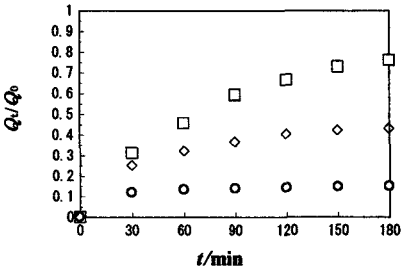


Fig.4 Time dependences of relative releases from HA-PHEA gel in water. □, NaBA; ○, CPHCl; ◇, BZTCl.

Effects of adding NaCl on the releases of cationic solutes CPHCl and BZTCl were measured to elucidate the effects of electrostatic interactions. The ionic strengths were adjusted to I = 0.01, 0.1 and 0.15 mol dm^{-3}. With increasing I, the release of CPHCl was enhanced but that of BZTCl was suppressed. The results of Q_t/Q_0 which attained to constant values after 24 *h* were denoted by Q_∞/Q_0. The results of Q_∞/Q_0 of CPHCl from HA and HA-PHEA gels are shown in Fig. 5 as a function of I. The results of BZTCl are given in Fig.6 in the same manner. The amounts of CPHCl that released from both gels increased with increasing I while that of BZTCl decreased. The suppressions of releases of BZTCl with increased I suggest from the enhancement of the bindings such as intra- and intermicelle formation.

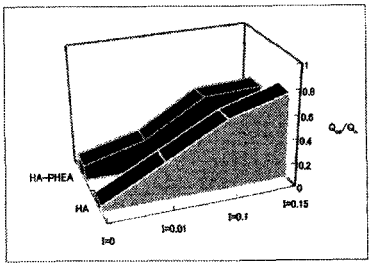


Fig.5 Effects of adding salts on relative releases Q_∞/Q_0 of CPHCl from HA and HA-PHEA gels.

CPHCl is reported to associate in solutions [11]. However, the amount of CPHCl, which released from the gel, increased by adding NaCl to the solution. These

results are quite different from that of BZTCl. The results suggest that CPHCl mainly interact with the polymer networks of HA and HA-PHEA gels through the electrostatic interaction. The effects of micell formation is less effective than that of BZTCl.

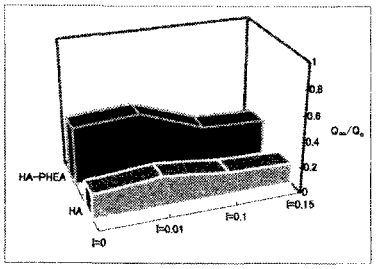


Fig.6 Effects of adding salts on relative releases Q_∞/Q_0 of BZTCl from HA and HA-PHEA gels

Table.III Effect of ionic strength on binding amount and diffusion coefficient of free solutes

I: ionic strength, Q_∞/Q_0: relative release. (Q_∞: maximum released amount, Q_0:initial adsorption amount.), n_f: free amounts in the gels, n_b: binding amounts in the gels.

Solute	I/moldm^{-3} (NaCl)	Q_∞/Q_0 HA	Q_∞/Q_0 HA-PHEA	$n_f=Q_\infty/10^{-6}$mol HA	$n_f=Q_\infty/10^{-6}$mol HA-PHEA	$n_b/10^{-6}$mol HA	$n_b/10^{-6}$mol HA-PHEA
NaBA	0	1	0.88	0	3.8	10	0.5
CPHCl	0	0.07	0.13	6.6	5.0	90	29
	0.01	0.32	0.26	47	10	99	28
	0.1	0.59	0.56	58	19	40	15
	0.15	0.72	0.58	71	20	28	14
BZTCl	0	0.19	0.45	18	26	78	26
	0.01	0.24	0.49	29	25	90	26
	0.1	0.18	0.30	18	13	80	31
	0.15	0.16	0.28	17	12	92	31
0.0015(triton)		0.32	0.63	43	33	89	19

4. DISCUSSION

Interactions of NaBA and cationic solutes with HA and HA-PHEA gels and their releases

In the case of NaBA, the values of n_e/n_c of HA and HA-PHEA gels were less than 1 as shown in Table 2, but in the cases of the cationic solutes CPHCl and BZTCl the results were much larger than 1. The amount of carboxylic groups n_c in the HA gel (83×10^{-6} mol) was larger than that of HA-PHEA gel (17×10^{-6} mol). The values of n_e/n_c of NaBA and cationic solutes are controlled by Donnan equilibrium as shown in the previous paper [10]. The value of n_e/n_c of NaBA in HA gel was smaller than that in HA-PHEA gel according to Donnan effect. However, the values of n_e/n_c of the cationic substances in HA gel were smaller than that in HA-PHEA gel. These results strongly suggest the presence of the hydrophobic interaction between these substances and the polymer network of gel. It is, therefore, shown that the releases of NaBA and CPHCl are mainly affected by the electrostatic interaction of these substances and the gel matrixes. On the other hand, the release of BZTCl is affected both by the electrostatic and the hydrophobic interaction.

The adsorbed substances can exist in the gels as free state as well as bound states. The bound substances cannot diffuse out of the gel while free substances diffuse out of the gel. The amount of substance, which released from the gel Q_∞, corresponds to the amount of substance in the free state in the gel n_f. These values, Q_∞, n_f, and n_b (= n_e - n_f), are given in Table 3. In the case of I = 0, the result of n_b of NaBA was 0 for HA and 0.5 for HA-PHEA gels. The results of CPHCl and BZTCl for HA gel were 90×10^{-6} and 78×10^{-6} mol which were almost equal to the value of n_c (=83×10^{-6} mol). Those for HA-PHEA gel were 29×10^{-6} and 26×10^{-6} mol which were slightly larger than the value of n_c (=17×10^{-6} mol). The hydrophobic interaction with PHEA chains may be responsible for this discrepancy. The binding amount of BZTCl was expected to be larger than CPHCl because of the hydrophobic interaction. However, the results indicate that the values of n_e and n_f of BZTCl is much larger than that of CPHCl. These results indicate that the equilibrium constant of micell formation in the gel is relatively small.

The substance, which bound to the polymer by the electrostatic interaction, dissociate by the addition of the salt to the solution. In the case of CPHCl, the values of n_f increasd with the concentration of salt. These results again indicate that CPHCl and the gel interacts each other through the electrostatic interaction. On the other hand, in the case of BZTCl, the values of n_b increased with the concentration of salt. The molecules of BZTCl can exist in the gel in three states; dissolved monomer, micelles, and the bound state to the polymer network. It has been well established that the C.M.C. of BZTCl solution is lowered by the presence of salt. Therefore, the molecules of BZTCl that is in the free state within the gel tend to form the micelle with the polymer network of the gel. The amount of BZTCl that released from the gel, therefore, decreased with the concentration of the salt.

5. CONCLUSION

1. HA-PHEA gel was stiffer than HA gel, and the drying-swelling processes were reversible.
2. The releases of CPHCl from HA and HA-PHEA gels were enhanced by adding salts. CPHCl interacted electrostatically with carboxylic groups of HA in them.
3. The releases of BZTCl were suppressed by adding salts due to the enhancement of the intra- and intermicellar formation.

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