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Network structure of spontaneously forming physically cross-link hydrogel composed of two-water soluble phospholipid polymers

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Abstract

Hydrogels containing 2-methacryloyloxyethyl phosphorylcholine (MPC) moieties were prepared from aqueous solutions with water-soluble poly[MPC-*co*-methacrylic acid (MA)] (PMA) and poly[MPC-*co*-*n*-butyl methacrylate (BMA)] (PMB). We had found that the hydrogel would swell under acidic pH and dissociate under neutral and alkaline conditions. Investigating these properties together with the complex modulus and the calculating cross-link molecular weight, we tried to determine how the network is constructed. The hydrogel showed a low swelling and a low elastic modulus E as the PMA feed ratio increases. However, a slow dissociation behavior was shown at the same time. This is due to the formation of the intramolecular cross-links caused by hydrogen bonds provided by carboxyl groups inside the hydrogel, which does not contribute to the swelling or elastic property. However, they had affected the ionization of the carboxyl groups. The average cross-link molecular weight decreased with the higher PMA feed ratio, indicating that the network is actually denser. The calculated cross-link molecular weight for the high PMA feed ratio is low, but the number of cross-links is also low, indicating that there are many intramolecular cross-link networks, but fewer valid entanglements for high mechanical strength. Therefore, denser networks are formed for higher the PMA feed ratio, but they do not function as cross-link junctions. This could be confirmed by Fourier transform infrared spectroscopy (FTIR). When the release behavior of the loaded drugs was tested, it showed that the drugs with molecular weights higher than the cross-link molecular weight would be slower than that of drugs with lower molecular weights.

Keywords: Spontaneously forming hydrogel; Hydrogen bond; Polymer network

1. Introduction

We have recently developed a spontaneously forming physical hydrogel as a drug reservoir with excellent biocompatibility and a 100% drug loading efficiency [1]. The hydrogel is based on the 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer, which shows very a good biocompatibility [2–5]. The MPC polymer, specifically copolymerized with *n*-butyl methacrylate (BMA), retained a higher free water content in comparison with conventional hydrophilic polymers, such as poly(2-hydroxyethyl methacrylate) [5–7]. Possession of hydrophobic side groups such as BMA enables the MPC polymer association with the hydrophobic core, which is utilized to load the hydrophobic drugs. The spontaneously forming hydrogel is prepared by the hydrogen bonds of poly (MPC-*co*-methacrylic acid) (PMA) that have been induced in the hydrophobic core, indicating a low permittivity by poly (MPC-*co*-BMA) (PMB). The hydrogel possesses carboxylic groups, which react as pH responsive side groups, but also serve as cross-link junctions by formation of hydrogen bonding. Therefore, the hydrogel would remain stable at low pH values, but dissociate at higher pH values. This hydrogel possesses over 90% free water, which avoids the denaturization process of the loaded polypeptide drugs. Therefore, we have been studying in order to make use of this hydrogel as an oral delivery carrier [1,8–10].

We had so far investigated how the MPC hydrogel would behave in the gastrointestinal (GI) tract. We have found that the hydrogel would mainly be surface eroded under neutral pH conditions but swells under acidic pH conditions. The drug release would be suppressed at low pH values and promoted at higher pH values. This behavior can be altered by controlling the hydrophobicity of the loaded drugs,

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polymer concentration, or polymer composition [7–10]. However, we still did not have any information on the network structure of the hydrogel.

The calculation method to obtain information on the network structure was introduced by Bray and Merill, and reinforced by Peppas et al. [11,12]. The calculation of the average cross-link molecular weight, cross-link density, and mesh size of the poly(vinyl alcohol) and poly(2-hydroxyethyl methacrylate) (PHEMA) network was done using the swelling property of the hydrogel in an aqueous or organic solvent. The network of the physically cross-link hydrogel was also executed by Hennink et al. using the dextran hydrogel [13,14]. They managed to calculate the cross-link molecular weight and the mesh size, which allowed them to predict the actual release property of the loaded drugs. Recently, Kim and Peppas succeeded in calculating the network structure of the poly(methacrylic acid-*co*-2-methacryloxyethyl glucoside) hydrogel [15].

In this paper, we report the network structure of the hydrogel by investigating the swelling under acidic pH conditions and dissociation under neutral pH conditions together with the elastic property of the MPC hydrogel, and release behavior of the polypeptide model drugs. Furthermore, we have calculated the average cross-link molecular weight and cross-link density in order to mathematically characterize the structure of the hydrogels.

The term erosion is used to imply the decrement of the size of the hydrogel body, dissolution to indicate the polymer moving out from the hydrogel (or diffuse out), and dissociation to imply the phenomenon of the carboxyl groups being divided into carboxylate anions and protons.

2. Experimental

2.1. Preparation of hydrogels

The preparation method of the phospholipid polymer hydrogel has been described in our previous report [1,7–10]. In short, two kinds of water-soluble phospholipid polymer poly(MPC-*co*-methacrylic acid) (PMA, MPC mole fraction: 0.3, $\bar{M}_n = 2.7 \times 10^5$, $\bar{M}_w = 6.8 \times 10^5$) and poly(MPC-*co*-*n*-butyl methacrylate) (PMB, MPC mole fraction: 0.8, $\bar{M}_n = 1.7 \times 10^5$, $\bar{M}_w = 1.7 \times 10^6$) 10 wt% aqueous solutions were chosen. The molecular structures of PMA and PMB are shown in Fig. 1. To make a 10 wt% hydrogel, 10 wt% PMA and PMB aqueous solutions were placed in a vial and vigorously mixed for 10 s. The feed ratio of PMA and PMB was adjusted by volume to 2:1, 1:1, and 1:2 to make the A2B1, A1B1, and A1B2 hydrogels. These hydrogel were used to characterize the structural formation and physical behavior under pH conditions.

2.2. Mechanical property of hydrogel

The mechanical property of the hydrogels was measured using a rheometer (Reolograph micro, Toyoseiki, Japan). The hydrogels were prepared according to the methods described above, and these hydrogels were put onto a plate for the compression test. The frequency and the amplitude used for the experiment were 5 Hz and 200 μm. The oscillation continued for 20 s to obtain the complex elastic modulus E and viscosity η of each hydrogel.

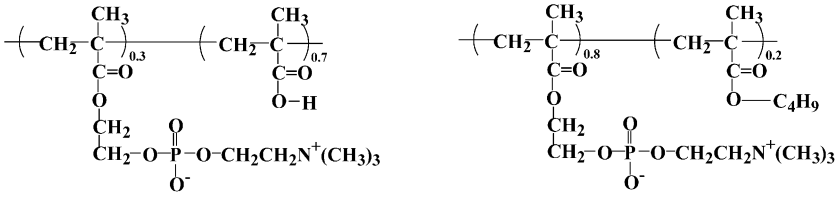
2.3. Swelling and dissociation behavior of hydrogels

The hydrogels were fabricated into a disk shape (radius: 7.5 mm, height: 6 mm). To observe how the hydrogel would behave in the gastrointestinal tract (GI tract), each hydrogel was characterized in a pH 1.8 aqueous solution (stomach condition) and a pH 6.8 phosphate buffer solution (PBS) (small intestine condition) [16,17]. In order to maintain the pH conditions, the volume ratio of the hydrogel and the buffer was controlled in the ratio of 1:99. The sample bottles with PBS were changed every 30 min in order to maintain a clean environment. Between changing the sample bottle, the weight of the hydrogel was measured until full dissociation occurred. The swelling ratio, water absorption ratio, polymer loss percentage of the respective hydrogels under acidic pH conditions was calculated using the equation written below:

$$\text{Swelling ratio, } S = \frac{W_{a,s} - W_{a,r}}{W_{a,r}} \tag{1}$$

$$\text{Water absorption ratio, } W_{ch} = \frac{(W_{a,s} - W_{p,s}) - (W_{a,r} - W_{p,r})}{W_{a,r} - W_{p,r}} \tag{2}$$

$$W_{w,s} + W_{p,s} = W_{a,s}$$



(a) (b)

Fig. 1. The chemical structure of (a) PMA and (b) PMB.

$$W_{w,r} + W_{p,r} = W_{a,r}$$

S indicates the swelling ratio, $W_{a,s}$ indicates the total weight of the hydrogel after swelling and $W_{a,r}$ indicates the total weight of the hydrogel before swelling. W_{ch} implies the pure water absorption ratio. $W_{p,r}$ and $W_{p,s}$ indicate the polymer weight before and after swelling, respectively. $W_{w,r}$ and $W_{w,s}$ are the pure amount of water in the hydrogel before and after swelling, respectively. The swelling ratio S indicates the total weight change of the hydrogel, water absorption ratio W_{ch} implies the change in the water content during swelling, and the polymer loss indicates the loss of the polymer portion during swelling.

To measure the polymer and the water content between the time, the hydrogels were removed from the solution and put into a nylon mesh bag to measure their weights. The hydrogel was completely then lyophilized overnight and the weight was measured to determine the weight gain of the remaining polymer. From this, the weight of the polymer and water content was calculated along with the polymer percentage of the hydrogel versus time. All the experiments were repeated 5 times. The swelling experiment was continued for 3 h and dissociation experiment was continued until complete dissociation of the hydrogel.

2.4. Determination of polymer volume fraction

Initially, the volume of the hydrogel before swelling $V_{g,r}$, and after swelling $V_{g,s}$, were calculated as follows [11,12,18,19]:

$$V_{g,r} = \frac{W_{a,r}}{\rho_w} \tag{3}$$

$$V_{g,s} = \frac{W_{a,s}}{\rho_w} \tag{4}$$

where ρ_w indicates the density of water at 25 °C. $W_{a,r}$ is the weight of hydrogel, and $W_{a,s}$ is the weight after swelling. The volume of the dry polymer V_p was calculated as:

$$V_p = \frac{W_{a,d}}{\rho_p} \tag{5}$$

where $W_{a,d}$ is the dried weight of the hydrogel and ρ_p is the density of the polymers mixed together in a certain ratio.

The polymer volume fraction of the hydrogels in both the relaxed and swollen states $\upsilon_{2,r}$ and $\upsilon_{2,s}$, respectively, were determined using the following equations:

$$\upsilon_{2,r} = \frac{V_p}{V_{g,r}} \tag{6}$$

$$\upsilon_{2,s} = \frac{V_p}{V_{g,s}} \tag{7}$$

The calculation of the molar volume of the polymers was done using the values that had been reported by Fedor [20].

2.5. Determination of molecular weight between cross-links

With the values calculated from Eqs. (3)–(7), it is possible to determine the number average molecular weight between the cross-links, $\bar{M}_c$. The equation for $\bar{M}_c$ was developed by Peppas and Merrill [11–14]:

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} - \frac{(\bar{v}/V_1)\left[\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi(\upsilon_{2,s})^2\right]}{\upsilon_{2,r}\left[\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)^{1/3} - \frac{1}{2}\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)\right]} \tag{8}$$

$\bar{M}_c$ is the number average molecular weight of the uncross-link polymer, χ is the Flory-swelling agent thermodynamic interaction parameter, υ is the specific volume of the polymers, and V_1 is the molar volume of water. This equation is applied to loosely cross-link networks, where the number of repeating units is large enough so that the chains can be represented by a Gaussian distribution.

An expression similar to Eq. (8) had been developed for isotropic, highly cross-link networks by Lucht and Peppas [21]:

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} - \frac{(\bar{v}/V_1)\left[\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi(\upsilon_{2,s})^2\right]\left[1 - \frac{1}{N}\upsilon_{2,s}^{2/3}\right]^3}{\left(\upsilon_{2,s}^{1/3} - \frac{1}{2}\upsilon_{2,s}\right)\left(1 + \frac{1}{N}\upsilon_{2,s}^{1/3}\right)^2} \tag{9}$$

This equation can be extended to include cross-link in solution.

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} - \frac{(\bar{v}/V_1)\left[\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi(\upsilon_{2,s})^2\right]\left[1 - \frac{1}{N}\upsilon_{s,r}^{2/3}\right]^3}{\upsilon_{s,r}\left(\upsilon_{s,r}^{1/3} - \frac{1}{2}\upsilon_{s,r}\right)\left(1 + \frac{1}{N}\upsilon_{s,r}^{1/3}\right)^2} \tag{10}$$

where the term $\upsilon_{s,r}$ denotes the ratio $\upsilon_{2,s}/\upsilon_{2,r}$. The parameter N, representing number of links of the chain, can be expressed by

$$N = \frac{2\bar{M}_c}{M_r} \tag{11}$$

where M_r is the molecular weight of the polymer repeating unit. Eq. (10) describes the swelling of a highly cross-link, moderately swollen polymeric network. Both Eqs. (8) and (10) are used to calculate $\bar{M}_c$. The thermodynamic interaction parameter χ is dependent on both the temperature and equilibrium polymer volume fraction, $\upsilon_{2,s}$. Therefore, it is adequately represented as a function of the polymer volume fraction at 37 °C by expression written below.

$$\chi = 0.220 + 0.904\upsilon_{2,s} \tag{12}$$

The cross-link density ρ_x was determined from the

molecular weight between cross-links calculated from Eq. (10).

$$\rho_x = \frac{1}{\bar{v}\bar{M}_c} \tag{13}$$

2.6. Infrared spectroscopy measurement

A1B2, A1B1, and A2B1 hydrogels were all lyophilized to measure Fourier transform infrared spectroscopy (FTIR; FT/IR-680, Jasco, Tokyo, Japan) in the wavelength range of 400–4000 cm^{-1} [22]. To express transmittance intensities of the peaks for the hydrogen bonds between carboxyl groups, the spectrum between 1000 and 2000 cm^{-1} was chosen.

2.7. Release behavior of polypeptide from hydrogel

The release tendency of the hydrophilic polypeptide drugs during swelling of the hydrogels was measured using vancomycin hydrochloride (M_w 1485, Wako Chemicals, Osaka, Japan: vancomycin) and cytochrome c (M_w 12,384, Sigma, St Louis, MO, USA). Vancomycin and cytochrome c was loaded into the PMB aqueous solution, respectively. The PMA aqueous solution was then added to the PMB-polypeptide solution (PMA:PMB = 1:1 (v/v)). The concentration of the PMA and PMB aqueous solutions was controlled to 10 wt% and then vigorously mixed for 10 s with vortex mixer to prepare a hydrogel loaded with the polypeptide. The release of the vancomycin and cytochrome c under acidic pH conditions was measured with a UV spectrometer (V-650, Jasco, Tokyo, Japan) (λ = 280 nm for vancomycin and λ = 409 nm for cytochrome c) every 30 min for the first 6 h and every 1 h afterward for 24 h. In order to maintain the buffer conditions, the ratio of the hydrogel and the buffer was controlled at the ratio of 1:99 (v/v). The released FITC-insulin remained soluble under acidic pH conditions for one week and remained soluble under neutral pH conditions for 48 h. A small aliquot was removed and placed in the quartz cell to measure the fluorescent and intensity UV absorbances. The aliquot was then put back into the sample solution. Using the acquired data, we calculated the diffusion exponent for the drug-loaded hydrogels under acidic pH conditions. To analyze the release mechanism, a simple semi-empirical equation presented by Peppas was used: [23–25]

$$\frac{M_t}{M_\infty} = kt^n \tag{14}$$

where the M_t/M_∞ is the fractional release, k is the kinetic constant, and n is the diffusion exponent. For cylinder, $n = 0.45$, $0.45 < n < 0.89$, or $n = 0.89$ indicates a Fickian release, anomalous transport (non-Fickian), or case II transport kinetics [26,27].

To obtain the diffusion coefficient of the hydrogels with diverse drugs by adopting the geometrical parameter into the fractional release, the diffusion coefficient in terms of time was calculated using the following equation [28,29].

$$\frac{M_t}{M_0} = kt^n = 4\left(\frac{Dt}{\pi l^2}\right)^{1/2} \tag{15}$$

where D is the diffusion coefficient, and l is the thickness or length of the hydrogel matrix.

3. Results

The mechanical strength of the hydrogel was evaluated by measuring the complex elastic modulus E using a rheometer. Fig. 2 shows the E values of the respective hydrogels. A1B2 had the highest E value, while the lowest E was for A2B1. The increase in PMB produced an increase in the complex modulus and the viscosity.

Fig. 3 shows the results of the swelling ratio S, water absorption ratio W_{ch}, and polymer loss under acidic conditions. The swelling ratio was the highest for the A1B2 hydrogel while the lowest swelling ratio was shown for the A2B1 hydrogel. The water absorption ratio for the hydrogel was slightly lower compared to the swelling ratio, but their water absorption tendency was the same as that of the swelling ratio. For polymer loss, the highest polymer loss percentage increased with the PMA feed ratio. The hydrogel was not eroded away for the equilibrium state after 1 week.

Fig. 4 shows the change in the polymer concentration of the hydrogel versus time. It can be seen that the decreases in the polymer concentration are very similar to each other. The hydrogel concentration decrease slows down and gradually reaches one-tenth of its original concentration after 1 week.

Fig. 5 shows the result of the change in the surface area and volume of the respective hydrogels under neutral pH conditions. It shows that the decrease in the surface area and volume is the fastest for the A1B2 hydrogel and slowest for the A2B1 hydrogel. The A2B1 hydrogel showed the longest complete dissociation time.

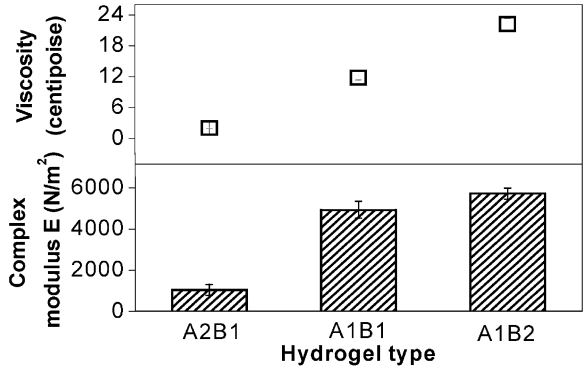


Fig. 2. Complex modulus and viscosity of the respective hydrogels. Each value represents the mean ± SD (n = 5).

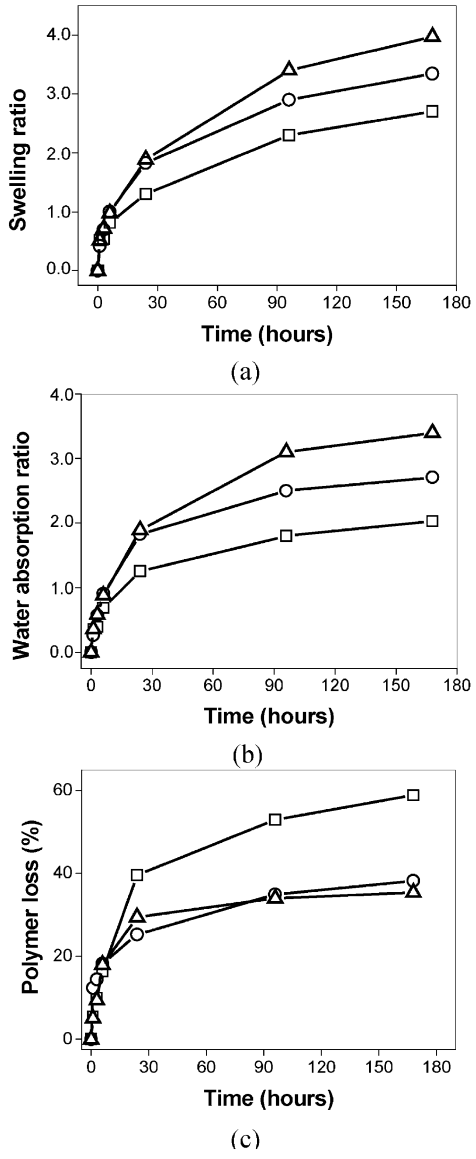


Fig. 3. The change in the swelling ratio (a), water absorption ratio (b), and polymer loss from the hydrogels (c) under pH 1.8 aqueous conditions. (□) A2B1, (○) A1B1, and (△) A1B2. Each value represents the mean ± SD ($n=5$).

The polymer loss percentage is shown in Fig. 6. The fastest polymer release was for A1B2, while the slowest polymer release was for A2B1.

Table 1 shows information for the respective hydrogels. We can see that the molar volume decreases with the increasing PMA moiety. The polymer volume fraction in the relaxed state would decrease with the PMB moiety while that in the swollen state increases.

Table 2 shows the results of $\bar{M}_c$, and ρ_x for each hydrogel. The $\bar{M}_c$ ranges from 2000 to 3500. The $\bar{M}_c$ tends to decrease as the PMA moiety increased while the ρ_x increased. The number of cross-link point, i.e. the N value drops down for as the PMA moiety increased.

Fig. 7 shows the Fourier transform infrared (FTIR) spectra. We had detected an increase in the internal hydrogen bonds by carboxyl groups (1650–1670 cm^{-1}) for the increased PMA feed ratio.

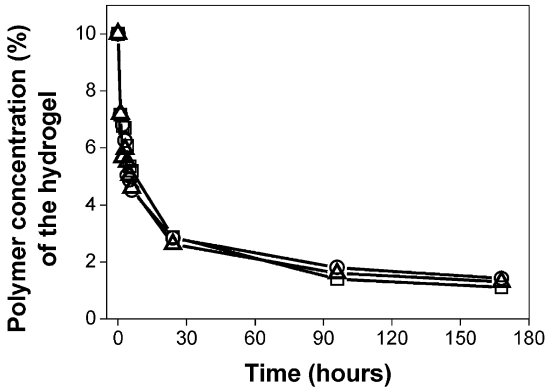


Fig. 4. Change in polymer concentration of 10 wt% hydrogel according to time under acidic pH conditions. (□) A2B1, (○) A1B1, and (△) A1B2. Each value represents the mean ± SD ($n=5$).

Fig. 8 shows the release behavior of vancomycin and cytochrome c loaded into the A1B1 hydrogel under acidic pH conditions. The release of vancomycin is much faster than that of cytochrome c.

Table 3 shows the diffusion exponents and diffusion coefficients of the vancomycin and cytochrome c. By calculating the diffusion exponent, it can be seen that the release would be all non-Fickian. The diffusion exponent shows that the release would be much faster for A2B1, which has a lower swelling ratio value.

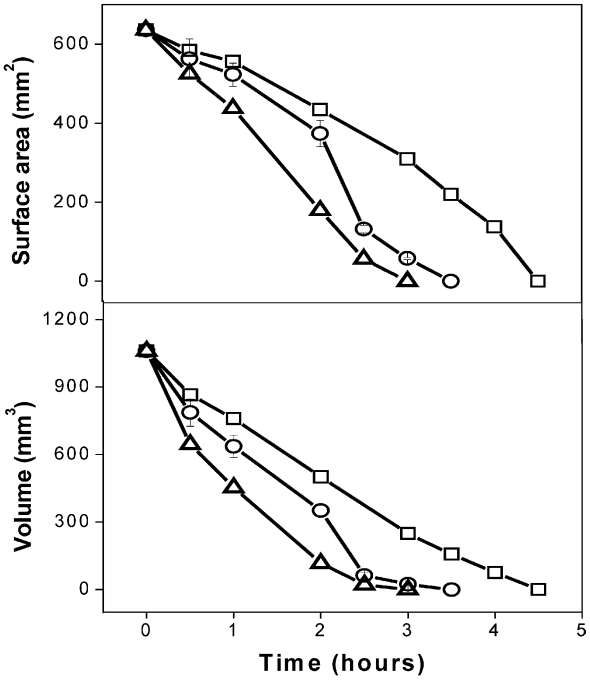


Fig. 5. Change in surface area and volume of respective hydrogels under neutral pH conditions. (□) A2B1, (○) A1B1, and (△) A1B2. Each value represents the mean ± SD ($n=5$).

Table 1
Several structural characteristics of hydrogels

Hydrogel	V_m [a]	M [b]	$\bar{v}$	$v_{2,r}$	$v_{2,s}$	χ
A1B2	185	226	0.819	0.162	0.047	0.262
A1B1	170	207	0.822	0.145	0.050	0.265
A2B1	155	187	0.826	0.110	0.056	0.270

[a] Calculated by Fedor's table [20].
[b] Average of PMA and PMB under homogeneous phase.

4. Discussion

4.1. Mechanical strength, swelling and dissociation behavior

Fig. 2 shows that the complex elastic modulus E and viscosity increased as the PMB increased. We have indicated in our earlier paper that the mechanical strength of the hydrogel depends on the viscosity of the hydrogel which is provided by PMB [9]. Furthermore, it was thought that the cross-link junction density for the higher PMB feed ratio was much higher than any hydrogels with a lower PMB feed ratio. Therefore, it is possible to suggest that the mechanical strength of the hydrogel depends on the viscosity provided by the PMB. This means that the cross-link network inside the hydrogel would be minimal, and this would avoid the hydrogel from having a high swelling ratio.

The A1B2 hydrogel, which had the highest amount of the hydrophobic moiety among the other hydrogels, would swell the most under acidic pH conditions (Fig. 3(a)). This result was opposite from what was expected. According to Fyfe and Blazek, the swelling of the matrix can be attributed to the disruption of the hydrogen bonding among the polymer chains [30]. The penetrating water fills the voids between the polymer chains and diffuses into the denser region of the polymers. The mobility of the polymer chains then increases leading to a high water diffusion rate. The A2B1 hydrogel, which possesses the lowest viscosity, showed that the hydrogel would absorb the least water.

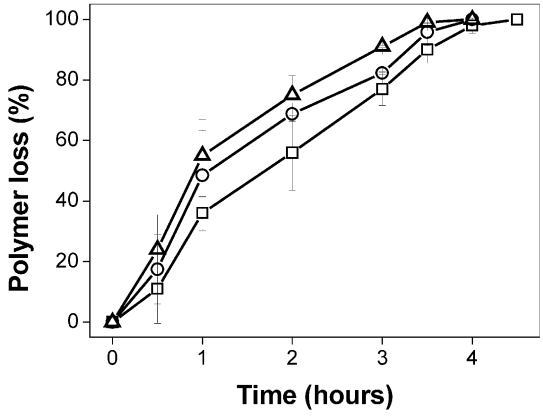


Fig. 6. Polymer being dissoluted out from the hydrogel shown in percentage. (□) A2B1, (○) A1B1, and (△) A1B2. Each value represents the mean ± SD (n = 5).

This can also be supported by the pure water absorption ratio (Fig. 3(b)), which showed that the water absorption was the highest for the A1B2 hydrogel and the lowest for the A2B1 hydrogel. This phenomenon can be explained by the fact that the polymer loss is the highest of all the hydrogels (Fig. 3(c)).

The hydrogel with high water absorption and low polymer loss and hydrogel with a low water absorption and high polymer loss are balanced. Fig. 4 shows that although the water absorption and polymer loss are different, the polymer concentration changes were very similar to each other, indicating that the swelling mechanism would be the same.

When the hydrogels were immersed under neutral pH conditions, the A1B2 hydrogel showed the fastest dissociation and fastest polymer loss, while the A2B1 hydrogel showed exactly the opposite behavior as shown in Fig. 5. From this, it can be postulated that, at least, the mechanical strength is not the major factor that governs the dissociation.

If the low the swelling of A2B1 hydrogel under acidic pH conditions is due to the breakdown of the hydrogel, the breakdown also has to be same under neutral pH conditions (Fig. 3). However, as shown in Fig. 6, the total dissociation of the hydrogel was the slowest for A2B1 and the fastest for A1B2. Furthermore, the polymer that is dissoluting out from the hydrogel is the slowest for A2B1 and the fastest for A1B2. Since the dissolution of the polymer starts as the water is being absorbed, how fast the hydrogel dissociates will depend on the water penetration through the hydrogel network. The water absorption would be higher for A1B2, indicating that the water penetration would be the easiest of all. For the A2B1 hydrogel, the relative difficulty in water penetration made the hydrogel slowly dissociate. However, one question arises: why for A1B2, which possesses the highest viscosity, would it be much easier for the water to penetrate into?

To solve this problem, the concept of intramolecular hydrogen bond networks and intermolecular hydrogen bond

Table 2
Average cross-link molecular weight $\bar{M}_c$, number of cross-link N, and cross-link density ρ_x of respective hydrogels

Hydrogels	$\bar{M}_c$ [Eq. (7)]	N	$\bar{M}_c$ [Eq. (9)]	ρ_x ($\times 10^4$ mol/cm^3)
A1B2	3120 ± 70	28	3480 ± 60	3.5 ± 0.2
A1B1	2640 ± 60	26	2950 ± 60	4.1 ± 0.1
A2B1	1630 ± 80	17	1990 ± 70	6.1 ± 0.2

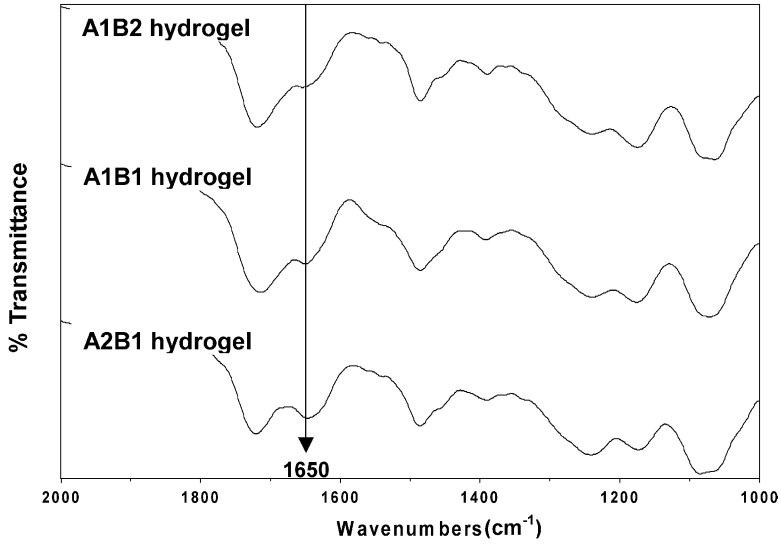


Fig. 7. The FTIR spectra of A2B1, A1B1, and A1B2.

networks was adopted. Intramolecular hydrogen bonds and intermolecular hydrogen bond networks are formed between PMA chains. The formation of the hydrogen bond was already introduced and was proved by our group and Kimura et al. [7,10,22], but type of hydrogen bond was never discussed before. The intramolecular hydrogen bond networks do not effectively function as cross-link junctions, because they do not hold the PMB chains together.

The breakdown of the hydrogel due to the polymer loss starts as the water penetrates into the hydrogel. The carboxylate anions turn into carboxyl groups, producing hydrogen bonds between the carboxyl groups. This phenomenon spreads out as the water proceeds deeper into the hydrogel where the polymers denser region is located.

However, in the case of dissociation under neutral pH conditions, the penetration of water makes the carboxyl groups into carboxylate anions. The dissociation process occurs by surface erosion. That is, the penetrating water would fill the micron-sized air voids between the polymer chains and diffuse into the denser region of the polymer. Since the hydrogel is homogeneous, the water ionizes the carboxyl groups located at the outermost region of the hydrogel and gradually proceeds inward, that is, like the opening of a zipper. It must ionize all the carboxyl groups to completely dissociate. It is required to ionize all the carboxyl groups to dissociate. As shown in the swelling test, although the polymer moiety has leaked out, the hydrogel did not lose the network, which made the hydrogel retain its form and absorb water. For the denser network, 'including the network formed by intramolecular hydrogen bonds', prohibits easy penetration of water.

So, it can be said that the swelling and dissociation would depend on the formation of intra- and intermolecular hydrogen bond networks inside the hydrogel.

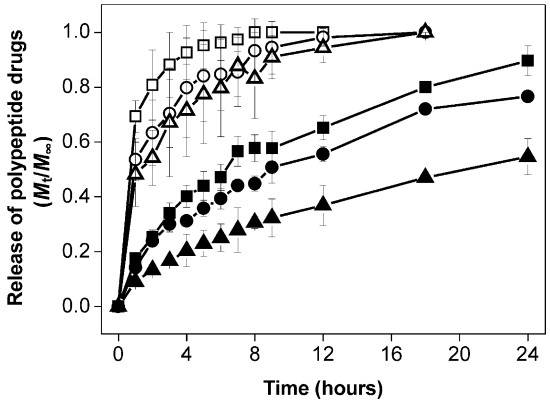


Fig. 8. The release profile of vancomycin (open symbol) and cytochrome c (closed symbol) from respective hydrogels under acidic pH conditions. (■, □) A2B1, (●, ○) A1B1, and (▲, △) A1B2. Each value represents the mean ± SD ($n = 5$).

4.2. Hydrogel characterization

The hydrogel would be cross-link by hydrogen bonds as the carboxylate anion groups turn into carboxyl groups. It is generally thought that the cross-link junctions would be produced between hydrogen bonds where the permittivity and polarity are low. For the physically cross-link hydrogels, there exist intramolecular forces together with intermolecular forces. It is the intermolecular forces, which contribute to the swelling. The formation of intramolecular cross-links is predominantly formed in the dilute solution. The intermolecular cross-links would become dominant as the polymer solution is being concentrated.

In order to calculate the cross-link molecular weight $\bar{M}_c$, we made the assumption that the hydrogel is homogeneous. We indicated in our earlier paper that the hydrogel remains homogeneous once they are mixed in aqueous solution [1,7]. Therefore, based on this assumption, we calculated the various parameters of the respective hydrogel using

Table 3
Diffusion exponent, kinetic constant, and diffusion coefficient of respective hydrogels loaded vancomycin (VAN) and cytochrome c (Cyc)

Hydrogels	Diffusion exponent, n		Kinetic constant, k		Diffusion coefficient D ($\times 10^{-5}$ cm^2/s)	
	VAN	Cyc	VAN	Cyc	VAN	Cyc
A1B2	0.59	0.56	0.31	0.09	21.0	1.46
A1B1	0.60	0.49	0.29	0.16	35.7	3.16
A2B1	0.69	0.57	0.27	0.17	77.9	4.71

Eqs. (2)–(12). Table 1 shows the results for the respective hydrogels.

Based on the information in Table 1, we calculated $\bar{M}_c$, N, and ρ_x of each hydrogel. The $\bar{M}_c$ ranges between 2000 and 3500 which is about 9–14 monomer repeating units. Highly cross-link hydrogels are those with fewer than 100 repeating units. The $\bar{M}_c$ tends to decrease while ρ_x increases as the PMA moiety increased. The decrease in $\bar{M}_c$ and increase in ρ_x indicates that the cross-link junctions are provided by the carboxylic groups of PMA. The number of cross-link points N decreases for A2B1. This means that the valid entanglement for the mechanical strength is low. We have already shown that the mechanical strength is low for A2B1 hydrogel.

We have mentioned that the there is an intramolecular hydrogen bond network and an intermolecular hydrogen bond network inside the hydrogel. The amount of these networks would be different according to the PMA and PMB feed ratio. The $\bar{M}_c$ equation provides information on the $\bar{M}_c$ value of the intramolecular cross-links together with the intermolecular cross-links [14]. We already mentioned that A1B2 would possess a higher mechanical strength than that of A2B1. Although the A2B1 possesses lower mechanical strength and lower swelling ratio, it has a lower $\bar{M}_c$. The lower $\bar{M}_c$ value and higher ρ_x value for A2B1 indicates that there are many cross-links that are intramolecularly cross-link. Therefore, the decrease in the $\bar{M}_c$ value would increase the ρ_x value, which makes the water relatively difficult to penetrate deep into the hydrogel. Furthermore, this result implies that the hydrogen bonds that occurred in the hydrophobic domain are mainly intermolecular hydrogen bonds. Fig. 9 shows a schematic picture of how A1B2 and A2B1 would be formed.

It shows that the A1B2 hydrogel has an abundant intermolecular network while the A2B1 hydrogel possesses many loops formed by the intramolecular network. The intramolecular cross-links do not contribute to the elastic effectiveness of the networks and the swelling of the hydrogel or the mechanical strength of the hydrogel. On the other hand, the intermolecular cross-links may support the network more firmly and swell more. Therefore, it could be said that the weak mechanical strength, but low swelling ratio of A2B1 was due to the intramolecular cross-link networks between the carboxyl groups. Also, the lower value of $\bar{M}_c$ and higher value of ρ_x made the swelling difficult. Furthermore, the loops had to be totally ionized to dissociate under neutral pH conditions, which delayed the time to reach complete dissociation for the A2B1 hydrogel. Consequently, intramolecular cross-links reduced the $\bar{M}_c$ values. The FTIR spectra support this result. Kaczmarek et al., have used FTIR to investigate the stabilization effect of poly(acrylic acid)–poly(vinylpyrrolidone) complexes with different stoichiometric ratio and found that increase of stabilization can be achieved by increment of intermolecular hydrogen bonds of the polymer structure [31]. We can clearly see that the peak at 1650 cm^{-1}, which indicates the number of intermolecular hydrogen bonds would be decreased as the PMA moiety increases (Fig. 7). This also supports the earlier assumption that the cross-links between the carboxyl groups in the hydrophobic region would be intermolecular. From this, we can conclude that the intramolecular hydrogen bond network increases as the PMA feed ratio increases, resulting in a weaker mechanical strength and lower water absorption ability which suppresses the swelling.

4.3. Release property of loaded drugs

The effect of $\bar{M}_c$ on the release has been investigated under acidic pH conditions. As shown in Fig. 8, the release of the vancomycin was much faster than cytochrome c and the release was suppressed as the PMB moiety increased. Within 24 h, the release of vancomycin was almost finished, while the release of cytochrome c was 70% of its loaded amount. The low molecular weight vancomycin is thought to have released from the hydrogel faster than the high molecular weight cytochrome c. We have indicated in our earlier paper that the release during the first 5 h would be highly affected by the leaking out of the polymer moiety from the hydrogel. However, since vancomycin has a lower molecular weight than the $\bar{M}_c$, the polymer is thought to be diffused out from the hydrogel, making the release very fast. When the release of model drugs are compared among A1B2, A1B1, and A2B1, it can be seen that the release from A2B1 would be the highest of all. The diffusion exponent and diffusion coefficient based on this result were calculated and summarized in Table 3.

The diffusion exponent result showed that the release is non-Fickian for the vancomycin and cytochrome c loaded hydrogels. As the PMA moiety increases, the diffusion coefficient increases, implying that the release of the loaded drugs would be faster. For the vancomycin loaded hydrogels, the release tendency according to the PMA/PMB feed ratio was the same as that of the cytochrome c loaded

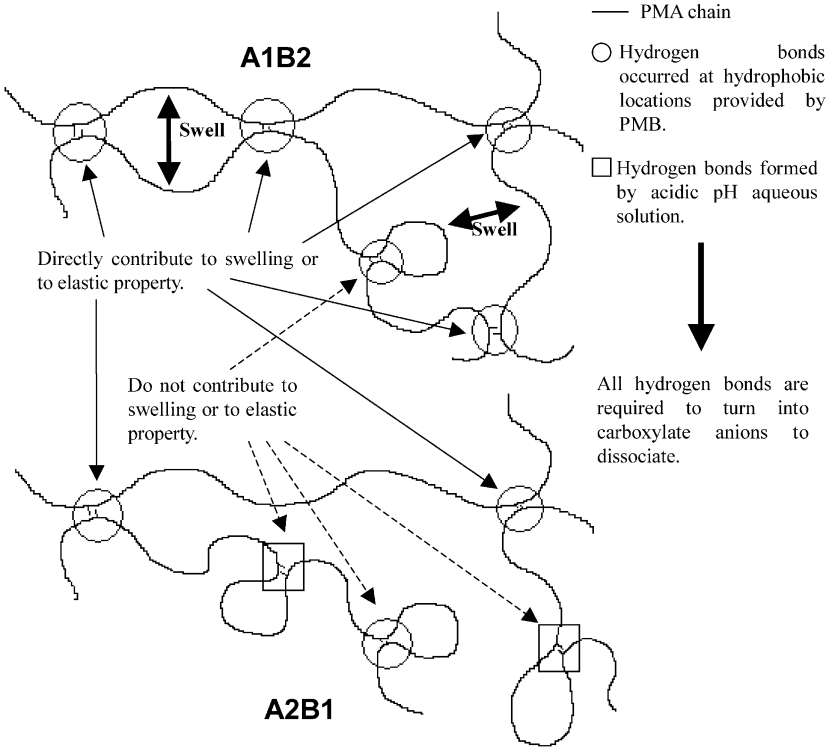


Fig. 9. Schematic picture of intermolecular hydrogen bond cross-links and intramolecular hydrogen bond cross-links. The intramolecular hydrogen bond cross-links do not contribute to the swelling but needs to be ionized in order to be dissoluted.

hydrogels. We have mentioned in an earlier section that A1B2 would swell the greatest. The swelling caused by the absorption of water would make the polymer leak out. At the same time, the loaded drugs would be released, but relying on the leaking out of the polymer instead of water absorption. Drugs such as cytochrome c possess a higher molecular weight than $\bar{M}_c$. The release would be interfered although the hydrogel swells, but drugs with a molecular weight lower than $\bar{M}_c$ would let the drugs out, resulting in a much faster release of the drugs. Similar result was observed by the experiment done by de Jong et al. and Ramkissoon-Garnorkar et al. [14,32].

5. Conclusion

We investigated the properties of two-polymer composed hydrogel by assuming that the hydrogel is homogeneous. When the PMA feed ratio increases, the mechanical strength and swelling ratio under acidic pH conditions decreases, but its dissociation time under neutral pH conditions is longer. This implies that the hydrogel network consisted of intramolecular cross-links. The calculated $\bar{M}_c$ and ρ_x values were low for A2B1, for intramolecular cross-links brought the values down. The release experiment showed that the release would be slow for drugs with a molecular weight higher than $\bar{M}_c$. And the release of model drugs from different hydrogels showed that the increase in the intramolecular cross-link would increase the release rate of the model drugs.

Therefore, it can be concluded that: (i) the cross-link junction is formed by intermolecular hydrogen bonds between the PMA chains, (ii) as the PMA feed ratio increases, the intramolecular hydrogen bond cross-link increases, which produces the weak formation of the hydrogel, and (iii) the release depends on both the polymer loss and diffusion of the loaded drugs, but would be different based on the molecular weight of the drugs.

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