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Preparation and Stimuli-Sensitive Characterization of Polyelectrolyte Complex Films Derived from Chitosan and Polyalkyleneoxide-Maleic Acid Copolymer

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Stimuli-sensitive materials with biocompatibility and biodegradability have enormous potential in various applications, especially in biomedical field, and have been investigated by many researchers to achieve controlled drug/protein/DNA delivery to specific-site. In this study, polyelectrolyte complex (**PEC**) films were prepared from a cationic polymer, chitosan (**CS**), and an anionic polymer, polyalkyleneoxide-maleic acid (**PAOMA**) copolymer, by casting/solvent evaporation method. Those PEC films prepared from CS and PAOMA copolymer had pH- and temperature-sensitivity. Additionally, the reversibility and response of the swelling process were confirmed at different pH-conditions. From permeation tests, the PEC films were proved to change drug permeability as environmental pH and temperature conditions altered. Permeation studies demonstrated that an increase in temperature from 25 ℃ to 50 ℃ yielded an increase in the rate of drug permeation because of shrinking of the PAOMA copolymer in the film. Among three model drugs tested, glucose showed the lowest effective diffusion coefficient because of the largest molecular weight and the strongest hydrophilicity.
Key words: Polyelectrolyte complex film, Chitosan, Polyalkyleneoxide-maleic acid, Stimuli-sensitivity

1. INTRODUCTION

Stimuli-sensitive hydrogels with biocompatibility and biodegradability have enormous potential in various applications, especially in biomedical field, and have been investigated extensively by many researchers to achieve controlled drug/protein/DNA delivery to specific-site. Temperature-sensitive hydrogels are one of the most commonly studied classes of stimuli-sensitive systems in drug delivery research [1]. Poly(*N*-isopropylacrylamide) (**PNIPAAm**) is the most typical polymer among the temperature-sensitive polymers investigated up to now, which has lower critical solution temperature (**LCST**) in the range of 25-32 °C, close to the body temperature. However, the clinical applications of PNIPAAm hydrogels have limitations due to the carcinogenic or teratogenic toxicity of the monomer and crosslinkers used in the PNIPAAm synthesis. In addition, PNIPAAm and its derivatives are not biodegradable [2].

Chitosan (**CS**), a naturally occurring cationic polysaccharide, is one of the major biodegradable and biocompatible polymers used in the drug delivery systems [3]. Polyalkylenoxide-maleic acid (**PAOMA**) copolymer, a polymeric anion with excellent bio-compatibility, has unique amphiphilic property: the increasing hydrophobicity with increase in temperature. Especially, a series of PAOMA has also various LCST with changing the alkyleneoxide (AO) chain composition.

Electrostatic interaction between cationic and anionic polyelectrolytes results in the formation of a polyelectrolyte complex (**PEC**). Thus, PEC films can be obtained from the electrostatic interaction of CS and PAOMA copolymer and show pH-responsive and temperature-responsive properties (swelling, drug permeation and drug release). As the PEC films would have high biocompatibility and biosafety, the films have potential for therapeutic usage as drug/protein/DNA carrier with stimuli-sensitivity.

In this study, PEC films were prepared from a cationic polymer, CS, and an anionic polymer, PAOMA copolymer, by casting/solvent evaporation method. The pH- and temperature-sensitive swelling behaviors in various buffer solutions were examined. The permeation and release profiles of model drugs, salicylic acid, phenol and glucose, from the PEC film were examined at different pH and temperature.

2. EXPERIMENTAL

2.1 Materials

CS (degree of acetylation, 0.085; average molecular weight, 10^6) was provided from Kyowa Tecnos Co. Ltd., Japan. PAOMA copolymer (AEM-0530, AKM-0530) was supplied from Nippon Oil & Fats Co., Japan. The structures of the polymers used are shown in **Figs. 1** and **2. Table I** shows the PAOMA copolymer composition. Model drugs, salicylic acid, phenol and glucose, were purchased from Wako Pure Chemical Industries, Ltd., Japan. All other reagents were of analytical grade.

2.2 Preparation of PEC Films

PAOMA solution (30 wt%) was purified by dialysis membrane (MWCO:3500, SPECTRUM) against distilled water for 4 days. The PAOMA solution (2 wt%), dissolved in distilled water, and the CS solution (2 wt%), dissolved in 36 wt% aqueous acetic acid, were mixed in same molar ratios. Then, the mixture was cast on a polystyrene petridish (135×95 mm) and dried at 50 °C until constant weight. The dried films were cut into disks (ϕ 10 mm) and stored at refrigerator.

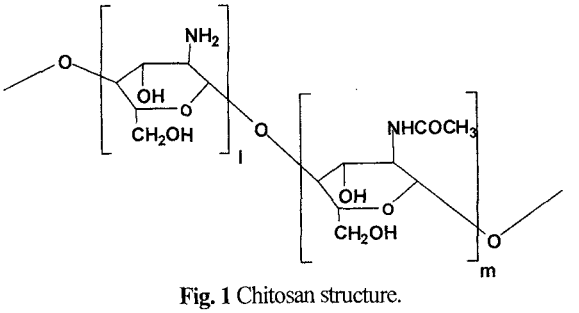
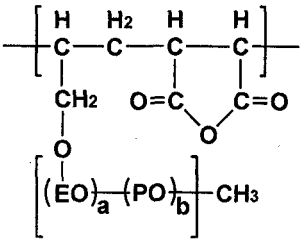


Fig. 1 Chitosan structure.



EO= CH_2-CH_2-O PO= $CH_2-CH(CH_3)-O$

Fig. 2 PAOMA structure.

Table I PAOMA copolymer composition

	a/(a+b)	M.W.	Cloud point
AEM0530	1.0	20,000	35℃
AKM0530	0.4	20,000	Over 70℃

2.3 Swelling Tests

Dried PEC films were carefully weighed and immersed in media (50 cm^3) with pH values from 1.2-7.5 at 37℃, or with temperature from 0 °C – 60 °C at pH 3.8. At predetermined time intervals (12 h), swollen films were taken out, and the excess water was blotted with filter paper from the surface, and then weighed on a sensitive balance (AEU-210, Shimadzu).

For stimuli-responsive swelling tests, the PEC films prepared from chitosan and AEM 0530 (**CS/AEM films**) were exposed to the different environments repeatedly. The pH- responsive swelling was investigated at pH 6.2 and pH 3.8. The temperature-responsive swelling was investigated at 50 and 25 °C. The following equation was used to determine the swelling degree (D_S).

$$D_S=(W_{et}-W_0)/W_0$$

Here, W_0 and W_{et} are the film weights of dry and swollen films.

2.4 In vitro Drug Permeation Tests

The permeability of model drugs through CS/AEM films was measured at constant temperature (25 and 50 °C) by modified diffusion-vessels method as shown in **Fig. 3** [4]. Filter papers were used to support the PEC film in this method. The initial donor concentration was 2.0 g/L and the effective surface area for flux was 0.785 cm^2. The solutes concentration in the receiver cell was measured with UV absorbance at 234 nm (U-3210, Hitachi, Japan) at pre-determined time.

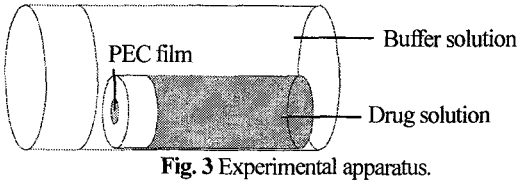


Fig. 3 Experimental apparatus.

3. RESULTS AND DISCUSSION

3.1 pH-dependent swelling

From the preliminary experiment, the weight of all films increased rapidly and the swellings were equilibrated within 6 h. As shown in **Fig. 4**, the D_S of PEC films were dependent on pH. The results indicate that PEC films swell significantly at low pH; as the pH value increases, the D_S decreases sharply in all cases. This can be explained by the protonation of amino groups in CS and carboxyl groups in PAOMA copolymer. The pK_a of CS was 6.5 and the pK_a of PAOMA copolymer was 4.8. At a low pH region, most of amino groups in CS are in the form of NH_3^+ and most of carboxyl groups in PAOMA copolymer are in the form of COOH. Therefore, the repulsive force between positive charges of CS molecules made the intermolecular distances much longer and electrostatic potential more hydrophilic. As the pH of the medium increases, the carboxylic acid groups are ionized, and electrostatic interaction between NH_3^+ and COO^- get strong gradually so that D_S decreases gradually with increase in pH. In the range of pH 4.8 to 6.5, PEC films shrink because electrostatic interaction between NH_3^+ and COO^- is very strong.

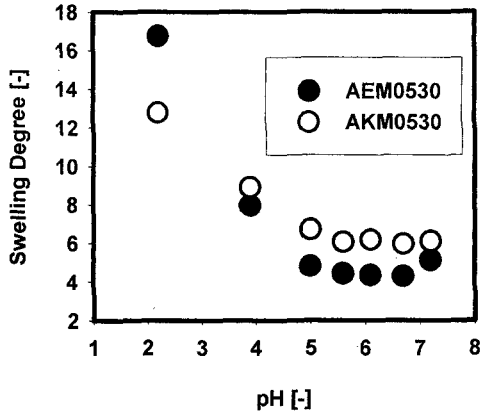


Fig. 4 pH-dependent swelling of PEC films prepared from different types of PAOMA-copolymer.

3.2 Temperature-dependent swelling

The swelling behavior of PEC films prepared from CS and PAOMA copolymers (AEM0530, AKM0530) was investigated as a function of temperature. As shown in **Fig. 5**, when AKM0530 was used as polyainon, the D_S values were independent of temperature. In contrast, D_S of PEC films prepared with AEM0530 were obviously dependent on temperature between 30 and 60 °C. The AEM0530 aqueous solution has LCST at *ca.* 35 °C, which is indicated by a reversible phase transition from soluble to insoluble states in response to temperature at *ca.* 35 °C. The temperature-responsive soluble-insoluble transition of AEM0530 can be explained by the dissociation of ordered water molecules surrounding hydrophobic propylene oxide groups over 35 °C. Due to this phenomenon, the PEC films composed of CS and AEM0530 showed a volume phase transition around LCST of AEM0530. On the other hand, LCST of AKM0530 is over 70 °C so that the D_S values weren't changed in the tested temperature.

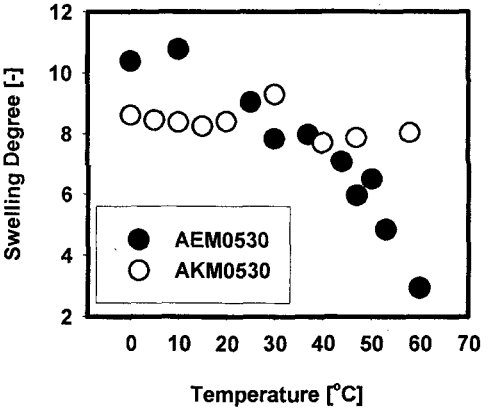


Fig. 5 Temperature-dependent swelling of PEC films prepared from different types of PAOMA copolymer.

3.3 Stimuli-responsive swelling behavior

In addition to the equilibrium swelling experiments, stimuli-responsive swelling tests were consecutively carried out at the different conditions of pH (6.2 and 3.8) and temperature (25 and 50 °C) to confirm the response and reversibility of the swelling process. The results of stimuli-responsive swelling test are shown in **Figs. 6** and **Fig. 7**. In response to the pH, the swelling was changed reversibly. As a result, the film could be expected to control drug permeability immediately in response to environmental pH. When the temperature-responsive swelling test was carried out at pH 3.8, the swelling was also changed reversibly in regard to temperature. We note, however, that the difference in swelling degree between 25 °C and 50 °C was not so significant.

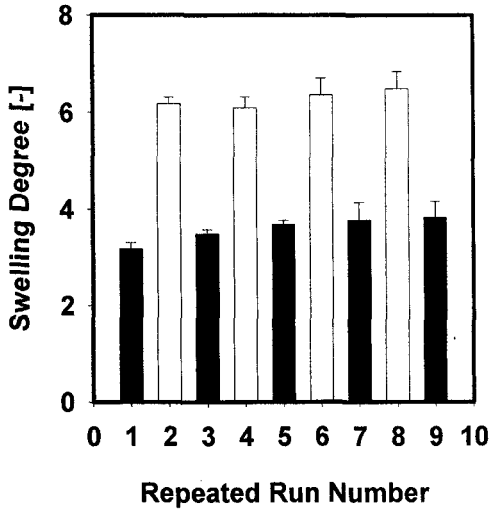


Fig. 6 pH(pH 6.2 or pH 3.8)-responsive swelling of CS/AEM film at 37℃.

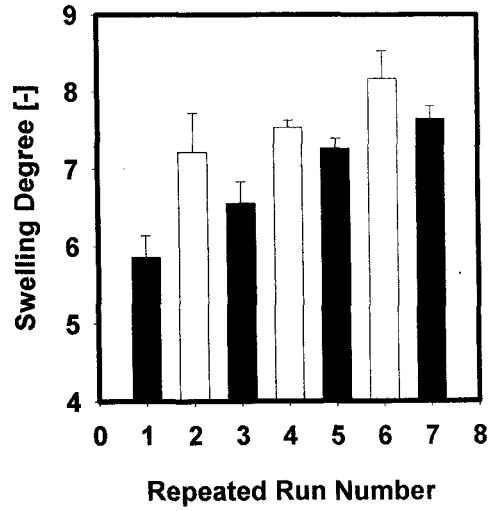


Fig. 7 Temperature (50 ℃ or 25 ℃)-responsive swelling of CS/AEM film at pH 3.8.

3.4 In vitro Drug Permeation Tests

It is assumed that the permeation of drug is in accord with Fickian law, so the permeability coefficients were determined based on the following equation:

$$\ln\left(\frac{C_0 - C_{B,t}}{C_0}\right) = -k \cdot A\left(\frac{1}{V_A} + \frac{1}{V_B}\right)P_f t$$

where $C_{B,t}$ is the solution concentration in the receptor vial at time t, C_0 is the initial solute concentration of the donor vial, V_A and V_B is the volume of donor and receptor vial respectively (V_A=80 cm^3, V_B=33 cm^3), A is the effective area of permeation (A=0.785 cm^2), P is the film permeability coefficient and k is the solute partition coefficient calculated from the following equation:

$$k = \frac{c_f}{c_s}$$

where c_f and c_s are the concentration of the solute in the membrane and in the surrounding solution at equilibrium.

Fig. 8 shows a typical permeation behavior of salicylic acid permeation through the PEC films at different temperature. From the slope of the linear line in Fig. 8, P was calculated. The values of P are listed in **Table II**.

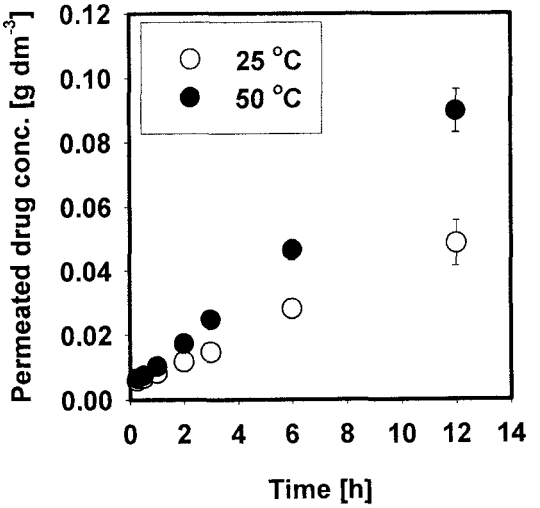


Fig. 8 The concentration of salicylic acid in permeate through the PEC films prepared from CS and AEM0530 at different temperature in pH 3.8 media.

For further elucidation of salicylic acid permeation through PEC film, the effective diffusion coefficient was calculated using the following equation:

$$D = P_f l$$

where D is the effective diffusion coefficient, l is the film thickness in the swollen state at constant pH and temperature. The values of D are also summarized in Table II.

It can be seen that for the same drug in different temperature media, P and D show an increasing trend along with the increase of temperature. The dependence of P and D on temperature is attributed to the hydrophobicity of the AEM0530. As shown **Fig. 9**, AEM0530 in PEC films swells under LCST. The swollen AEM0530 was expected to block the pores of CS as if a valve is closed. The lower rate of drug permeation at 25 °C could be attributed to the blocking effect of swollen AEM0530. Whereas, the rate of drug permeation at 50 °C was higher because the AEM0530 molecule shrank in the PEC film, resulted in the increasing in void space in the film. [6]

Also, it can be seen in Table II that for the same drug in different pH media, P and D show a decreasing trend along with the increase of pH. The dependence of P and D on pH is explained by free volume in the film. In polymer hydrogel, effective free volume is essentially the free volume of water, and the transport of solutes is presumed to permeate through the free-water region

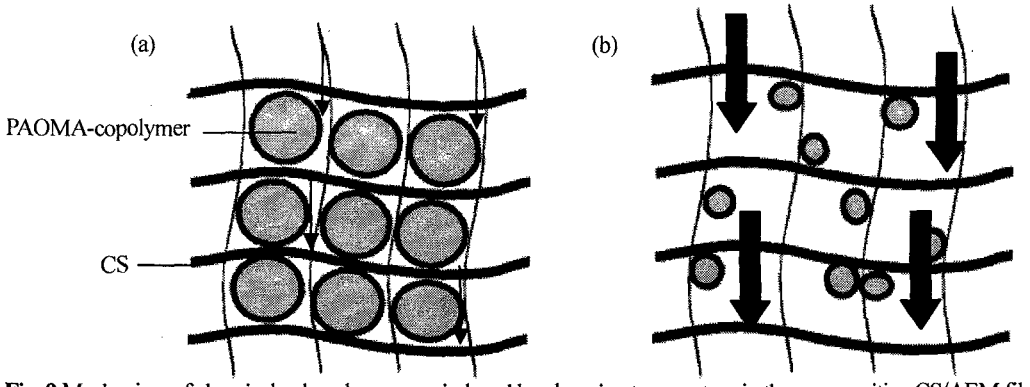


Fig. 9 Mechanism of chemical valve phenomena induced by changing temperature in thermo-sensitive CS/AEM films (a) swollen state of the PAOMA-copolymer at temperature < LCST (b) shrunken state of the PAOMA-copolymer at temperature > LCST

in the swollen film [7]. As shown Fig. 4, PEC films swells at low pH. Consequently, as the total amount of pores or channels in a unit volume of film increase, the amount of solute transport increase. To the contrary, as the amount of pores decrease at high pH, *P* and *D* decreased.

The model drugs such as phenol (hydrophobic), salicylic acid (a slightly hydrophobic), glucose (hydrophilic) were used for permeation tests to investigate the effect of drug molecular weight and hydrophobicity. The molecular weight and hydrophilicity of three drugs are in order of phenol < salicylic acid < glucose. As shown in Table II, *D* of phenol was the largest and *D* of glucose was the smallest among them. This result means that the rate of drug permeation is much higher at the lower molecular weight and hydrophilicity of drugs. In other words, this phenomenon is considered due to the increase of effective molecular radius through the hydration process.

4. CONCLUSION

PEC films were prepared from CS and PAOMA copolymer. Equilibrium pH-swelling studies showed that the film swelled at low pH and shrank at high pH regardless of a type of PAOMA copolymer. On the other hand, Equilibrium temperature-swelling studies of CS/AEM films showed that the film swelled at 25 °C and shrunk at 50 °C. From the results of stimuli-responsive swelling tests with alternating pH between 6.2 and 3.8, the reversibility and response of the swelling process were confirmed at different pH-conditions. However, the difference in swelling degree between 25 °C and 50 °C was not so significant. Also, PEC films prepared from CS and AEM0530 were proved to change drug permeability immediately as environmental pH and temperature condition altered. Permeation studies demonstrated that an increase in temperature from 25 °C to 50 °C yielded an increase in the rate of drug permeation because of shrinking of the PAOMA copolymer in the film. Among three model drugs tested, effective diffusion coefficient of phenol was the highest because of the smallest molecular weight and the strongest hydrophobicity.

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REFERENCES

[1] L. E. Bromberg, E. S. Ron, *Adv. Drug Deliv. Rev.*, **31**, 197-221 (1998)
[2] Y. Qiu, K. Park, *Adv. Drug Deliv. Rev.*, **53**, 321-339 (2001)
[3] E. Khor, *Cur. Opi. in Sol. Mat. Sci.*, **6**, 313-317 (2002)
[4] T. Inoue, G. Chen, K. Nakamae, A. S. Hoffman, *J. Contr. Release*, **51**, 221-229 (1998)
[5] T. Kajiuchi, Y. Shin-ya, H. Nagai, K. Fujiwara, *J. Chem. Tech. Biotech.*, **77**, 495-499 (2002)
[6] S. G Hirsch, R. J. Spontak, *Pol.*, **43**, 123-129 (2002)
[7] Y. E. Fang, Q. Cheng, X. B. Lu, *J. Appl. Pol. Sci.*, **68**, 1751-1758 (1998)

Table II Permeability and effective diffusion coefficients

pH	Temperature[℃]	Model drug	Effective area[cm^2]	$P \times 10^{-5}$ [cm/s]	Thickness [mm]	$D \times 10^{-7}$ [cm^2/s]
3.8	25	Salicylic acid	0.785	1.12 ± 0.02	0.46	5.18 ± 1.45
3.8	50	Salicylic acid	0.785	3.21 ± 0.62	0.37	11.9 ± 2.49
6.2	25	Salicylic acid	0.785	0.44 ± 0.03	0.57	2.51 ± 0.16
6.2	50	Salicylic acid	0.785	1.92 ± 0.63	0.51	9.80 ± 3.22
3.8	25	Phenol	0.785	1.43 ± 0.22	0.43	6.15 ± 0.97
3.8	50	Phenol	0.785	4.49 ± 0.99	0.40	18.0 ± 3.98
3.8	25	Glucose	0.785	0.39 ± 0.04	0.42	1.66 ± 0.15
3.8	50	Glucose	0.785	0.68 ± 0.11	0.62	4.23 ± 0.67

P is permeability coefficient. *D* is effective diffusion coefficient.

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