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Research paper

pH- and temperature-sensitive release behaviors from polyelectrolyte complex films composed of chitosan and PAOMA copolymer

Toru Yoshizawa, Yoshitsune Shin-ya*, Kyung-Jin Hong, Toshio Kajiuchi

Department of International Development Engineering, Tokyo Institute of Technology, Tokyo, Japan

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Abstract

This paper describes the pH and temperature effects on drug release from polyelectrolyte complex (PEC) films composed of a cationic polymer, chitosan, and an anionic polymer, polyalkyleneoxide–maleic acid copolymer (PAOMA). In this study, we prepared and investigated PEC films in terms of the drug release properties as pH- and temperature-sensitive drug carriers. Drug release rates were tested at pH 3.8 and 7.2, and at 25 and 50 °C. Salicylic acid and phenol were selected as model drugs. An increase in pH from 3.8 to 7.2 resulted in an increase in the rate of drug release because of the repulsive forces between carboxyl groups in PAOMA and anionic groups in model drugs. When the hydrophobic PAOMA was used as a polyanion, the drug release rate increased at 50 °C. This is attributed to the increase of release area due to the phase transition of PAOMA and the increase of repulsive forces between carboxyl groups in PAOMA and anionic groups in model drugs.

Keywords: Chitosan; Drug release; Polyalkyleneoxide–maleic acid copolymer; pH- and temperature-sensitivities; Polyelectrolyte complex film

1. Introduction

Much interest has been focused on polymer systems that show a phase transition in response to external stimuli such as temperature, pH, ionic strength and electric potential. Stimuli-sensitive biodegradable and biocompatible films and hydrogels have recently shown enormous potential in controlled release drug delivery applications [1–3]. Temperature-sensitive hydrogels are one of the most commonly studied classes of stimuli-sensitive systems [4,5]. Poly(*N*-isopropylacrylamide) (PNIPAAm) is a typical polymer among the temperature-sensitive polymers investigated to date because it has a lower critical solution temperature (LCST) in the range of 25–32 °C [6–8]. However, the clinical applications of PNIPAAm hydrogels are limited due to the carcinogenic or teratogenic toxicity of the monomer and crosslinkers used in PNIPAAm synthesis [1]. In addition, PNIPAAm and its derivatives are not biodegradable [1].

Chitosan (CS), poly-β-(1-4)-2-amino-2-deoxy-D-glucose, is a naturally occurring biodegradable and biocompatible cationic polysaccharide derived from the *N*-deacetylation of chitin. CS has found wide utility in applications ranging from tissue engineering to drug delivery [9–14]. At acidic pH ranges, the ionizable amino groups in CS molecules are protonated. Therefore, the formation of polyelectrolyte complexes (PEC) with polyanionic molecules have been widely reported [13,14]. Polyalkylenoxide–maleic acid copolymer (PAOMA) is an anionic polymer with excellent biocompatibility and unique amphiphilic properties. Especially, a series of PAOMA have various LCST with changing the alkyleneoxide (AO) chain composition. Using these unique polymeric materials, enzyme modification [15,16] and protein separation [17] have been conducted.

Electrostatic interactions between the cationic CS and the anionic PAOMA result in the formation of a PEC [2]. The swelling, drug permeation and release properties of

* Corresponding author. Department of International Development Engineering, Tokyo Institute of Technology, 2-12-1-I4-18 Ookayama, Ishikawadai 4th Building, Meguro-ku, Tokyo 152-8550, Japan. Tel.: +81 3 5734 3246; fax: +81 3 5734 3315.
E-mail address: yshin-ya@kuramae.ne.jp (Y. Shin-ya).

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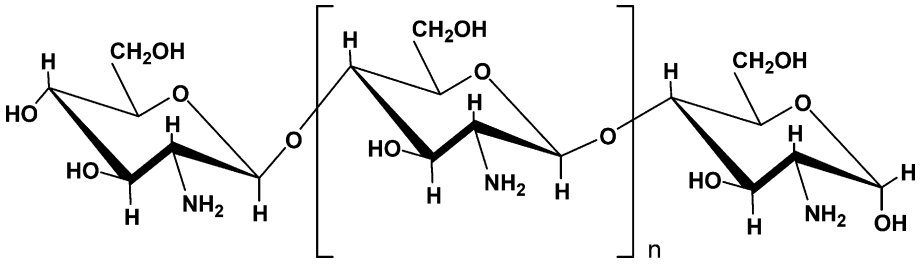

Fig. 1. Chitosan structure.

PEC can be controlled by pH- and temperature-changes. The high biocompatibility of PEC films provides potential for therapeutic applications as a stimuli-sensitive drug delivery carrier.

In previous study [18], we prepared PEC films composed of CS and PAOMA and investigated the pH- and temperature-responsive permeation from the films using model drugs. As a result, we confirmed that those PEC films have pH- and temperature-sensitivity for equilibrium swelling. In addition, the increase in temperature from 25 to 50 °C and the decrease in pH from 7.2 to 3.8 yielded an increase in the rate of drug permeation.

In this study, we prepared the model drug-loaded CS/PAOMA films and investigated the effect of pH and temperature on drugs release. The model drugs used were salicylic acid and phenol. In addition we observed the surface morphology of the films by SEM to correlate the drug release mechanism with the microstructure of the films.

2. Materials and methods

2.1. Materials

CS (degree of acetylation, 0.085; average molecular weight, 10^6) was provided from Kyowa Tecnos Co. Ltd, Japan. PAOMA (AEM-0530, AKM-0530) were supplied from Nippon Oil and Fats Co., Japan. The structures of the polymers used are shown in Figs. 1 and 2. Table 1 shows the PAOMA copolymer composition, molecular weight and cloud point. Cloud point was measured as follows: PAOMA aqueous solution (2 g dm^{-3}, 7 cm^3) was prepared and settled in a temperature-constant bath. Temperature of the bath was increased at a rate of 1–3 °C/10 min. The LCST was defined as the temperature at which the solution started to be cloud. Salicylic acid and phenol were purchased from Wako Pure Chemical Industries, Ltd, Japan. All other reagents were of analytical grade.

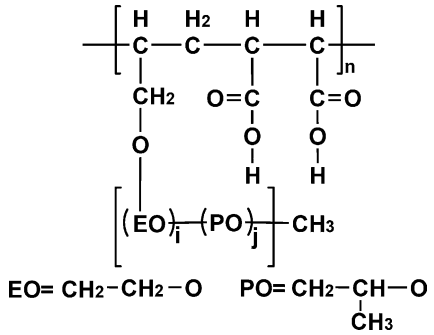

Fig. 2. PAOMA structure.

2.2. Methods

2.2.1. Preparation of PEC films

PAOMA solution (30 wt%) was purified by dialysis (MWCO:3500, SPECTRUM) against distilled water for 4 days. The dialyzed PAOMA solution (90 g dm^{-3}, 18.5 cm^3) dissolved in distilled water and CS solution (2 wt%, 50 g) dissolved in 36 wt% aqueous acetic acid were mixed and stirred for 3 days. The molar ratio of carboxyl group in PAOMA to amino group in CS was set at 1.0. The mixture was cast on a polystyrene petridish (135×95 mm) and dried in vacuo at 50 °C until the film reached a constant weight. The dried films were cut into disks (∅10 mm) and stored in a refrigerated desiccator until use.

2.2.2. Preparation of drug-loaded PEC films

The mixture of CS and PAOMA were prepared by mixing same molar ratios of dialyzed PAOMA solution (90 g dm^{-3}, 18.5 cm^3) and CS solution (2 wt%, 50 g), and then stirred for 1 day. Then, 0.1 g of model drugs, salicylic acid and phenol, were added into the mixture and stirred for 3 days [19]. The mixture was cast, dried and stored as described in Section 2.2.1.

2.2.3. In vitro drug release tests

Dried drug loaded films were incubated at 25 or 50 °C in buffer solution (40 cm^3) with pH 3.8 and 7.2 at a frequency of 80 strokes/min. At predetermined time intervals, 2.0 cm^3

Table 1
PAOMA copolymer composition

	EO ratio[a]	MW	Cloud point (°C)
AEM-0530 (hydrophobic)	0.4	20,000	35
AKM-0530 (hydrophilic)	1.0	20,000	Over 70

[a] EO ratio means molar fraction of ethylene oxide in alkylene oxide chain defined as the following equation; $i/(i+j)$, where i and j are the random copolymerization degree of PAOMA copolymer shown in Fig. 2.

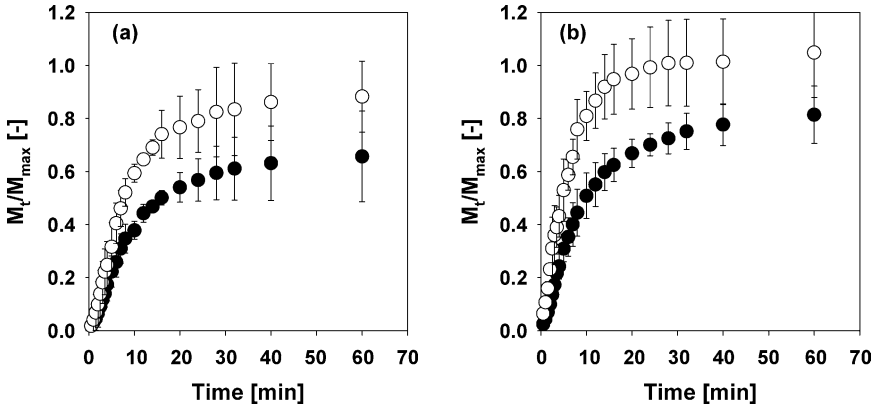

Fig. 3. Effect of pH on salicylic acid release characteristics from (a) CS/AEM-0530 film, (b) CS/AKM-0530 film at pH 3.8 (●) and 7.2 (○) at 25 °C.

aliquots were withdrawn and assayed for the amount of released drug. The amounts of salicylic acid and phenol released in the solutions were determined using a UV–vis spectroscopy (U-3210, HITACHI, Japan) at 234 and 270 nm, respectively. Three independent experiments were done and the results expressed as the mean of the three independent results.

2.2.4. Kinetic analysis of release data [20]

The characteristics of a drug delivery system are generally evaluated using the first 60% release data. Release of drug from film can be considered as one-dimensional if it happens predominantly from the two surfaces. The following equation, so-called power law equation, was used to evaluate the release mechanism:

$$M_t/M_0 = at^n \quad (1)$$

where M_t is the amount of drug released time t, M_0 is the initial amount of drug, a is the rate constant, and n is a constant called the diffusional exponent. The diffusional exponent (n) was calculated from the fitted linear regression lines of double logarithmic plots between fractional drug released and time. The slope was taken from the linear portion of the graph (at most 60% of drug was released). A slope of 0.5 indicates a diffusional square-root-of-time release, a slope between 0.5 and 1.0 indicates an anomalous non-Fickian transport and a slope of 1.0 indicates zero-order release kinetics.

2.2.5. Scanning electron microscopy

The surface characteristics of the freeze-dried hydrogel films were observed by SEM. Small pieces of swollen films were plunged in liquid nitrogen and the vitrified samples were quickly applied to lyophilization. The resulting dried films were sputter-coated with Pt/Pd using a vacuum evaporator (E-1030, HITACHI) and examined using scanning electron microscope (JSM-5310, JEOL) at 15 kV accelerating voltage.

3. Results and discussion

Both dried PEC films and model drug-loaded films were easily peeled off from petri dishes. Those freshly prepared films were transparent and had a thickness of ca. 60 μm. The surface morphology of dried film was quite smooth and uniform, and the cross-sectional morphology was equally dense and homogeneous.

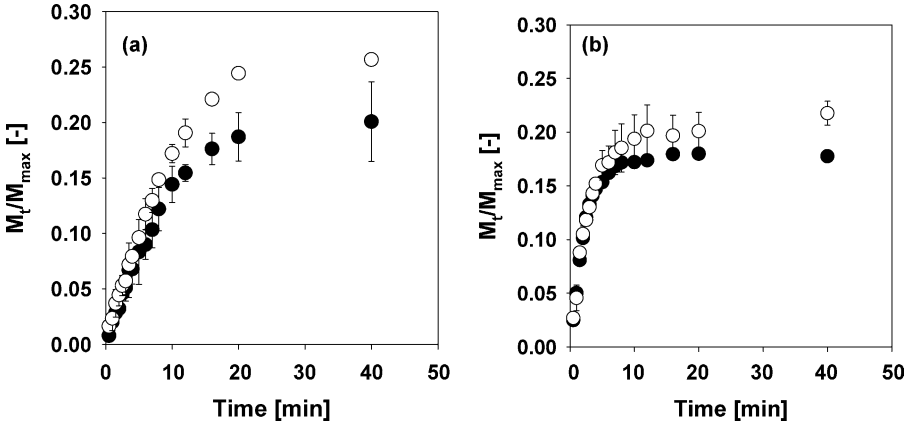

Fig. 4. Effect of pH on phenol release characteristics from (a) CS/AEM-0530 film, (b) CS/AKM-0530 film in pH 3.8 (●) and 7.2 (○) media at 25 °C.

Table 2
Release exponents (n), kinetic constants (a) and determination coefficients (R^2) following linear regression of release data from CS/PAOMA films

pH	Temperature (°C)	n		a		R^2	
		Sal.	Phenol	Sal.	Phenol	Sal.	Phenol
3.8	25	1.15	0.99	0.039	0.069	0.992	0.972
7.2	25	1.12	0.85	0.054	0.101	0.998	0.992
3.8	50	0.89	0.91	0.068	0.099	0.991	0.984

Sal. means salicylic acid.

3.1. Effect of pH on drug release

The release profiles of salicylic acid from CS/PAOMA films in different pH media at 25 °C are shown in Fig. 3. The results indicated that the release rate of salicylic acid increased at pH 7.2 using both hydrophobic and hydrophilic films. Besides, the pH-sensitive release of phenol from the CS/PAOMA films in different pH is shown in Fig. 4. Though the high release rate was observed at pH 7.2, there is not so much of a difference between pH 3.8 and 7.2.

For further elucidation of pH effect on drug release, the power law equation (Eq. (1)) was used to determine the release kinetics of the drugs. The results are listed in Table 2. The values of n were close to 1.0 in both pHs tested, indicating that the mechanism of drug release follows case II transport due to polymer relaxation [21].

The factors involved in the release rate change due to pH are elucidated by the microscopic network structures of CS/AEM-0530 films. Fig. 5 shows the SEM images of CS/AEM-0530 film swollen in buffer solutions of (a) pH 3.8 at 25 °C, (b) pH 7.2 at 25 °C and (c) pH 3.8 at 50 °C. The microscopic film structure at pH 3.8 has a relatively flat appearance and larger pores (10–25 μm) as shown in Fig. 5(a). In contrast to the film at pH 3.8, the one at pH 7.2 showed apparently isolated and irregularly distributed pores, which are supposed to be formed as a result of CS insolublization at pH 7.2 above the pK_a of CS (pK_a = 6.5). The increase in drug release at pH 7.2 seems to be due to the increase of effective diffusion surface area derived from pore formation.

In addition, the reason for the significant differences of salicylic acid release between pH 3.8 and 7.2 is explained by the anionic property of salicylic acid. At high pH, most of carboxyl groups in PAOMA and salicylic acid are in the forms of COO^-. Therefore the repulsive force between negative charges of PAOMA and salicylic acid results in the increasing of drug release. As the pH of medium decreases, carboxyl groups are protonated, and the repulsive forces reduce, and then the drug release rate decreases. On the other hand, it was reported that salicylate was formed due to the interaction between the carboxyl group of salicylic acid and the amino group of CS at weekly acidic pH [22]. Therefore, the release of salicylic acid at pH 7.2 was significantly faster than at pH 3.8. On the contrary, phenol is

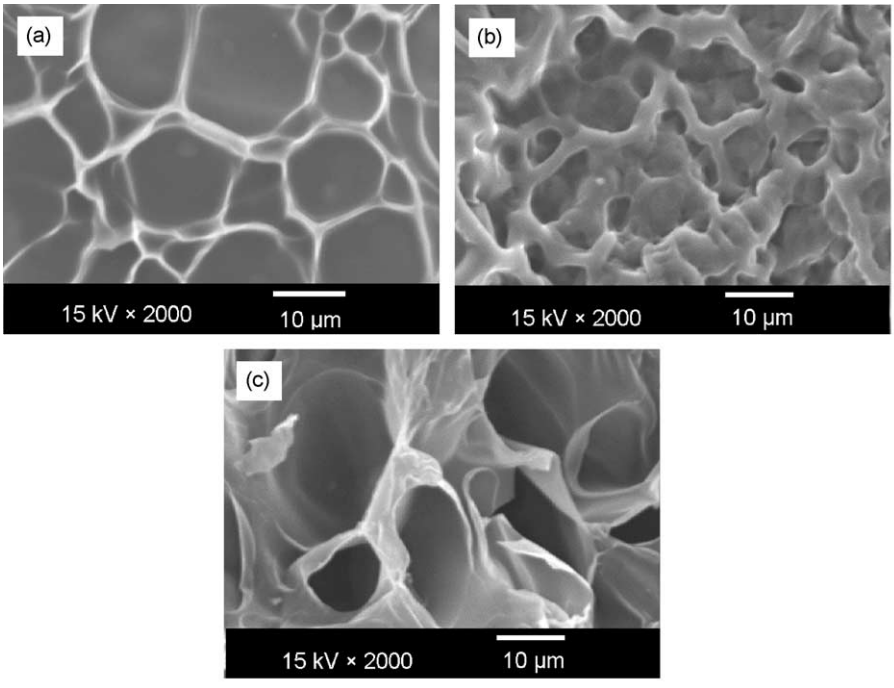

Fig. 5. SEM micrographs of CS/AEM-0530 film in (a) pH 3.8 media at 25 °C, (b) pH 7.2 media at 25 °C, (c) pH 3.8 media at 50 °C.

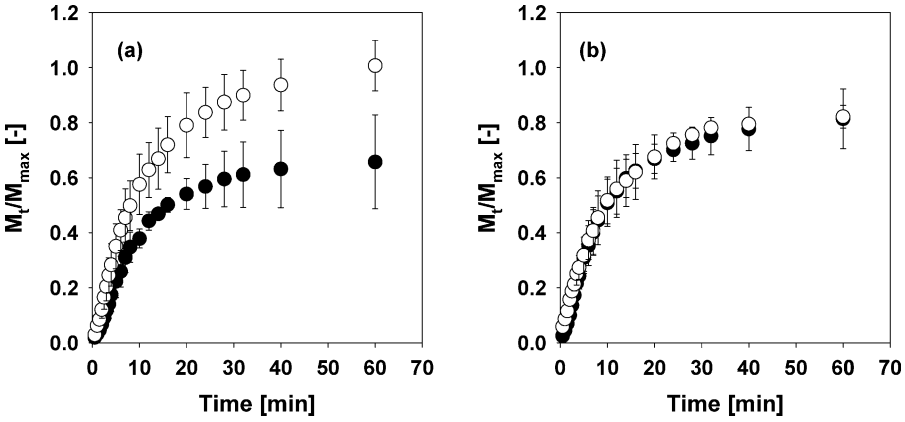

Fig. 6. Effect of temperature on salicylic acid release characteristics from (a) CS/AEM-0530 film, (b) CS/AKM-0530 film in pH 3.8 media at 25 °C (●) and 50 °C (○).

a relatively weaker acid than salicylic acid so that it seems that there is not so much of a difference in release rate between different pHs.

3.2. Effect of temperature on drug release

The release profiles of salicylic acid from CS/PAOMA films in pH 3.8 at different temperature are shown in Fig. 6. The results indicated that the release rate of salicylic acid increased at 50 °C using CS/AEM-0530 films (Fig. 6(a)), while there is almost no variation between 25 and 50 °C using CS/AKM-0530 films (Fig. 6(b)). The temperature-sensitive release of phenol from the CS/PAOMA films in pH 3.8 media is shown in Fig. 7. These results are also identical with the case of using salicylic acid as a model drug. Applying Eq. (1) to gain information about the release mechanism for variants, a value of 0.89–1.15 (Table 2), was found for the exponent n. These n values over 1.0 compared to those found by others [23–25], indicating the drug release mechanism is affected by polymer relaxation.

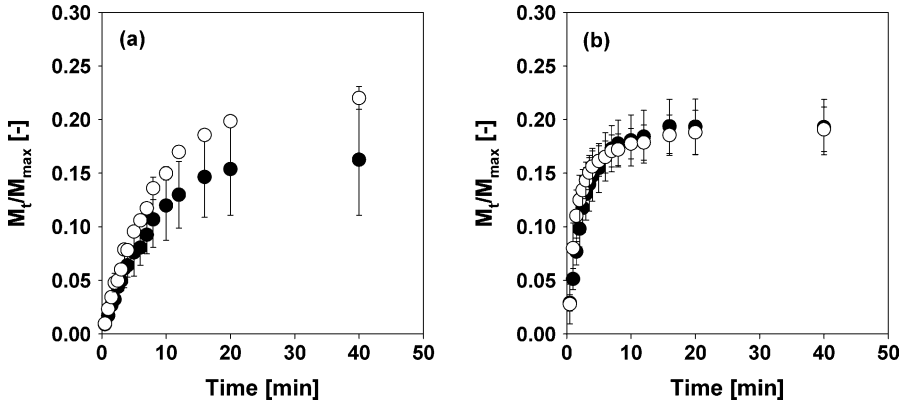

Fig. 7. Effect of temperature on phenol release characteristics from (a) CS/AEM-0530 film, (b) CS/AKM-0530 film in pH 3.8 media at 25 °C (●) and 50 °C (○).

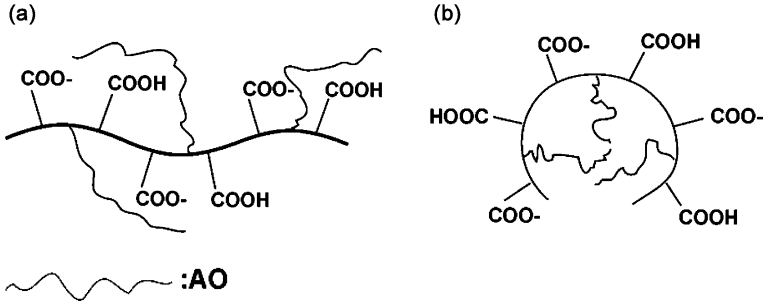

Fig. 8. Conformation of hydrophobic PAOMA molecule at (a) 25 °C and (b) 50 °C.

These temperature-sensitive releases can be explained by the change of free volume in the PEC films in response to temperature. Scanning electron micrographs of CS/AEM-0530 film swollen in pH 3.8 media at different temperature are shown in Fig. 5(a) and (c). As can be seen, the film surface swollen at 25 °C has comparatively flat pores (Fig. 5(a)). One remarkable characteristic of the film surface swollen at 50 °C was the deep pore and rough surface (Fig. 5(c)). In general, the effective free volume in polymer hydrogels is derived from the free volume of water, and the transport of solutes is presumed to permeate through the free-water region in the swollen film [26]. In this case, as the total volume of pores or channels in the surface of PEC film at 50 °C, resulting in the increase in the effective diffusion area, the amount of solute transport increases.

Besides, these release trends are influenced by the hydrophobicity of PAOMA and the anionic property of model drug. The AEM-0530 aqueous solution has LCST at ca. 35 °C, as indicated by a reversible phase transition from soluble to insoluble states. The temperature-responsive soluble–insoluble transition of AEM-0530 can be explained by the dissociation of ordered water molecules surrounding hydrophobic propylene oxide groups over 35 °C. Above LCST, the hydrophobic AO chain in PAOMA could form a micelle-like structure and the conformation of PAOMA molecules could turn to be compact, as shown in Fig. 8. As a result of these conformational changes with heating, the carboxyl groups in PAOMA could align on the PAOMA molecules resulting in an effective repulsion of PAOMA and anionic drugs. On the contrary, the drug release rate of CS/AKM-0530 did not change at the range of temperature tested because LCST of AKM-0530 is over 70 °C.

4. Conclusion

Drug release characteristics of PEC films prepared from CS and PAOMA were studied. Salicylic acid and phenol were used as model drugs. These PEC films were shown to change drug release rate in response to changes in environmental pH and temperature. The drug release was higher in pH 7.2 media compared to pH 3.8 because of the increase of repulsive force between carboxyl groups in PAOMA and anionic groups in model drugs and the increase of effective diffusion area. Temperature-sensitive drug release studies showed that the increase in temperature from 25 to 50 °C yielded the increase in the rate of drug release when using AEM-0530. This is attributed to the increase of release area due to the phase transition of PAOMA and the increase of repulsive forces between carboxyl groups in PAOMA and anionic groups in model drugs. On the contrary, there was almost no variation between 25 and 50 °C using CS/AKM-0530 films. In conclusion, we have shown that the drug release rate can be regulated by altering the temperature when CS/PAOMA films were used as a novel drug carrier.

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