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Characterization of Polyelectrolyte Complex Films Composed of Cys-PEOMA and Chitosan as a Drug Carrier

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Chitosan (CS) is a naturally occurring polysaccharide with excellent biodegradability and biocompatibility. CS is a pH-sensitive polycation so that it is insoluble at neutral and alkaline pH. Drug Delivery Systems (DDS) using CS has extensively investigated due to its unique characteristics. Polyethylenoxide-maleic anhydride (PEOMA) copolymer becomes a polyanion through the ring-opening hydrolysis in aqueous solution. The electrostatic attraction between the anionic and cationic polyelectrolytes results in the formation of a polyelectrolyte complex (PEC). L-Cysteine (Cys) is one of essential amino acids, which has a thiol (SH) group in its side chain. The SH groups form disulfide bonds in the presence of oxygen. Thus, the disulfide bond formation is used for gelation of SH-containing polymer. In this study, Cys was covalently conjugated with PEOMA through the amide bond formation between maleic anhydride groups in PEOMA and amino groups in Cys. PEC films with disulfide bond were prepared from Cys-PEOMA conjugate and CS by casting/solvent evaporation method. Cys-PEOMA/CS film was characterized by SEM observation and thermo-gravimetric analysis, swelling degree. The potential for pharmaceutical use was examined by model drug release profiles.
Key words: polyelectrolyte complex, chitosan, thiol-polymer, drug carrier

1. Introduction

Chitosan (CS) is a naturally occurring polysaccharide with excellent biodegradability and biocompatibility [1, 2]. CS is a pH-sensitive polycation so that it is insoluble at neutral and alkaline pH. Drug Delivery Systems (DDS) using CS has extensively investigated due to its unique characteristics [3]. Polyethylenoxide-maleic anhydride (PEOMA) copolymer becomes a polyanion in aqueous solution [4], which has been known as a modifier of several enzymes [5]. The electrostatic attraction between the anionic and cationic polyelectrolytes results in the formation of a polyelectrolyte complex (PEC) [6].

L-cysteine (Cys) is one of essential amino acids, which has a thiol (SH) group in its side chain. It is well known that the SH groups form a disulfide bond in a protein molecule to maintain the 3-Dstrucuture. The disulfide bond formation is also used for gelation of synthetic polymer [7].

In this study, Cys was covalently conjugated with PEOMA through the amide bond formation between maleic anhydride groups in PEOMA and amino groups in Cys. PEC films with disulfide bond were prepared from Cys-PEOMA conjugate and CS by casting/solvent evaporation method. The Cys-PEOMA/CS film was examined in terms of the swelling degree and the drug release profiles at different pH.

2. Materials and Methods

2.1. Materials

CS (degree of acetylation, 0.076; average molecular weight, 10^5) was provided from Kyowa Tecnos Co. Ltd., Japan. PEOMA copolymer (AKM-0530) was supplied from Nippon Oil & Fats Co., Japan. The structure of CS and PEOMA are shown in **Figs. 1** and **2**, respectively. Salicylic acid as a model drug was purchased from Wako Pure Chemical Industries, Ltd., Japan. All other reagents were of analytical grade.

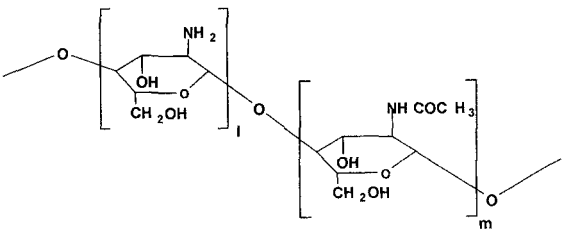


Fig. 1 Chitosan structure

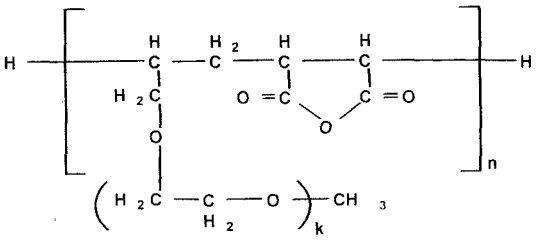


Fig. 2 PEOMA structure

2.2. Synthesis of Cys-PEOMA Conjugates

The covalent attachment of Cys to PEOMA was achieved by formation of amid bonds between the primary amino group of the amino acid and maleic anhydride groups of the PEOMA.

PEOMA (2 g) was added to Cys solution (4 wt%, 36.3 ml) in 0.2 mol/l borate buffer (pH 8.5). The reaction mixture was incubated for 2 h under constant mixing at 4 °C. The pH of the reaction mixture was maintained at 8.5 by adding 1 mol/l NaOH drop by drop. Then, pH of the reaction mixture was adjusted to 5.5 by adding 1 mol/l HCl to avoid unexpected disulfide bond formation. Unreacted Cys was removed by dialysis (MWCO, 1000; SPECTRUM) against 0.05 mol/l acetate buffer (pH 5) in the dark at 4 °C.

2.3. Evaluation of Degree of Modification

The amount of remaining free Cys was quantified *via* TNBS method. The degree of modification (DM) was calculated by following equation:

$$DM\,[-] = (N_m/N_t) = (N_r/N_t)$$

where N_m was the number of conjugated carboxylic groups in Cys-PEOMA conjugate, N_t was the number of free carboxylic groups in unconjugated PEOMA, N_r was the number of bonded amino group in Cys. In brief, 2 ml of Cys-PEOMA conjugate (before dialysis) was incubated with 4 ml of 0.15 mol/l borate buffer and 1 ml of 0.01 mol/l Na_2SO_4 and 1 ml of 0.10 % 2,4,6-trinitrobenzenesulfonic acid (TNBS, Sigma, Japan) at 37 °C. After 1 h incubation, the absorbance was measured at 420 nm (UV2100, Hitachi, Japan).

The DM was also confirmed by determining SH group quantification in Cys-PEOMA solution by iodometric titration method. Cys-PEOMA/CS conjugate (2 ml) was mixed with 20ml of 0.01 wt% starch solution. Then, 1 mmol/l iodine solution was titrated with vigorous agitation. The titration end point was determined when the mixture was colored in purplish red. The SH group in Cys-PEOMA conjugate was estimated with calibration curve using Cys as a standard.

2.4. Preparation of Film

Cys-PEOMA conjugate solution (3 wt%) and CS solution (3 wt%) in 1 wt% aqueous acetic acid were mixed in a molar ratio of 3/7 (amino groups in CS/carboxylic groups in PEOMA), and were incubated with stirring for 24 h at 50 °C to allow the formation of disulfide bonds. Then they were cast on a polyethylene petri dish (135×95 mm) and dried at 50 °C for 24 h. The dried films were cut into disks (φ10 mm).

2.5. Surface Observation

The surface morphology of films was examined by using a scanning electron microscope (JEOL SEM-4000, Japan). The acceleration voltage was in the range of 1.8-2.0 kV, the pressure of sample stage was ~0 Pa and the secondary electron image was observed.

2.6. Thermo Gravimetric Analysis of Films

Thermo gravimetric analysis (TGA) was performed on a TG8120 (RIGAKU, Japan) under a helium atmosphere. A temperature range of 30–850 °C was used in each scan at a scan rate of 15 °C /min.

2.7. Swelling Test

Dried films (15.5 ± 1 mg) were carefully weighted and immersed in media (50 ml) with pH values from 1.2 to 7.5 at 37 °C. At pre-determined time (1 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, Japan). The following equation was used to determine the swelling degree (*DS*):

$$DS\,[-] = (W_w - W_d)/W_d$$

Here, W_d and W_w are the film weights of dry and swollen films.

2.8. In vitro Drug Release Test and Analysis of Drug Release Kinetics

Drug-loaded films were prepared by immersing films in salicylic acid solution (0.2 g/l) for 1 h. Then the swollen films ware taken out, and the excess water was blotted with filter paper from the surface, and then moved into media (10 ml) with pH values from 1.2 to 7.5 at 37 °C. At appropriate time intervals, the solution was withdrawn and the content of salicylic acid was determined by measuring UV absorbance at 234 nm.

Semiempirical equation (Korsmeyer-Peppas model) was used to analyze the data of drug release from PEOMA/CS film or Cys-PEOMA/CS film at the initial stage [8].

Korsmeyer-Peppas model:

$$f_R = M_t/M_\infty = 2(Dt/l^2)^{1/2}$$

Here, f_R is the fractional drug released; M_t and M_∞ are the absolute cumulative amount of drug release at time t and infinite time, respectively; D is the effective diffusion coefficient; l is film thickness (l = 0.04 cm).

3. Results and Discussion

3.1. Degree of Modification

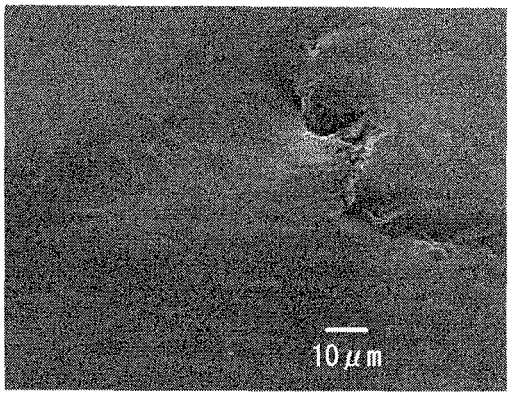
The covalent attachment of Cys to PEOMA was confirmed by TNBS method and also iodometric titration method. The resultant Cys-PEOMA conjugate had DM of 10% and was used for the following experiments.

3.2. Surface and Cross-Section Morphology

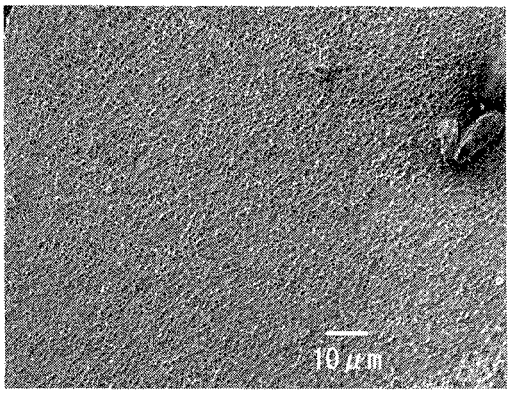
The film surface and cross-section images were shown in **Fig. 3**. PEOMA/CS film (a) showed uniform surface. It might be indicate that the film formed by electrostatic attraction between PEOMA and CS has homogeneous structure. On the other hand, Cys-PEOMA/CS film (b) had rugged surface. The additional crosslinking by disulfide bond between Cys-PEOMAs might produce disorderly 3-D structure polymer network. This also confirmed by the section image of films (c) and (d).

3.3. Thermal behavior of films

The thermal behavior of PEOMA/CS and Cys-PEOMA/CS films was shown in **Fig. 4**. While PEOMA/CS has two decomposition stages with one starting at around 210 °C and



(a)



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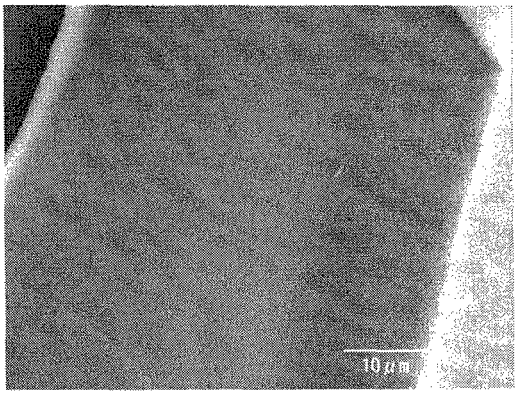
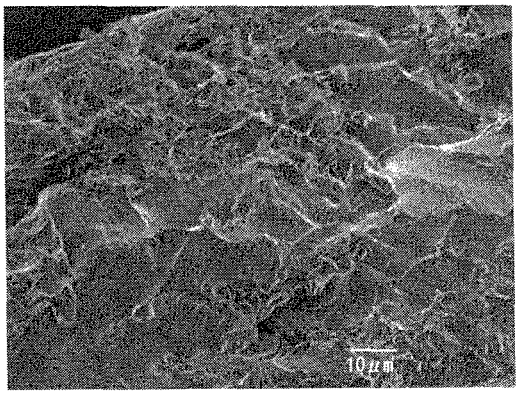


(c)



(d)

Fig. 3 SEMs of surface of (a) PEOMA/CS film, (b)Cys-PEOMA/CS film, section of (c) PEOMA/CS film, (d) Cys-PEOMA/CS film

another starting at 430 °C, the Cys-PEOMA/CS exhibit three decomposition stages. They start at 220, 380 and 640 °C. Because of the disulfide bond, the difference might appear in the thermal behavior of PEOMA/CS and Cys-PEOMA/CS.

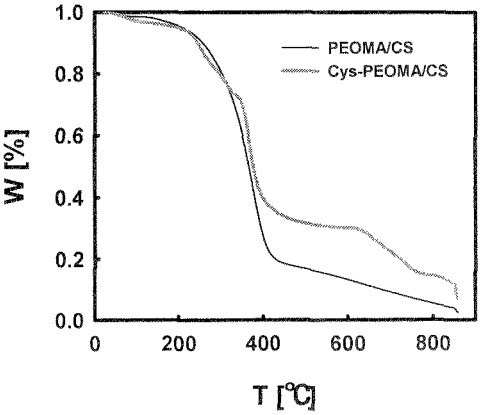


Fig. 4 Thermo gravimetric behavior

3.4. Film Swelling Behavior

The swelling behaviors of PEOMA/CS or Cys-PEOMA/CS films might have a great impact on their stability and drug release. The Cys-PEOMA/CS film showed less *DS* than PEOMA/CS film at all pH tested. The disulfide bond formed in Cys-PEOMA/CS film made the film structure more stable than PEOMA/CS film. The result was shown in **Fig. 5**.

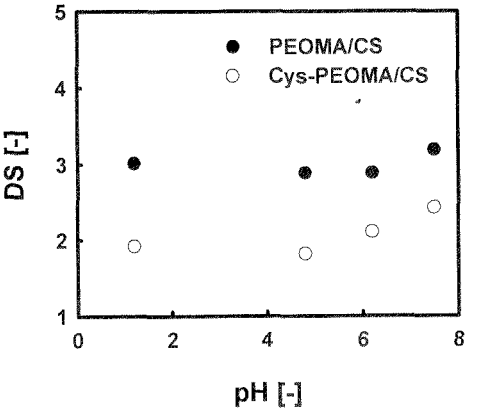


Fig. 5 Swelling degree (*DS*) of films at different pH.

3.5. In vitro Drug Release

The release profiles of salicylic acid from PEOMA/CS or Cys-PEOMA/PEOMA film at pH 1.2-6.2 were illustrated in **Fig. 6-9**. In the case of Cys-PEOMA/CS film, the relatively sustained release was observed. The effective diffusion coefficient, *D*, of film in different pH buffers were shown in **Fig. 10**. The film thickness, *l*, of each film was assumed to be 0.04 cm. In all pH solution examined, *D* values of Cys-PEOMA/CS film were lower than that of PEOMA/CS film. This indicated that Cys-PEOMA/CS films had more sustained drug release profile than PEOMA/CS films. If the *DS* of the film was lower, the film had still dense structure; so loaded drug was hard to seep out.

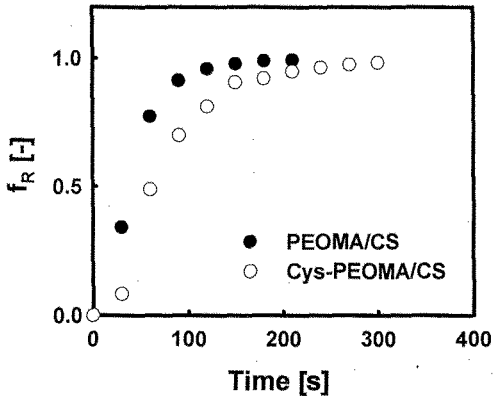


Fig. 6 Salicylic acid release profiles fro films (pH 1.2).

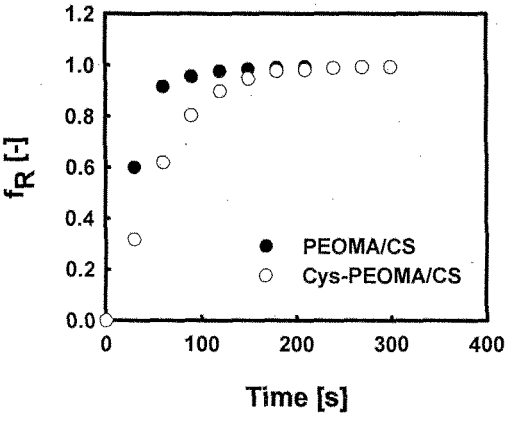


Fig. 7 Salicylic acid release profiles fro films (pH 4.8).

4. Conclusion

PEC hydrogel film based on Cys-PEOMA conjugate and chitosan was prepared by casting/solvent evaporation method. It became clear by surface observation by SEM and TGA analysis that the Cys-PEOMA/CS film has different character compared with the conventional PEOMA/CS film.

The swelling tests showed that *DS* of Cys-PEOMA/CS film was lower than that of PEOMA/CS. In accordance with this result, the effective diffusion coefficient, *D*, of Cys-PEOMA/CS film was lower than that of PEOMA/CS film. In comparison with PEOMA/CS system, Cys-PEOMA/CS system showed more sustained drug release profile because additional disulfide bond formed more stable cross-linking structure. To get a much more practical film with sustained release profile, Cys-PEOMA conjugation, Cys-PEOMA/CS mixing ratio and curing time of mixture should be needed to be optimized.

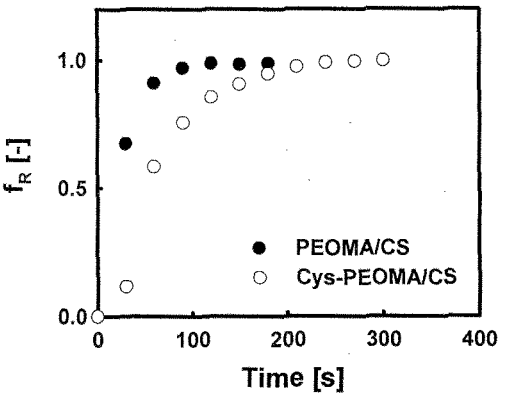


Fig. 8 Salicylic acid release profiles fro films (pH 6.2).

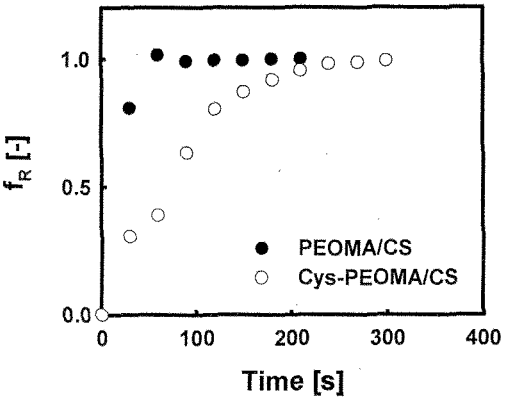


Fig. 9 Salicylic acid release profiles fro films (pH 7.5).

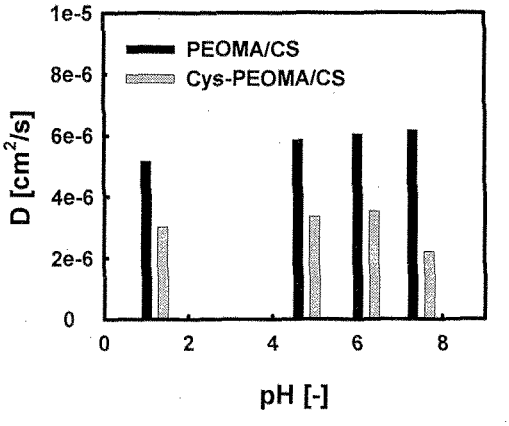


Fig. 10 Effective diffusion coefficient (D) of films at different pH.

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