

Effect of Interpolymer Complex Formation on Bioadhesive Property and Drug Release Phenomenon of Compressed Tablet Consisting of Chitosan and Sodium Hyaluronate

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The bioadhesion property of tablets consisting of chitosan (CS) and sodium hyaluronate (HA) was investigated using a lyophilized porcine dermis as a model of mucous membrane. Release phenomena of brilliant blue FCF (BBL) from the CS–HA tablets were also studied. BBL was employed as a model compound of water-soluble drugs. Strong adhesion forces were observed when the tablets were prepared from HA alone or a physical mixture of CS and HA. The adhesion of CS tablets was also obtained but it was rather weak. No effect of pH values in the media was observed on the adhesion force in these tablets. On the other hand, the release rate of BBL from CS–HA tablets was greatly affected by the change of the polymer mixing ratio, suggesting a possible interaction between CS and HA in the tablet following water penetration into the tablet.

Keywords bioadhesion; drug release; compressed tablet; chitosan; sodium hyaluronate; interpolymer complex; turbidity; viscosity; infrared spectroscopy

Introduction

An incorporation of bioadhesive polymers in the formulation has been considered as a useful method to localize the dosage forms at a specific site and to prolong a drug release from the formulation.^{1,2)} Among various candidate bioadhesive polymers, polyacrylic acids have been found to have significant interaction with a mucous membrane.^{3–7)} In previous papers,^{8,9)} we investigated factors affecting the bioadhesion property and drug release phenomenon of compressed tablets consisting of hydroxypropyl cellulose (HPC) and carboxyvinyl polymer (CP), polyacrylic acid derivative. It has been found that the bioadhesion force of the tablet and drug release from the tablet are greatly affected by the interpolymer complex formation between HPC and CP. The lowest adhesion force and the lowest drug release were observed when the tablet was prepared with a physical mixture of these polymers in a weight ratio of 3:2 (HPC:CP). Interestingly, this was the stoichiometric ratio of the solid complex. Thus, the interpolymer complex formation observed between candidate adhesive polymers may afford their additional possibilities for more precisely controlled bioadhesive drug delivery systems. In practical application, however, safety of these polymers to the living body should be taken into consideration. Therefore, an attempt to find and characterize new bioadhesive polymers of which biological safety has been established seems to be important.

In this study, we investigated bioadhesion properties and drug release phenomena of tablets consisting of a mixture of chitosan (CS) and sodium hyaluronate (HA), considering a possible interaction of these polymers. For the study of drug release, brilliant blue FCF (BBL) was used as a model compound of water-soluble drugs. Bioadhesion properties of HA were well evaluated in the application to ophthalmic formulations and excellent muco-adhesion has been detected.^{10,11)} On the other hand, the application of CS has been mainly examined in the development of controlled systems for drug release.^{12–14)} In addition, both CS and HA seem to have high biocompatibility and biodegradability as natural polymers.

Experimental

Materials CS, derived from crab shell chitin with about 95% deacetylation, was generously supplied from Dainichi Seika Color & Chemicals Mfg. Co., Ltd. CS was used after shattering by ball-mill and passage through a 100 mesh sieve. The relative viscosity of a 0.2% CS aqueous solution (at pH 2.0) was 4.11 and 37 °C as determined with an Ubbelohde viscometer. The average molecular weight of CS was determined to be 4.4×10^5 , using gel-permeation chromatography.¹⁵⁾ HA, average molecular weight 2.17×10^6 , was generously supplied from Kyowa Hakko Kogyo Co., Ltd. HA was used after passage through a 100 mesh sieve. The relative viscosity of a 0.2% HA aqueous solution (at pH 2.0) was 8.49 at 37 °C as determined with the same apparatus described above. CP marketed as "Carbopol 934" was purchased from B. F. Goodrich Co. CP was used after passage through a 100 mesh sieve. Other chemicals used were of reagent grade.

Turbidity Measurement CS solution (2.5 ml; 0–0.004%) was mixed with HA solution (2.5 ml; 0–0.004%). The mixture solution was then incubated at 37 °C for 1 h to obtain a stable turbidity in the sample solution. Buffer solutions (pH 2.0, 3.0, 4.0 and 5.0), prepared from 0.05 N HCl, 0.05 M CH₃COONa, 0.05 M KH₂PO₄, and/or 0.05 M Na₂HPO₄, were used to dissolve samples. Total polymer concentration was fixed at 0.004% in all samples. The turbidity of each sample solution was determined at 500 nm, where there was no absorption due to the polymers in solution, using a Hitachi 200-20 spectrophotometer. The buffer solutions used in this measurement were also employed for the following experiments.

Viscosity Measurement CS solution (10 ml; 0–0.2%) was mixed with HA solution (10 ml; 0–0.2%). The sample solution was then incubated at 37 °C for 2 d to complete the complex formation. Total polymer concentration was fixed at 0.2% in all samples. After centrifugation for 20 min at 15000 rpm in a Hitachi SCR 20B centrifuge, the viscosity of the supernatant solution was determined at 37 °C by the use of an Ubbelohde viscometer. Buffer solutions (pH 2.0, 3.0, 4.0 and 5.0) were used to prepare the sample solutions.

Preparation of Solid Complex The CS solution (50 ml; 0.01%) was mixed with the HA solution (50 ml; 0.01%). The buffer solution (pH 3.0) was used to prepare the sample solution. The sample solution was then incubated at 37 °C for 2 d. After the removal of water in the sample solution, the remaining solid complex was dried in vacuum for 3 d at room temperature.

Infrared Absorption Spectroscopy Fourier-transform infrared (FT-IR) spectra of the CS–HA solid complex, intact CS and HA were measured using a JASCO model FT/IR-5 spectrophotometer (KBr disk method).

Preparation of Compressed Tablet Unless otherwise stated, tablets with a diameter of 10 mm were prepared by compressing 100 mg of a mixture of CS and HA in various mixing ratios at a pressure of 50 kg/cm² using a Shimadzu hydraulic press. Tablets consisting of CP powder were also made as a control for the adhesion force measurement. For the dissolution test, 10% of BBL was mixed into each polymer mixture before tablet preparation.

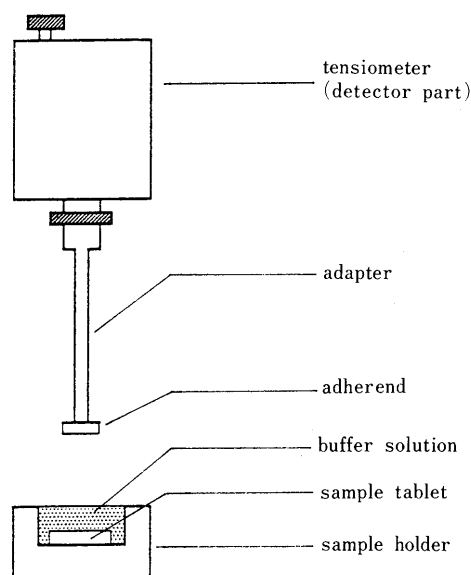


Fig. 1. Schematic Representation of Experimental Setup for Determination of Bioadhesion Force

Adhesion Force Measurement The adhesion properties of tablets consisting of various mixing ratios of CS and HA were evaluated by measuring the force required to separate the tablet from an adherend. An apparatus used for the measurement is schematically represented in Fig. 1. The tablet was attached to the holder with cyanoacrylate type adhesives. Buffer solutions (2 ml; pH 2.0, 3.5 and 5.0) were gently added on the tablet to hydrate the tablet surface for 5 min at 25°C. Unless otherwise stated, a lyophilized porcine dermis (Alloask D, Taiho Pharmaceutical Co., Ltd.) and rabbit peritoneal membrane excised from male New Zealand white rabbits (3.0–3.5 kg) were used as adherends. The rabbits were killed by pentobarbital injection, the peritoneal membrane was extracted and stored at -10°C. Before the experiment, it was thawed at 4°C in an isotonic saline solution. The residual water on the surface was removed with filter paper. The porcine dermis or rabbit peritoneal membrane, which were cut into a round shape with a diameter of 5 mm, were attached to the tip of an adapter in tensiometer (Fudoh Kogyo Co., Ltd.) with cyanoacrylate type adhesive. The adherends were then immersed in buffer solutions (pH 2.0, 3.5 and 5.0) for 5 min at 25°C before the measurement. The holder wearing the sample tablet was then lifted up in contact with the adherend which was preliminarily hydrated in the buffer solutions by applying a loading pressure of 250 g/cm². The tablet and the adherend were kept in contact with each other for 3 min. The tablet was then stretched from the adherend at an extension rate of 4 mm/s and the force required to detach the tablet from the adherend was recorded.

Release Test Unless otherwise stated, a Toyama Sangyo NTR-VS type dissolution tester (paddle method) was used at 50 rpm paddle speed with 500 ml of the dissolution medium at 37°C. Buffer solutions (pH 2.0, 3.5 and 5.0) were employed as the dissolution media. Samples (5 ml) were withdrawn at appropriate intervals through a Fine Filter F (Ishikawa Seisakusho Co., Ltd.) and immediately replaced with an equal volume of the test medium. Samples were analyzed spectrophotometrically at 630 nm for BBL using a Hitachi 200-20 spectrophotometer.

Results and Discussion

Confirmation of Complex Formation Interpolymer complex formation was confirmed employing turbidity and viscosity measurements in media of various pH values (pH 2.0, 3.0, 4.0 and 5.0). The reason why the acid buffer solution was used in this study was that CS was not soluble in the neutral and alkali solutions. Figure 2 shows the turbidity of the CS-HA mixture solution as a function of the weight ratio of CS and HA. The maximum turbidity was found when the weight fraction of CS in the samples was 20–30%, indicating that the solid complex of CS and HA might be formed in these media. It was also observed that the binding

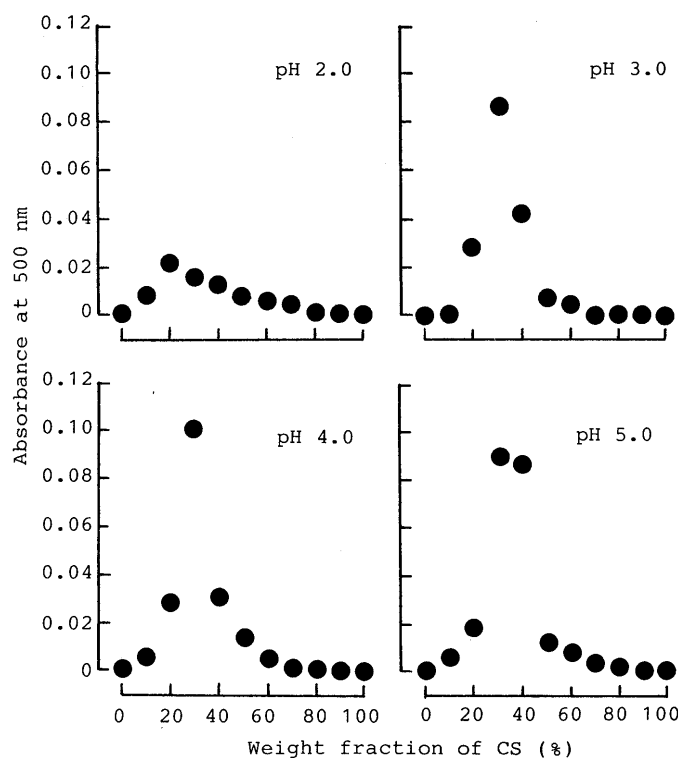


Fig. 2. Turbidity of CS-HA System as a Function of Polymer Mixing Ratio in Media of Various pH Values at 37°C

Each point represents the mean of three determinations.

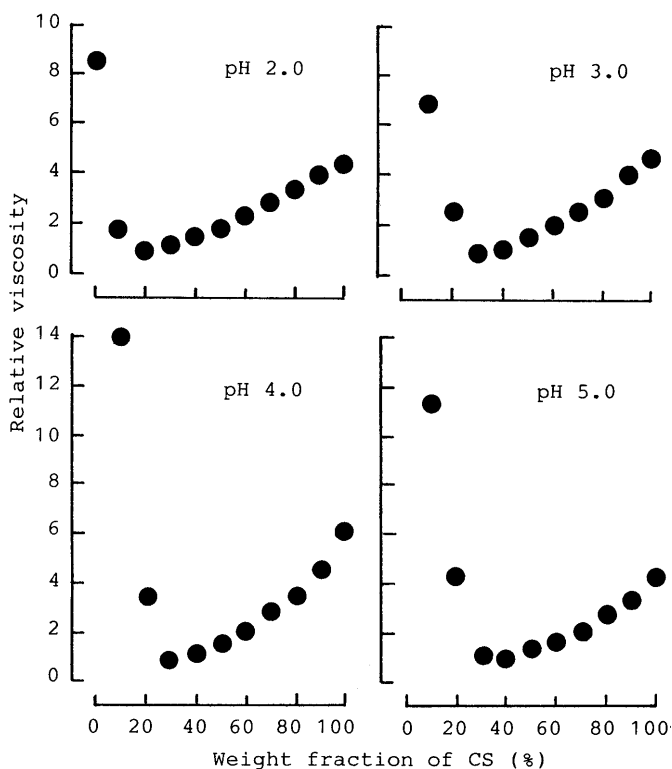


Fig. 3. Viscosity of Supernatant Solution in CS-HA System as a Function of Polymer Mixing Ratio in Media of Various pH Values at 37°C

Each point represents the mean of three determinations.

ratio of the complex was affected by pH, showing a change from 1:4 to 3:7 (CS:HA) with an increase of pH values from 2.0 to 5.0. Figure 3 shows the viscosity of supernatant

in the CS-HA mixture solution as a function of the weight ratio of CS and HA. No change of the pH value of supernatant in the mixture solution was observed as compared with the initial value before mixing of both polymer solutions. When the weight fraction of CS in the samples was 20–30% the viscosity of supernatant in the CS-HA solution was observed to be almost the same as that of the medium. Thus, the decrease of viscosity observed in the CS-HA mixture solution showed that the solid complexes were formed and they were removed by the centrifugation. The effect of pH in the media on the binding ratio of the complex was again observed similar to the result obtained in the turbidity measurement. Since the pK_a value of CS was reported to be 6.3,¹⁶⁾ most of the CS molecules were ionized in the media used in this study (pH 2.0–5.0). The binding ratio of CS-HA complex (at weight ratio 3:7) observed at a higher pH region corresponds to 1:1 at the unit molecular weight ratio, as shown in Figs. 2 and 3. In these pH regions, HA molecules are mostly ionized as estimated from the pK_a value of HA (2.9).¹⁷⁾ On the contrary, the degrees of ionization of HA molecules would decrease with decreasing pH values in the media. As a result, much larger amounts of HA molecules were required to interact with CS molecules in the media of low pH values. Accordingly, if the active sites on the polymer molecules for the complexation are not greatly affected by steric hindrance, the binding ratio should be altered with a change of pH values in the media. Similar phenomenon has been observed in the complex formation between CS and sodium polyacrylate as described previously.¹⁵⁾

Figure 4 shows FT-IR spectra of the CS-HA solid complex obtained as precipitates in the medium of pH 3.0. IR spectra of the solid complex were greatly different from those of CS alone, HA alone and a physical mixture of CS and HA at a weight ratio of 3:7. A broad peak, appearing at around 2500 cm^{-1} , might be assigned to the ammonium absorption band which was brought about by the interaction of the amino groups of CS with the carboxyl groups of HA. Together with the fact that the binding ratio of the complex was affected by the change of pH values in the

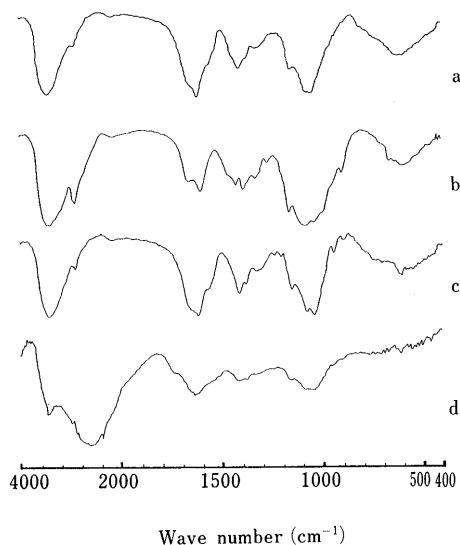


Fig. 4. FT-IR Spectra of CS-HA Physical Mixture (3:7) (a), CS Alone (b), HA Alone (c) and CS-HA Complex (d) (KBr Disk Method)

media (Figs. 2 and 3), the ionic bonding seems to be a primary binding force for the complex formation between CS and HA.

Bioadhesion Property of CS-HA Tablets Bioadhesion properties of tablets consisting of a physical mixture of CS and HA were measured, comparing with those of CP tablets. All experiments were performed in the acidic media (pH 2.0, 3.5 and 5.0) in order to know the effect of interpolymer complex formation on the tablet surface following water penetration into the tablet. For the purpose of finding a suitable model of the mucous membrane, we tried to use a lyophilized porcine dermis as an adherend. Figure 5 shows the adhesion force of CP tablet to the rabbit peritoneal membrane of porcine dermis in the media of various pH values. The adhesion force of the CP tablet was very strong in a medium of low pH value. However, an increase of pH in the media led to a remarkable decrease of the adhesion force. Similar phenomena obtained with polyacrylic acid hydrogels have been reported by Park and Robinson.³⁾ The adhesion force of CP tablets to the porcine dermis was observed to be very close to that obtained in the rabbit peritoneal membrane, suggesting that the porcine dermis is

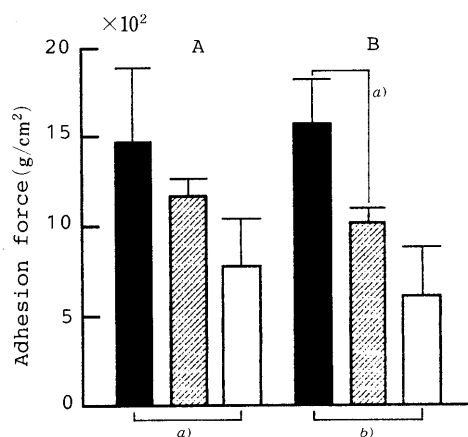


Fig. 5. Adhesion Force of Compressed CP Tablet to Rabbit Peritoneal Membrane or Porcine Dermis after Hydration of Tablet Surface in Media of Various pH Values

A, rabbit peritoneal membrane; B, porcine dermis. Each datum represents the mean \pm S.D. of five determinations. a) Significantly different ($p < 0.01$); b) Significantly different ($p < 0.05$). ■, pH 2.0; ▨, pH 3.5; □, pH 5.0.

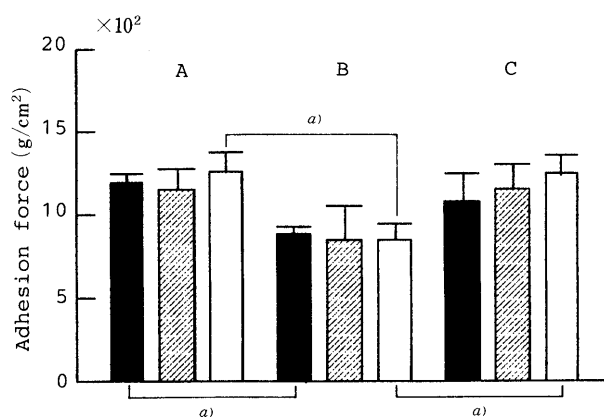


Fig. 6. Effect of pH Values of Buffer Solution Used for Hydration of Tablet Surface on Adhesion Force of Compressed Tablet

A, HA tablet; B, CS tablet; C, CS-HA tablet (3:7). a) Significantly different ($p < 0.01$). Each datum represents the mean \pm S.D. of five determinations. ■, pH 2.0; ▨, pH 3.5; □, pH 5.0.

suitable as a model of the mucous membrane. Thus, the lyophilized porcine dermis was used in the following experiments.

Figure 6 shows the adhesion properties of tablets consisting of HA alone, CS alone and a mixture of CS and HA at a weight ratio of 3:7 in the media of various pH values. Very strong adhesion corresponding to the force of the CP tablet was observed when the tablet was prepared from HA alone or a CS-HA mixture. Although the adhesion force of the tablet consisting of CS alone was somewhat weak, no significant change of the adhesion of CS-HA tablets was obtained when the tablet was prepared under a different mixing ratio of the polymers (3:7, 1:1 and 7:3). Therefore, the adhesion of CS-HA tablets may not be affected by the interpolymer complex formation which would be brought about by water penetration into the tablet. Furthermore, no effect of pH values in the media on the adhesion force was observed in any cases, thus deviating from the result obtained in the CP tablet. The adhesion property of HA to the mucous membrane has been reported by Saettone *et al.*^{10,11)} They showed no significant effect of pH in the media on the adhesion of HA to the mucous membrane.¹¹⁾ Accordingly, the adhesion mechanism of HA to the porcine dermis is considered to be different from that of CP. Figure 7 shows the effect of hydration time of the tablet surface on the adhesion of the CS-HA tablet. The adhesion force was relatively constant though a slight decrease was observed with increasing hydration time. On the other hand, the adhesion force of the CP tablet was rather sensitive and it decreased continuously with an increase of hydration time.

In order to investigate the adhesion behavior more precisely, the force required to detach the tablet from the gel layer was measured. For this experiment, the tablet consisting of a physical mixture of CS and HA in a weight ratio of 3:7 was employed as both adherend and sample tablet. As the adherend, especially, the tablet with a diameter of 5 mm was prepared by compressing 25 mg of a mixture of CS and HA. The buffer solution (pH 3.5) was used for hydration of the tablet surface. Other conditions

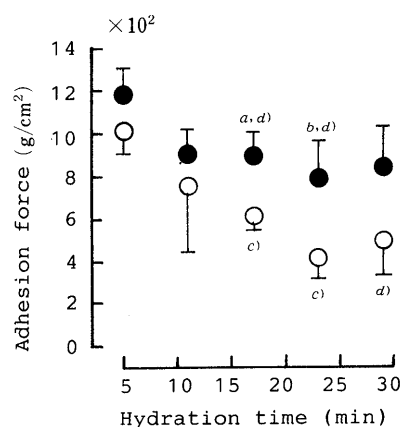


Fig. 7. Effect of Hydration Time of Tablet Surface on Adhesion Force of Compressed Tablet

●, CS-HA tablet (3:7); ○, CP tablet. Buffer solution (pH 3.5) was used for hydration of tablet surface. Each point represents the mean \pm S.D. for five determinations. a) Significantly different (compared with CP tablet, $p < 0.01$). b) Significantly different (compared with CP tablet, $p < 0.05$). c) Significantly different (compared with initial value, $p < 0.01$). d) Significantly different (compared with initial value, $p < 0.05$).

were the same as described above for the determination of the adhesion force of the tablet to the porcine dermis. As a result, a much stronger adhesion force ($17.5 \times 10^2 \pm 0.9 \times 10^2$ g/cm², as mean \pm S.D. of five determinations) was observed when it was compared with that obtained in the porcine dermis ($11.8 \times 10^2 \pm 1.5 \times 10^2$ g/cm², as mean \pm S.D. of five determinations), suggesting that the adhesion force of the tablet to the porcine dermis is mainly due to the force required to detach the dermis from the gel layer formed on the tablet surface. However, a little amount of polymer gel remained on the surface of porcine dermis after measuring the adhesion force. Therefore, the adhesion force of the CS-HA tablet to the porcine dermis could arise from a combination of plural factors such as the adhesion of the gel layer to the dermis, the cohesion between the gel layer and the tablet, and the thickness and strength of the gel layer formed at the boundary of the tablet and the porcine dermis. However, further study should be made to elucidate precisely the adhesion mechanisms of CS-HA tablets.

Release Phenomena of BBL from CS-HA Tablets Release phenomena of BBL from the tablets consisting of various mixing ratio of CS and HA were investigated considering the effect of interpolymer complex formation. For this measurement, the acid buffer solutions (pH 2.0,

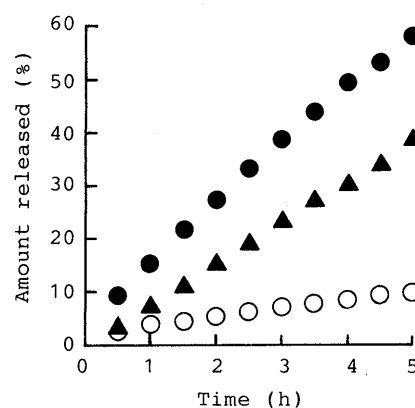


Fig. 8. Release Phenomena of BBL from Compressed Tablet Determined by Rotating Paddle Method in the Medium of pH 3.5 (50 rpm, 37°C)

○, CS-HA tablet (3:7); ▲, CS tablet; ●, HA tablet. Each point represents the mean of three determinations.

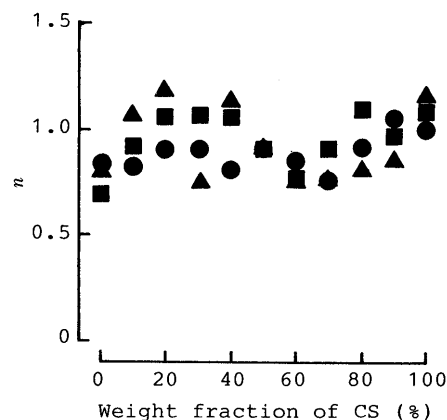


Fig. 9. Release Order, n , for BBL Release from CS-HA Tablet as a Function of Polymer Mixing Ratio in Media of Various pH Values

●, pH 2.0; ▲, pH 3.5; ■, pH 5.0.

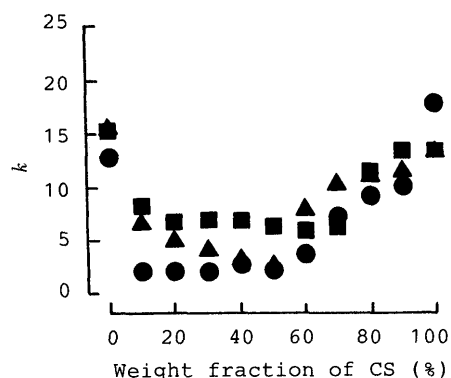


Fig. 10. Apparent Release Rate Constant, k , for BBL Release from CS-HA Tablet as a Function of Polymer Mixing Ratio in Media of Various pH Values

●, pH 2.0; ▲, pH 3.5; ■, pH 5.0.

3.5 and 5.0) were again employed to clarify the effect of the complex formation of CS and HA. Figure 8 shows the release phenomena of BBL from the tablets consisting of CS alone, HA alone and the physical mixture of CS and HA at a weight ratio of 3:7 (CS:HA). The release rate of BBL greatly decreased when the tablet was prepared by the mixture of CS and HA. The effect of stirring on the release behavior of BBL was hardly observed when the release rate from the tablet consisting of a mixture of CS and HA (3:7) was measured at pH 3.5 under the various paddle rotating speeds (25, 50 and 100 rpm). To investigate more precisely the effect of interpolymers complex formation on the release of BBL, results were analyzed according to the following equation.¹⁸⁾

$$M_t/M_\infty = kt^n \quad (1)$$

where M_t/M_∞ is the amount of BBL (%) released at time t (h), n is a diffusional exponent and k is the apparent release rate (%/h ^{n}). In all cases, n value was close to 1, indicating that the release of BBL from the tablet consisting of the mixture of CS and HA can be regarded as following an apparent zero-order mechanism (Fig. 9). In the case of insoluble and non-swelling polymer matrix, the drug release has generally been expressed by a Fickian diffusion mechanism, that is a time-dependence of the square-root of time ($n=0.5$ in Eq. 1). Therefore, the result obtained here, a non-Fickian release behavior, may suggest that the release of BBL for the tablet is controlled by a combination of a diffusion of BBL in the matrix and a swelling of the matrix followed by water penetration into the tablet. Similar

phenomena have been reported in the release behavior of metronidazole from tablets consisting of hydroxypropylmethyl cellulose and polyacrylic acid.⁶⁾ Figure 10 shows an apparent release rate, k , from CS-HA tablets. Concave relationships between k values and the mixing ratio of CS and HA in the tablet were observed in all the dissolution media, clearly indicating that the release of BBL was controlled by the three-dimensional network structure which was produced by the complex formation following water penetration into the tablet.

Based on the results obtained in this study, we can conclude that specific properties of the CS-HA system which would be brought about by the complex formation, such as strong and stable bioadhesion, and the control of drug release, should be applicable to the design of more precisely controlled bioadhesive drug delivery systems.

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