International Journal of Pharmaceutics, 61 (1990) 35–41
Elsevier

IJP 02039

Characteristics of polyion complexes of chitosan with sodium alginate and sodium polyacrylate

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(Received 31 July 1989)
(Modified version received 13 October 1989)
(Accepted 10 November 1989)

Key words: Polyion complex; Chitosan; Sodium alginate; Sodium polyacrylate

Summary

With a view to the application of chitosan (CS) for drug delivery formulations, formation of interpolymer complexes of CS with sodium alginate (AL) and sodium polyacrylate (AC) was investigated, employing viscosity measurement, Fourier-transform infrared (FT-IR) spectroscopy and elementary analysis. The effect of the molecular weight of AL or AC on complexation with CS was analyzed by gel-permeation chromatography. The binding ratio of a unit molecule of CS with AL was found to be approximately 1 : 1. This ratio was relatively constant in media of various pH values. On the other hand, the unit molecular binding ratio of CS with AC was greatly affected by the pH values of the media, showing a change from 1 : 4 to 1 : 1.7 with increase in pH values from 3.7 to 4.7. Results obtained from gel-permeation chromatography suggested that the lower molecules of AL bind with CS more selectively on complex formation, although the selectivity was unclear in the case of the CS-AC system. Based on the results of FT-IR spectra and elementary analysis, the binding ratio of each component in the solid complexes was very close to that observed in viscosity measurements.

Introduction

The use of chitosan (CS) for controlled drug delivery has been investigated using a mixture of various components (Nagai et al., 1984). Considering CS is a cationic polymer, if we use its polyionic interaction with anionic polymers, additional possibilities for CS in more precisely controlled drug delivery may be afforded. Polyion complexes, obtained as precipitates on mixing cationic polymers with anionic polymers in aqueous solutions, have often been reported (Fuoss and Sadek, 1949; Michaels, 1965; Tsuchida et al., 1974). A Coulombic force is believed to be the primary binding force for the formation of these complexes (Bekturov and Bimendina, 1981). In general, the stoichiometry of both components in the polyion complexes depends on the pH values of the media, the ionic strength and temperature (Tsuchida et al., 1980). In this study, we investigated basic properties of polyion complexes composed of CS with sodium alginate (AL) or with sodium polyacrylate (AC), taking their pharmaceutical applications into consideration. CS, selected as a cationic polymer, is readily derived by deacetylation of chitin which is available in large quantities and is widely distributed in nature. Additionally, it has

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high biocompatibility and biodegradability. Thus, the application of CS in the design of pharmaceutical preparations has been examined (Miyazaki et al., 1981; Sawayanagi et al., 1982; Hou et al., 1985; Kawashima et al., 1985; Inouye et al., 1987). On the other hand, sodium alginate (AL) and sodium polyacrylate (AC) were used as anionic polymers. The safety of AL and AC for the living body has already been established and they are widely used as additives in pharmaceutical formulations.

Materials and Methods

Materials

CS, derived from crab shell chitin of about 95% deacetylation, and AL were generously supplied from Dainichiseika Color and Chemicals Mfg. Co., Ltd. CS was used after shattering by ball-mill and passage through a 100 mesh sieve. AC, which was used as the food additive, was purchased from Wako Pure Chemical Industries, Ltd. Its degree of polymerization was 15000–20000. Average molecular weights of CS, AL and AC were determined to be 440000, 630000 and 1510000, respectively, using gel-permeation chromatography (GPC). Details of the GPC study are described later. Other chemicals were of reagent grade.

Viscosity measurement

CS solution (20 ml; 0–0.1%) was mixed with the AL solution (20 ml; 0–0.1%) or AC solution (20 ml; 0–0.1%) at constant temperature to prepare the sample solutions. The sample solution was then incubated at constant temperature for 48 h. A buffer solution, consisting of 0.1 N CH_3COOH and 0.1 M CH_3COONa, was used to prepare the sample solutions. Total polymer concentration was fixed at 0.1% in all samples. To investigate the effect of ionic strength, NaCl (0–0.5 M) was added to the sample solutions. After centrifugation for 20 min at 3000 rpm in a Hitachi SCR 20B centrifuge, the viscosity of the supernatant solution was determined at 37°C on an Ubbelohde viscometer.

Gel-permeation chromatography

The effect of the molecular weights of AL or AC on complex formation with CS was studied by gel-permeation chromatography (GPC). CS solution (4 ml; 0.1%) was mixed with AL or AC solution (16 ml, 0.1%; for both). The sample solutions were then incubated at 37°C for 48 h. A buffer solution (pH 4.0), consisting of 0.1 N CH_3COOH and 0.1 M CH_3COONa, was used to prepare the samples. After centrifugation for 10 min at 10000 rpm in a Hitachi SCR 20B centrifuge, the average molecular weight of AL or AC in the supernatant, remaining as the free form after complexation with CS, was determined by GPC. The average molecular weights of the intact AL and AC were also determined as controls. The GPC study was performed using an assembly of a constant flow pump (Tosoh model CCPE, Toyosoda, Co., Ltd.), a refractive index monitor (Shodex model RI SE-61, Showa-Denko, Co., Ltd.) and GPC columns (Shodex OH Pack models KB-800p and KB-806). These columns were eluted at room temperature with a mobile phase of 0.1 N NaCl solution. The flow rate was 0.5 ml/min. Column calibration was performed using a TSK standard poly(ethylene oxide) (Toyosoda, Co., Ltd.).

Preparation of solid complexes

A CS solution (20 ml; 0.1%) was mixed with AL or AC solution (both: 20 ml; 0.1%). Buffer solutions (pH 3.7, 4.2 and 4.7), consisting of 0.1 N CH_3COOH and 0.1 M CH_3COONa, were used to prepare these samples. The sample solution was then incubated at 37°C for 48 h. Total polymer concentration was fixed at 0.1% in all samples. After the removal of water from the sample solution, the remaining solid complex was dried under vacuum for 3 days at 37°C.

Infrared absorption spectroscopy

FT-IR spectra of the solid complexes in the CS-AL and CS-AC systems were measured using a Jasco model FT/IR-5 spectrophotometer (KBr disk method).

Elementary analysis

Elementary analyses of the CS-AL and CS-AC

solid complexes were performed using a Perkin Elmer model 240B elementary analyzer.

Results and Discussion

Confirmation of polyion complex

Polyion complex formation was confirmed by employing viscosity measurement. The equilibrium binding ratio for complex formation was determined in a 0.1% concentration of polymer solution to obtain equilibrium for a short time, which is in a condition of fairly low concentration compared with that in manufacturing of dosage forms. Fig. 1 shows the viscosity of supernatant in the CS-AL mixture solution as a function of the weight ratio of CS and AL in a medium of pH 4.2. When the weight fraction of AL in the samples was 60%, the viscosity of supernatant in the CS-AL solution was observed to be almost the same as that of the medium. In the case of CS alone or AL, the viscosity of these solutions increased continuously with increase in polymer concentrations. Similar phenomena were observed in the case of the CS-AC system, as shown in Fig. 2. Accordingly, the decrease in viscosity observed in the CS-AL and CS-AC mixtures showed that the solid complexes were formed at a weight ratio of 1 : 1.5 at pH 4.2, and were removed by centrifugation.

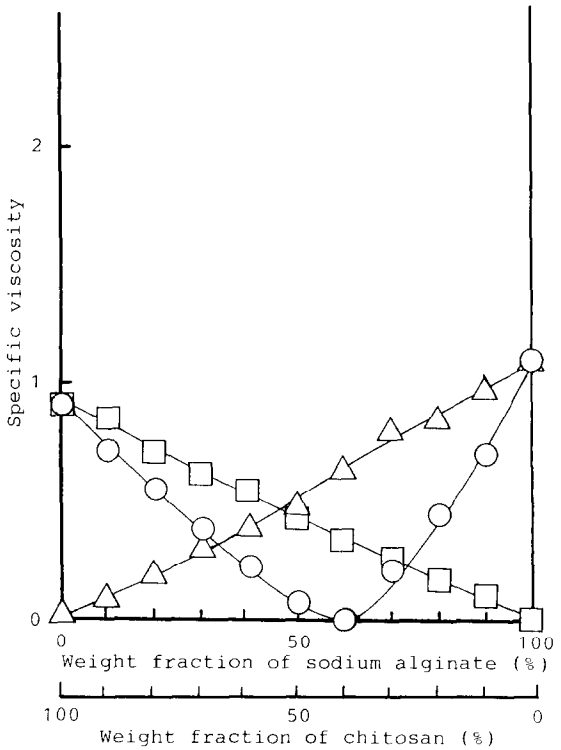

Fig. 1. Specific viscosity of supernatant solution of CS-AL systems as a function of polymer mixing ratio at pH 4.2 and 37 °C; CS-AL mixture solution (○); AL solution alone (△); CS solution alone (□). In the case of CS-AL mixture solution, the total polymer concentration in the supernatant solution was lower than 0.1% in most cases, since the solid complexes formed were removed by centrifugation.

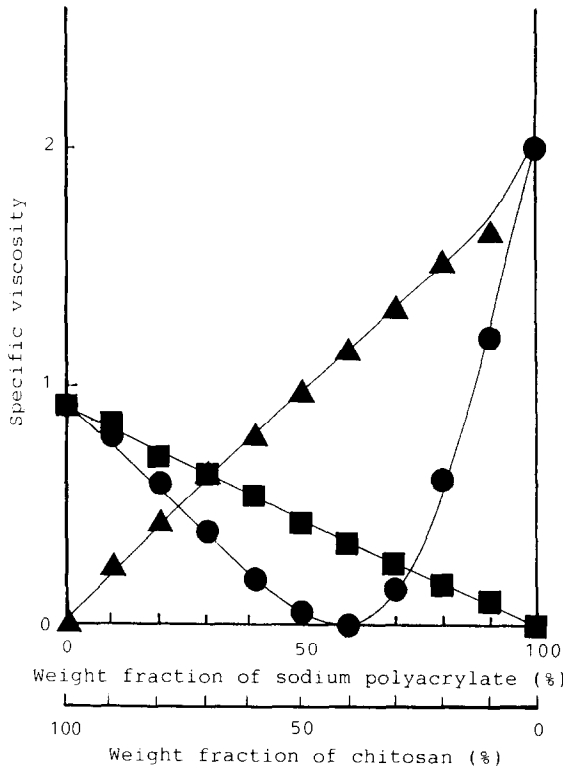

Fig. 2. Specific viscosity of supernatant solution of CS-AC systems as a function of polymer mixing ratio at pH 4.2 and 37 °C; CS-AC mixture solution (●); AC solution alone (▲); CS solution alone (■). In the case of the CS-AC mixture solution, the total polymer concentration in the supernatant solution was lower than 0.1% in most cases, since the solid complexes formed were removed by centrifugation.

Factors affecting complex formation

The effects of various pH values of the media on complex formation in the CS-AL and CS-AC

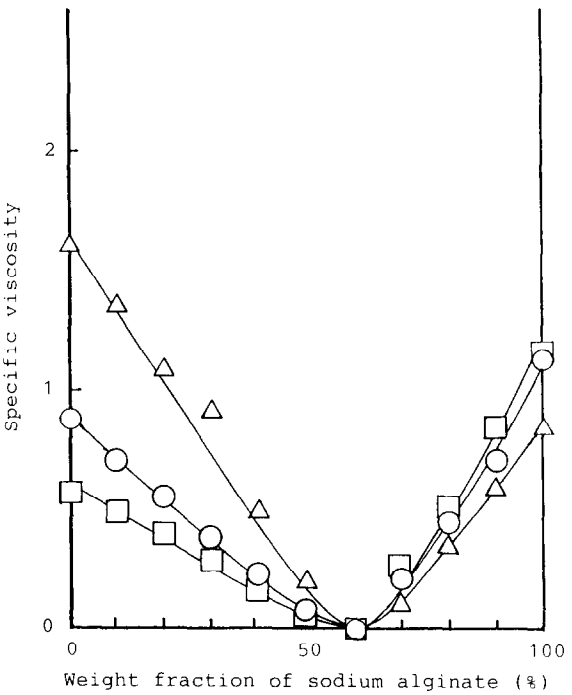

Fig. 3. Relationship between polymer mixing ratio and specific viscosity of supernatant solution of CS-AL systems in media of various pH values at 37°C; pH 3.7 (△); pH 4.2 (○); pH 4.7 (□).

systems are shown in Figs. 3 and 4. The reason why acidic buffer solution (pH 3.7, 4.2 and 4.7) was used in this study was that CS was not soluble in the neutral and alkali solutions. In the case of the CS-AL complex, the binding ratio was almost constant despite the change in pH values. The ratio of 1:1.5 (CS:AL) in weight is approximately equivalent to 1:1 of the unit molecular weight ratio. On the other hand, the unit molecular binding ratio of the CS-AC complex was greatly affected by pH, showing a change from 1:4 to 1:1.7 (CS:AC) with increase in pH from 3.7 to 4.7. Although we investigated the effects of ionic strength of the media (NaCl; 0–0.5 M) and temperature (27–47°C) on complex formation, the binding ratios of the CS-AL and CS-AC systems were not affected by these factors.

The degrees of ionization in both AL and AC molecules were considered to increase with increasing pH values in the media. In contrast, the tendency toward ionization of the CS molecules would decrease with increasing pH values. Accordingly, if polymer chains were sufficiently flexible and, thereby, the active sites on the molecules for the complexation not greatly affected by steric hindrance, the unit molecular binding ratio should alter with changing pH values in the media, as observed in the CS-AC complex. We believe that the polymer chain of AC is more flexible than that of AL, since AC comprises vinyl bonding of acrylic acid, though AL is built up of glycosidic bonding. Thus, the rigidity or flexibility of the polymer chains might play an important role in the formation of polyion complexes.

Selectivity of polymer chain length on complex formation

In order to elucidate more accurately the effect of rigidity (or flexibility) of the polymer chain on complex formation, GPC study was performed as follows: The CS solution was mixed with the AL or AC solutions in order to make the amounts of

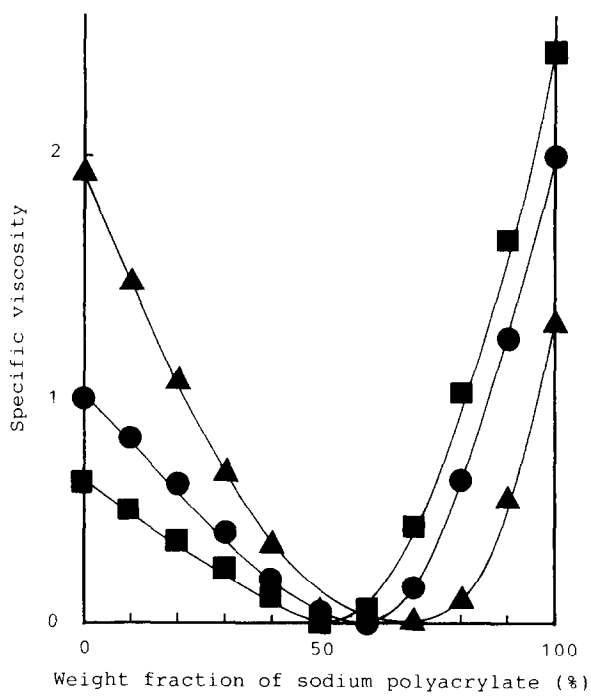

Fig. 4. Relationship between polymer mixing ratio and specific viscosity of supernatant solution of CS-AC systems in media of various pH values at 37°C; pH 3.7 (▲); pH 4.2 (●); pH 4.7 (■).

TABLE 1

Average molecular weights of sodium alginate (AL) or sodium polyacrylate (AC) remained as free forms after complexation with chitosan

Polymers	Average molecular weight ($\times 10^4$)
Intact AL	63.1
AL remaining as free form	75.9 (20.3%) [a]
Intact AC	151
AC remaining as free form	162 (7.3%) [a]

[a] Percent increase in molecular weight of polyanions remaining as free forms after complexation with CS.

AL or AC exactly 4-times greater than that of CS. The mixture solutions were then incubated and centrifuged. The average molecular weights of AL or AC (remaining as free forms in the supernatants) were determined by the GPC method. The results are summarized in Table 1. In the case of the CS-AL system, the average molecular weight of AL, remaining as the free form after the complexation, significantly increased when compared with that of the intact AL. This signifies that smaller molecules of AL bind more selectively with CS molecules. On the other hand, the selectivity of AC molecules was scarcely observed for complexation with CS molecules. The discrepancy in complex formation between the CS-AL and CS-AC systems might be due to the difference between the rigid structure of AL molecules and the flexible structure of AC. Namely, the binding activity of larger molecules of AL to CS seems to be weaker than that of smaller ones because of the rigidity of AL molecules. On the other hand, the activity of larger molecules of AC might be the same as smaller ones due to its flexibility. However, further studies taking account of rheological and electric properties should be performed in order to elucidate precisely the mechanisms of complex formation.

Structures of solid complexes

Fig. 5 shows FT-IR spectra of the CS-AC solid complex obtained as precipitates in media of various pH values. IR spectra of the solid complex were greatly different from those of the CS-AC physical mixture (1 : 1.5 weight ratio). New peaks, appearing at 1710 and 1580 cm^{-1}, were assigned to the carboxyl groups of AC bound with CS and amino groups of CS bound with AC, respectively. It has been reported that the amino groups of CS are capable of interacting with an anionic polymer which has carboxyl groups, such as carboxymethyl

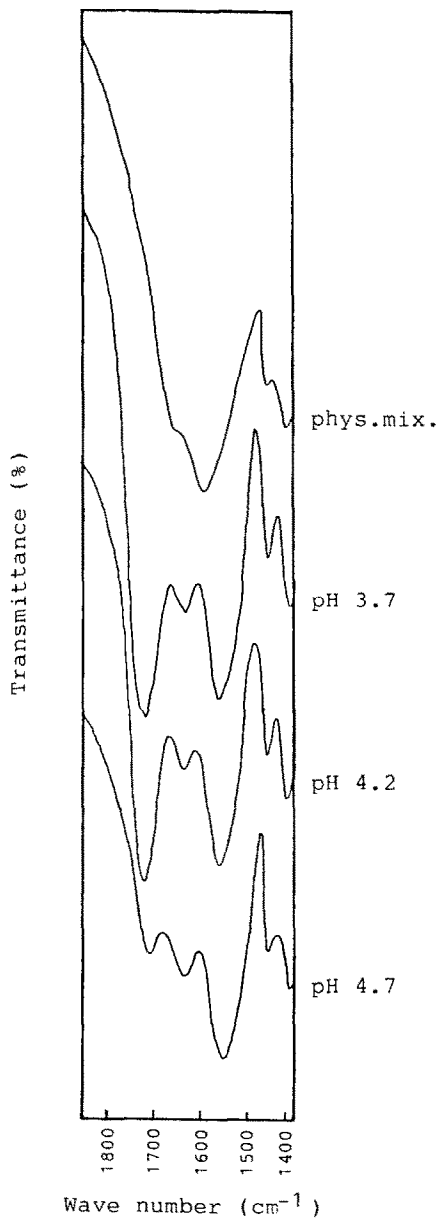

Fig. 5. FT-IR spectra of CS-AC solid complexes obtained as precipitates from media of various pH values.

cellulose (CMC), by ionic bonding as a primary binding force (Fukuda and Kikuchi, 1979). In the case of the CS-CMC complex, new peaks, assigned to the carboxyl and amino groups, have been observed in the IR spectra. These peaks were very close to the results obtained in the CS-AC complex (Fukuda and Kikuchi, 1979). Accordingly, we consider that the ionic bonding is a primary binding force for the complex formation between CS and AC.

Fig. 5 also shows that the increase in pH in the media in which the solid complex was obtained as the precipitate led to a decrease in the peak at 1710 cm^{-1} and to an increase in that at 1580 cm^{-1}. Therefore, the structure of the CS-AC complex changed with varying pH in the media similarly to observations in the viscosity measurement (Fig. 4). Although the IR spectra of the CS-AL solid complex were different from those of the physical mixture, a change in the spectra was scarcely observed on changing the pH in the media in which the solid complex was obtained.

Next, we calculated the binding ratio of each component in the solid complexes based on the results obtained by an elementary analysis. Fig. 6 shows the unit molecular binding ratio of AL or AC with CS in the solid complexes obtained in media of various pH values. The binding ratio of AL with CS was relatively constant and the ratio was found to be approx. 1 : 1. On the other hand, the binding ratios of AC with CS changed from 2.5 : 1 to 1.5 : 1 with increasing pH values. These phenomena were close to those observed in the previously described viscosity measurements (Fig. 4).

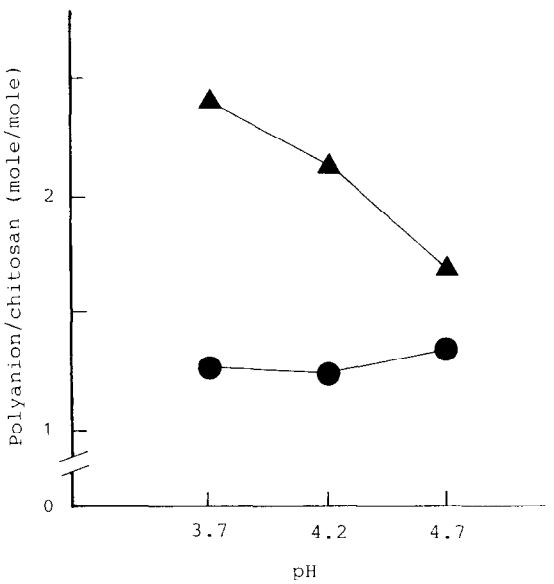

Fig. 6. Unit molecular binding ratios of CS-AL and CS-AC solid complexes obtained as precipitates from media of various pH values; CS-AL complex (●); CS-AC complex (▲).

In conclusion, the differences observed between the CS-AL and CS-AC systems were mainly due to the rigidity or flexibility of the polymer chains. The specific properties of the CS-AL complex, which is stable to pH change, and the CS-AC complex, which is quite sensitive to pH change, should be applicable to the design of more precisely controlled drug delivery systems.

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research on Priority Area, New Functional Materials-Design, Preparation and Control (No. 62604604), from the Ministry of Education, Science and Culture. The authors are very grateful to Showa-Denko, Co., Ltd. for supplying columns for the GPC study. The authors would like to thank Mr. Atsushi Harada for assistance in the experimental work.

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