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Formation and characterization of an insoluble polyelectrolyte complex: Chitosan-polyacrylic acid

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ABSTRACT

Chitosan and polyacrylic acid mixtures were prepared in different mole ratios and at different pH values and ionic strengths (0.025-0.300). Complex formation was detected by turbidity measurement and quantified by weighing the freeze dried pellet recovered by centrifugation. No insoluble complex formation at pH = 2 was detected. In the 3 to 6 pH range, the maximum complex formation occurred at different mole ratios. Quantitative analysis of the supernatant showed that pH affects the complex composition. Solution ionic strength in the 0.025-0.300 range did not affect complex formation.

Supernatant pH measurement showed that in the 3 to 5 pH range, the pH of the mixture decreased as the complex was formed. At pH = 6, the opposite behavior was observed. This information was used to propose a mechanism for complex formation which was confirmed by quantitative analysis of the supernatant and IR analysis of the insoluble complex. These studies showed that an electrostatic interaction between COO^- and NH_3^+ groups was involved in complex formation.

INTRODUCTION

Chitosan, the best known chitin derivative, is obtained by deacetylation of chitin. It consists of unbranched chains of β(1-4)2-amino-2-deoxy-D-glucan residues. Chitosan has great potential in food industry and biotechnology applications because of its unique cationic character. An understanding of the complex formation between chitosan and polyanions could be used to design improved systems for the recovery of proteins and other bioproducts.

Polyelectrolyte complex formation between chitosan and other polyanions such as sodium carboxymethylcellulose (1), heparin (2), and acidic glycosaminoglycans (3) have been previously reported. Researchers have also attempted to use chitosan as a water clarifying agent (4), and as a floculating agent in some industrial effluent treatment applications (5, 6). In this paper we present work on the elucidation of the insoluble complex formation mechanism between chitosan and a polyanion with a very simple structure, polyacrylic acid. A thorough understanding of interpolymer complex formation should lead to the control of complex formation and facilitate industrial chitosan applications by allowing the preparation of interpolymer complexes with specific functional properties.

In our model studies we considered the effect on complex formation of pH, ionic strength and mixing ratio of chitosan and polyacrylic acid. Turbidity was used as an index of insoluble complex formation. pH

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** To whom correspondence should be directed measurement and IR analysis were used to investigate the complex formation mechanism. Quantitative analysis of supernatants were done to determine the composition of complexes at different pH values. The properties of the complex itself such as solubility, porosity, charge density, etc. will not be discussed in this paper.

EXPERIMENTAL

Materials

Chitosan (CHI, Lot: 5112A) was purchased from Bioshell Inc., Albany, Oregon and was purified by dissolving it in 0.1N HCl, filtering through a medium porosity fritted disk Buchner type filtration funnel, reprecipitating with NaOH, rinsing with deionized water and then freeze-drying. The molecular weight of CHI (220,000) was determined at 25°C using a Cannon-Fenske viscometer with 27.5 g NaCl in 1000 ml of 1% acetic acid as the solvent. Polyacrylic acid (PAA) was purchased from Aldrich (Milwaukee, Wisconsin). Using dioxane as a solvent, its molecular weight was estimated to be 202,000 (7).

Complex formation

0.1 g CHI and PAA were dissolved in 100 ml hydrochloric acid and NaCl solutions, respectively. The ionic strength, 0.025 to 0.300, was controlled by adjusting the concentration of the hydrochloric acid and NaCl solutions. The pH of both reactants, 2.000 to 6.000, was adjusted by using hydrochloric acid or sodium hydroxide solutions. CHI was insoluble at pH values greater than 6.3. pH was measured with a combination pH electrode (Ross model 81550) and read to 0.001 pH units on a microprocessor pH/mV meter (Orion model 811). The amount of added pH adjusting solutions were recorded to calculate the final concentration of each reactant.

Reactant solutions with equal pH values were mixed in the following volumetric proportions (ml CHI:ml PAA): 0:40, 5:35, 10:30, 15:25, 20:20, 25:15, 30:10, 35:5, 40:0. A mixing ratio was defined as A/(A+B) where:

$$A = \frac{\text{weight of chitosan}}{\text{m.w. of monomer of chitosan}}$$

and

$$B = \frac{\text{weight of polyacrylic acid}}{\text{m.w. of monomer of polyacrylic acid}}$$

The mixture was shaken vigorously and left for 15 minutes before measuring turbidity in a Varian DMS 80 U.V./Visible Spectrophotometer (absorbancy at 420 nm). The insoluble complex was separated by centrifugation at 34,800 g for 40 minutes. The pellet was twice resuspended in distilled water and then centrifuged again. The washed complex was finally freeze dried, weighed and analyzed by IR using the KBr pellet technique (1). The pH of the supernatant was recorded and the CHI concentration was measured using the Nessler reagent method (8). By knowing the weight of the complex, the original reactants mixture and the CHI which was left in the supernatant, it was possible to calculate the amount of PAA left in the supernatant.

RESULTS AND DISCUSSION

At pH = 2 and at all ionic strengths tested, there was no insoluble complex formation. At this pH the PAA carboxylic groups do not have a charge density sufficiently high to form a complex with CHI (9). The insoluble complex formation occurred only in the pH 3 to 6 range.

Although turbidity was a good indicator for complex formation, under some experimental conditions it was not directly related to the amount of complex formed. This occurred when extensive complex formation resulted in sedimentation and lowered the turbidity of the mixture. For example, measurements of mixtures at pH 3 and ionic strength = 0.300 showed two turbidity maxima at 0.56 and 0.20 mixing ratio (Fig. 1) while missing the true maximum at 0.12 (Fig. 2). Note also that the mixing ratio for maximum insoluble complex formation increased with the initial pH of the solution (Fig. 2).

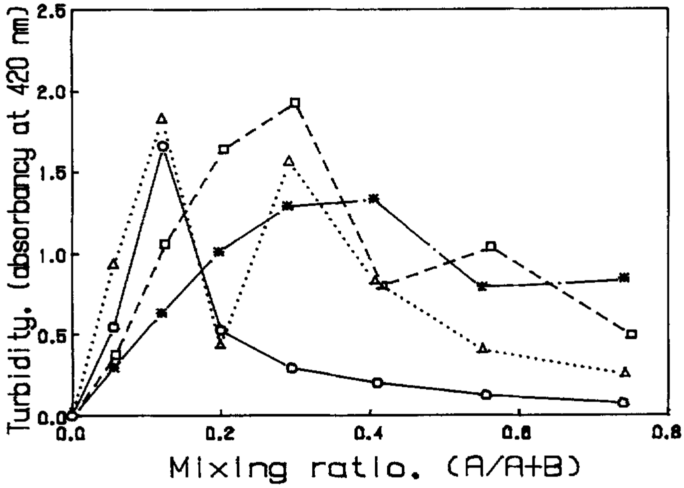

Figure 1: Effect of mixing ratio on complex formation: turbidity measurements. ○pH=3, △pH=4, □pH=5 and *pH=6

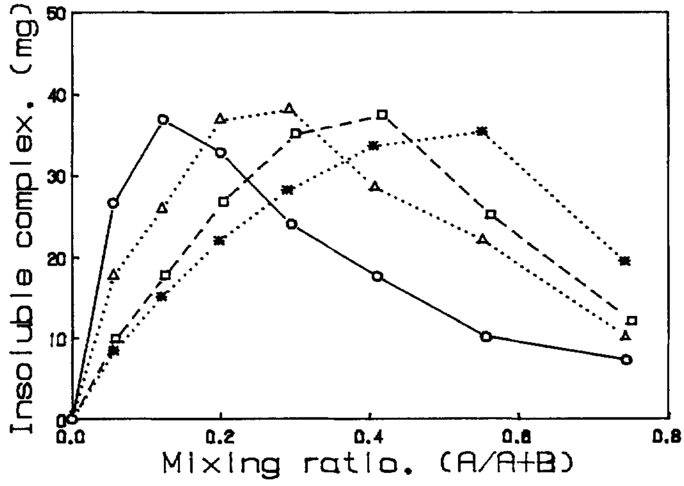

Figure 2. Effect of mixing ratio on complex formation: pellet weight determinations. ○pH=3 △pH=4 □pH=5 *pH=6

The ionic strength range covered in this study, 0.025 to 0.300, did not affect the amount of complex formation. Fig. 3 shows complex formation at pH 3 and 6 and only minor differences can be seen between curves at different ionic strengths at these two pH conditions even though the ionic strength changed more than one order of magnitude. The rest of this paper will refer specifically to experiments at ionic strength = 0.300.

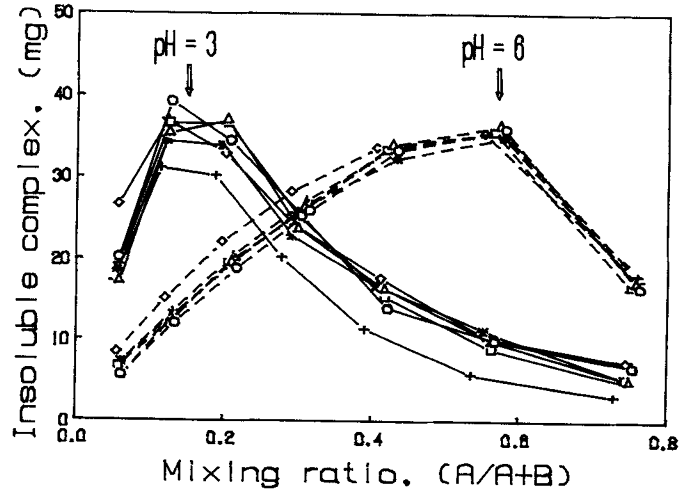

Figure 3: Effect of ionic strength on complex formation. Ionic strengths: ◇ 0.025, ○ .05, △ 0.1, △ 0.15, □ 0.25 and □ 0.30.

At pH 3,4 and 5 the degree of ionization of CHI was about 1.0, 0.95 and 0.85, respectively (10). At the same conditions the degree of ionization of PAA was about 0.1, 0.2 and 0.5, respectively (9). In other words, in the 3 to 5 pH range, most of the CHI amine groups are in the NH_3^+ form while most of the PAA carboxyl groups are in the COOH form. This suggests the following complex formation mechanism:

$$\begin{array}{lcll} NH_3^+ + HOOC & \longrightarrow & NH_3^+ \ {}^-OOC + H^+ & \quad (1) \\ \text{(chitosan)} + \text{(polyacrylic acid)} & \longrightarrow & \text{(complex)} & \end{array}$$

Eq. (1) suggests that complex formation should lower the supernatant pH. The relationship between the supernatant pH value and complex weight for mixtures at initial pH 3, 4 and 5 is shown in Figs. 4a, 4b and 4c, respectively. These figures show that indeed, the pH of the mixtures decreased as the amount of complex increased.

At pH = 6, the degree of ionization of ChI is reduced to about 0.6 (10) while that of PAA is about 0.8 (9); i.e. most of the amine groups are in the NH_2 form while most of the PAA carboxyl groups are in the COO^- form. This suggests the following complex formation mechanism:

$$\begin{array}{lcll} NH_2 + {}^-OOC & \xrightarrow{+H^+} & NH_3^+ \ {}^-OOC & \quad (2) \\ \text{(chitosan)} + \text{(polyacrylic acid)} & \longrightarrow & \text{(complex)} & \end{array}$$

Eq. (2) suggests that complex formation should decrease the concentration of free H^+, i.e. increase the pH of the supernatant. Fig. 4d shows the relationship between supernatant pH and amount of complex formed for mixtures with initial pH = 6. It shows that the supernatant pH behaved as predicted by Eq. (2).

The effect of pH on the mixing ratio for maximum insoluble complex formation noticed in Fig. 2 has been analyzed in more details in Fig. 5. We have incorporated information on the analysis of the supernatant fraction to indicate which reactant was present in trace amounts and which excess reactant supernatant concentration increases.

The supernatant pH changes are not only a function of the initial pH conditions and the amount of complex formed, but also of the buffering properties of the excess reactant present in the supernatant. Due to the CHI and PAA buffering capacities, the existence or disappearance of them in the supernatant should affect the change in the supernatant pH. For example, in Fig. 4a, when the amount of complex formed was 27.5 mg (points 1 and 3), the supernatant pH values (points 2 and 4) were different. This was due to the difference in the amount of excess reactant remaining in the supernatant (Fig. 5).

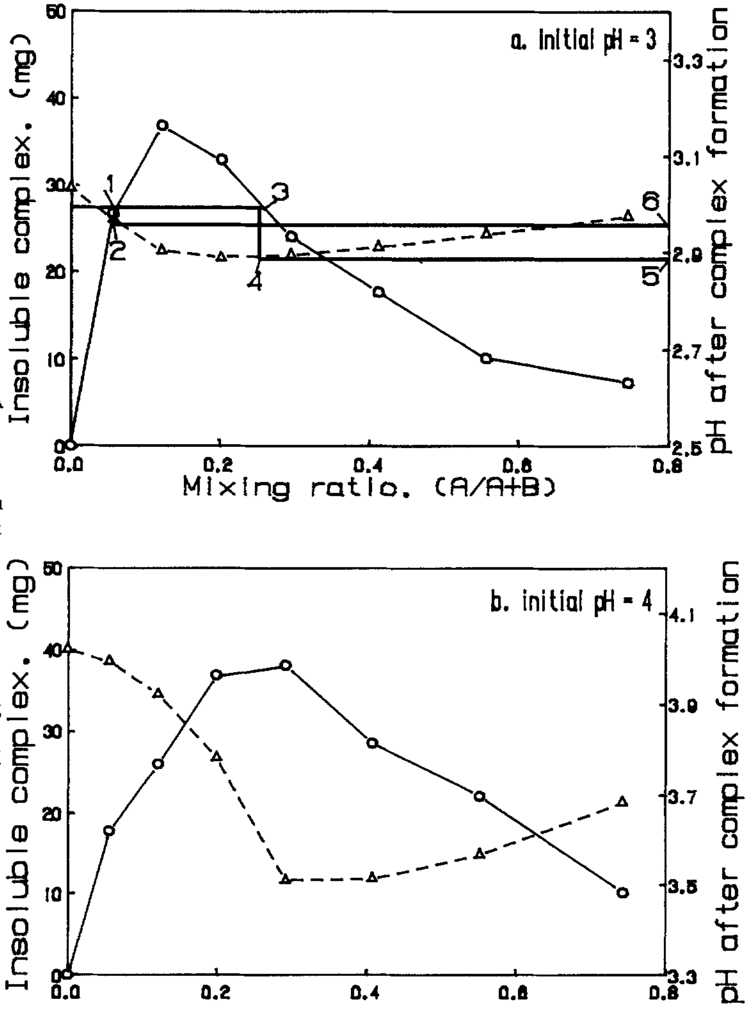

Figure 4: Effect of complex formation on supernatant pH. o = insoluble complex weight ▵ = pH

The maximum amount of complex formed, occurred at different mixing ratios depending upon the initial pH conditions. It should be noted that both reactants had different charge densities at different pH values. At low pH values, CHI had a high charge density while PAA had a low charge density. Therefore, complex formation at low pH values required a large amount of PAA to neutralize small amounts of CHI and form the complex. At higher pH values CHI had a lower charge density, and PAA had a higher charge density, therefore the complex formation needed more CHI and less were present in the complex and none left in the supernatant. Therefore, it was possible to assume that the mixing ratio at that point represents the composition of the complex formed at each pH value. The result shows that the higher the pH value the higher the CHI ratio in the complex.

An example of IR analysis is shown in Fig. 6 and indicates that the main differences, between the IR spectra of a reactants mixture in the same proportion found in the complex and the complex itself, occurred at 1410 cm^{-1} and around 1520-1600 cm^{-1}. The wavelengths of 1410 cm^{-1} and 1580 cm^{-1} correspond to the antisymmetrical and symmetrical valency vibration of the carboxylate anion present in the complex (11). The absorbancy at 1520 cm^{-1} has been reported to correspond to NH_3^+ groups present in the complex (1). These observations confirm that ionic bonding was involved in the complex formation reaction.

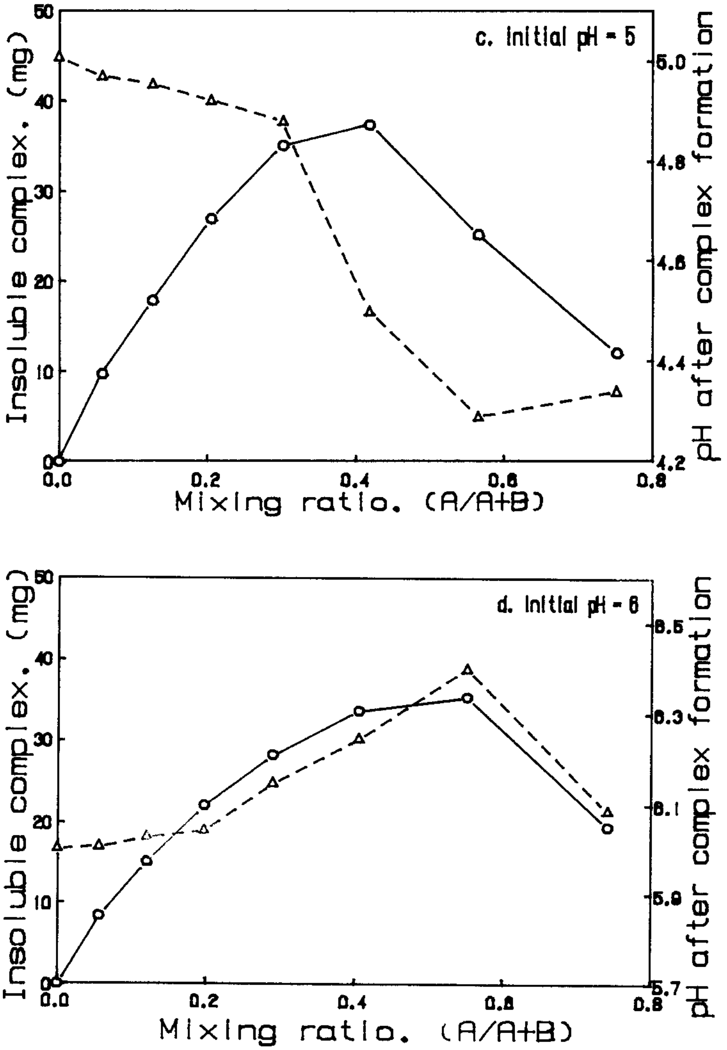

Figure 4: Effect of complex formation on supernatant pH.
○ =insoluble complex weight △ =pH

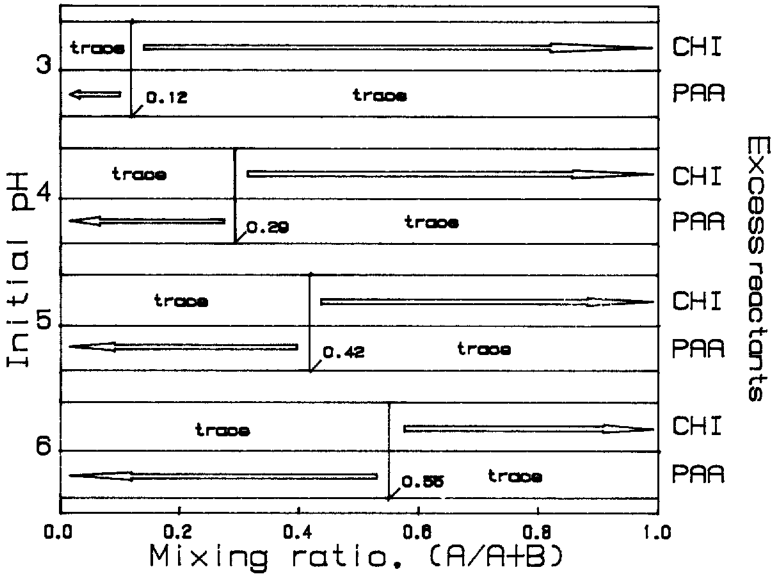

Figure 5: Effect of mixing ratio and initial pH on supernatant composition. See text for further details.

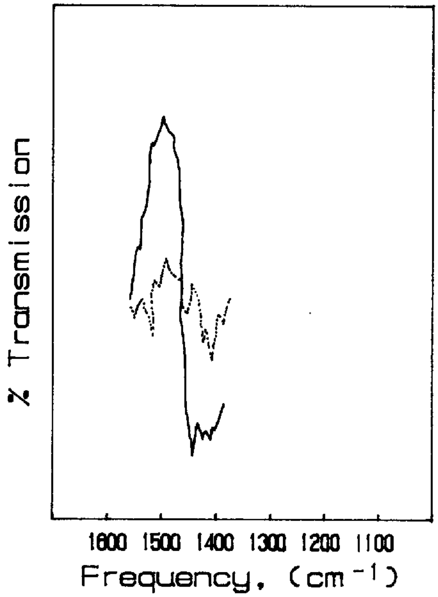

Figure 6: IR analysis of a mixture of chitosan and polyacrylic acid (—) and of a complex (---) formed at initial pH = 3 and mixing ratio = 0.122

CONCLUSIONS

Our experimental evidence indicates that the insoluble complex formed by reacting chitosan and polyacrylic acid are polyelectrolyte complexes. We have also shown that their composition is a function of the initial pH of the reaction mixture. This finding suggests that it is possible to prepare chitosan-polyacrylic acid complexes with specific and controlled properties.

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