Bioartificial Materials Based on Blends of Collagen and Poly(Acrylic Acid)

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ABSTRACT: The interactions between soluble collagen (C) from calf skin and poly(acrylic acid) (PAA) were studied. Mixing aqueous solutions of collagen and PAA, at various pH values (2.5–4), leads to the formation of complexes that precipitate in the form of insoluble aggregates. The effects of mixture composition, pH, and ionic strength on C/PAA complex formation were investigated by gravimetric, turbidimetric, and conductometric analysis. The experimental results indicate that the complexes form through electrostatic interactions. Homogeneous solid films with variable C/PAA ratios were obtained by casting from solutions in which the pH was adjusted just over the isoelectric point of collagen, thus avoiding the attractive ionic interactions responsible for the complexation of collagen and PAA molecules. A relevant result obtained is related to the possibility of restoring the ionic interactions between the two polymers inside the solid films. Mixture composition and pH appear to influence the thermal properties of both complexes and films. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 72: 971–976, 1999

Key words: collagen; poly(acrylic acid); polymer complexes; bioartificial polymeric materials

INTRODUCTION

The properties of bioartificial polymeric materials based on blends of soluble collagen with different water-soluble synthetic polymers were described in our previous articles. Most of the materials were studied in the form of thin films, obtained by casting, starting from polymeric aqueous solutions. Synthetic polymers such as poly(vinyl alcohol) or its copolymers showed that they could interact with collagen through hydrogen bonds. Although these interactions are weak, the resulting materials showed mechanical properties somewhat better than those of the single components, and the proteolytic stability of collagen in the blends had higher results with respect to that of pure collagen. A further improvement in the characteristics of such materials could reasonably be expected if interactions stronger than hydrogen bonds occurred between collagen and the synthetic component.

A preliminary investigation was carried out on blends of collagen with poly(acrylic acid) (C/PAA), in which PAA was used as a model for studying the interactions between collagen and carboxylic functional groups. That study showed the possibility of obtaining a high degree of compatibility between these two polymers thanks to the strong ionic interactions that occur between them.

The present work deals with the preparation and characterization of C/PAA blends, in which the interactions occurring between the two components were studied both in aqueous solution...
and in the solid state. In results from literature,\textsuperscript{5–7} it was found that PAA forms polyelectrolyte complexes with various polybases, either synthetic or biological. In the case of C/PAA system, ionic interactions occur between the two oppositely charged macromolecules and lead to the formation of a polycrystal that precipitation as an insoluble aggregate because the charged groups responsible for solubility (carboxylic groups of PAA in the form COO\textsuperscript{-} and aminic groups of collagen in the form of NH\textsubscript{3}\textsuperscript{+}) are involved in the complex.

On one hand, the phenomenon of complex formation demonstrates the occurrence of strong interactions between the two polymers, but on the other hand, does not allow the preparation of films by solution casting.

However, because the polyelectrolyte complexes form and precipitate in aqueous media only within a narrow pH range, homogeneous films can be obtained by casting from C/PAA aqueous solutions simply by adjusting their pH value over that corresponding to the isoelectric point of collagen (about 5.5).\textsuperscript{8} At pH = 6, the ionization degree of PAA is about 0.8,\textsuperscript{9} in other words, most of the carboxyl groups of PAA are in the COO\textsuperscript{-} form as well as the carboxylic groups of collagen, whereas the amino groups of collagen are mainly in the NH\textsubscript{3}\textsuperscript{+} form.

In these conditions the two polymers are both negatively charged; the repulsive forces prevent complex formation and no aggregate forms. Thus, water-soluble films, inside which no significant ionic interactions occur, can be obtained. Once these films are produced, it is possible to restore the ionic interactions between collagen and PAA chains with the use of a simple dipping method. In this way, the films preserve their shape and size and become insoluble in water, and their physicochemical and mechanical properties change.

In this work, the effects of mixture composition, pH, and ionic strength on the formation of C/PAA complexes are investigated; a procedure for the preparation of homogeneous C/PAA films is described and the thermal properties of both polycrystals and films are analyzed.

**EXPERIMENTAL**

Soluble collagen, type I from calf skin, was a Sigma (St. Louis, MO) product. PAA (M\textsubscript{w} = 250,000) was provided by Aldrich Chemie (Stemheim, Germany). Both polymers were used as received. Collagen solutions (1 mg/mL) in 0.5M acetic acid and PAA solutions (1 mg/mL) in distilled water were prepared at various pH values and ionic strength. Adjustments of the pH value, where required, were performed using acetic acid or sodium hydroxide solutions. Adjustments of the ionic strength values were performed using sodium chloride solutions.

**Complex Formation**

Complex formation was investigated by gravimetric, turbidimetric, and conductometric analysis. Complex formation was observed in all the experiments carried out at a pH value lower than that of the isoelectric point of collagen (pH = 2.5, 3.0, 3.5, 4.0, and 4.5). In these experiments, the ionic strength was varied using sodium chloride solutions with concentration ranging from 0.025 to 0.1M.

During each experiment, pH and ionic strength were kept constant. Volumes equal to 2 mL of the collagen solution were dispensed into test tubes. Volumes of the PAA solution ranging from 0.2 to 4.4 mL were added so that each tube contained a different amount of PAA. The final volume in each tube was brought to 10 mL by adding acetic acid at the selected pH. The tubes were shaken, settled for 1-h, and then centrifuged at 3000 rpm for 30 min. The supernatants were analyzed by a Shimadzu UV-2100 spectrophotometer with the absorbance measuring 313 nm. The precipitates were washed twice in acetic acid at the same pH of the experiment, dried, and weighted.

The amount of each precipitate was calculated using the following formula:

\[
\text{precipitate (wt %)} = \frac{\text{weight of precipitate}}{\text{weight of collagen} + \text{weight of PAA}} \times 100
\]

and was reported as a function of the PAA content.

During complex formation, conductometric measurements were also carried out to determine an electrochemical end point and to get additional information on the polycrystal formation through conductivity changes. All conductometric titrations were performed at 20°C with a Hanna Instruments HI-8033 conductimeter. A volume of 60 mL of a collagen solution (0.5 mg/mL) was titrated by adding a solution of PAA (3 mg/mL) drop by drop under stirring. After each addition of
the titrant, the conductivity was measured and recorded after the achievement of a constant value. The conductivity values were corrected taking into account the volume changes.

Film Preparation
Homogeneous solid films with various C/PAA ratios were obtained by casting at room temperature starting from collagen and PAA solutions having a pH = 6. These films were dipped for 24 h into an acetic acid solution at pH = 2.5 or 4.0, rinsed twice with distilled water, dried in air stream up to constant weight, and stored in a desiccator chamber until used.

Material Characterization
Complexes and films prepared as previously described were subjected to calorimetric analysis to investigate the effect of the interactions between collagen and PAA on the denaturation temperature of collagen. The calorimetric analysis was performed by a differential scanning calorimeter (DSC) (Perkin Elmer DSC-7) in the 20–150°C temperature range at a scan rate of 5°C/min using stainless steel pans.

RESULTS AND DISCUSSION
Complex Formation
In the 2.5–4.0 pH range, neither pure collagen nor pure PAA form a precipitate. Therefore, the formation of an insoluble product upon mixing their solutions would be the result of interactions occurring between the two polymers.

The results of gravimetric analysis (Fig. 1) show that the maximum amount of precipitate obtained is close to 77%, irrespective of pH. There is a remarkable influence of pH on the PAA content at which this maximum is observed. The PAA content corresponding to the maximum amount of precipitate decreases almost linearly with increasing pH (Fig. 2). As pH ranges from 2.0 to 4.0, the ionization degree of PAA in dilute aqueous solutions changes linearly from less than 0.1 to 0.2. Therefore, as the pH increases, the number of carboxylic groups of PAA in the COO⁻ form increases, while most of the amino groups of collagen are in the NH₃⁺ form. The higher the ionization degree of PAA, the smaller the number of PAA chains that are required for a complete pairing between COO⁻ and NH₃⁺ groups. In other words, the higher the pH value, the lower the amount of PAA required to have the maximum complexation.

These results indicate that in the C/PAA system, the influence of pH on the polycrystal composition is noticeable; the C/PAA (w/w) ratio corresponding to the maximum complexation decreases approximately from 80/20 at pH = 4.0 to 55/45 at pH = 2.5. With regard to the effect of the ionic strength, it was observed that the amount of complex formed at a given pH does not show relevant differences varying the NaCl concentration in the solutions (Fig. 3).

Turbidimetric analysis of the surnatants obtained after each PAA addition to the collagen

Figure 1 Precipitate amount as a function of PAA content in the mixture at different pH. pH = 2.5 (○), pH = 4.0 (■).

Figure 2 PAA content corresponding to the maximum yield of the complex as a function of pH.
solution was carried out at 313 nm to ascertain the presence of collagen not complexed with PAA. A preliminary study on collagen dilute solutions showed that at 313 nm there is a linear relationship between absorbance and collagen concentration. In Figure 4, the absorbance at pH 2.5 and 4.0 is reported as a function of PAA content in the mixture. In both cases, the amount of noncomplexed collagen is <10% of the initial collagen content. The absorbance decreases as the PAA content increases and reaches a rather constant value when the maximum complexation is achieved. It can be seen that the absorbance reaches its constant value for a PAA content that is in good agreement with that corresponding to the maximum amount of precipitate found by gravimetric analysis.

Conductivity measurements confirm the previous results. The C/PAA (w/w) ratios corresponding to the best complex formation result in 81/19, 70/30, 63/37, and 56/44 at pH 4.0, 3.5, 3.0, and 2.5, respectively. As an example, conductometric curves relative to pH values of 3.0 and 4.0 are shown in Figure 5(a) and (b). Both curves show a slope change corresponding to the maximum complexation.

Figure 3 Precipitate amount as a function of PAA content at pH = 3 at different ionic strength.

Figure 4 Turbidimetric analysis of the supernatant. Absorbance at 313 nm as a function of PAA content in the mixture. pH = 2.5 (○), pH = 4.0 (■).

Figure 5 (a) Conductometric titration of collagen by a PAA solution at pH = 3.0. (b) Conductometric titration of collagen by a PAA solution at pH = 4.0.
Thermal Characterization

The precipitates obtained by complexation in solution were analyzed by DSC without drying. Thermograms of the complexes obtained at different pH values (Fig. 6) show that collagen denaturation temperature ($T_d$) slightly depends on pH and ranges from approximately 43°C (at pH = 2.5) to 50°C (at pH = 4.0). Samples of swollen pure collagen also show a $T_d$ that slightly depends on pH but is appreciably lower and ranges from about 30°C (at pH = 2.5) to 35°C (at pH = 4.0) (Fig. 8). These values are in good agreement with those reported by Privalov and Dick and Nordwing. They found that for collagen from calf skin, a $T_d$ variation of no more than 7°C (from 32 to 39°C) varies the pH value from 1.0 to 7.0. The DSC results indicate that complexation of collagen with PAA induces an increase in its thermal stability and overcomes the effect of bulk water that normally lowers the denaturation temperature of the protein. DSC thermograms shown in Figures 8 and 9 refer to C/PAA films prepared by casting and dipped in solutions at pH = 2.5 and 4.0, respectively. The collagen $T_d$ values in the films have higher results than those in...
the complexes; this could be attributed to the lower water content of the films with respect to that of the complexes.

The water content in the films was about 20% (w/w), a value corresponding to primary water hydration.\textsuperscript{10,11} At pH = 2.5, \( T_d \) varies from 30°C for swollen collagen to about 85°C for collagen in the form of film. At pH = 4.0, the \( T_d \) variation results are even greater. These values are in agreement with the results reported by Luescher et al.\textsuperscript{8} on the thermal behavior of soluble collagen from calf skin as a function of water content. Both Figures 8 and 9 show that the thermal stability of collagen is enhanced by the presence of PAA. Therefore, by dipping the solid films into the acetic acid solutions, strong ionic interactions can be established between the two polymers.

CONCLUSIONS

The results obtained in this study on the interactions between collagen and PAA show that a complex of the two polymers is formed in aqueous solution, within a narrow pH range, through electrostatic interactions involving the \( \text{NH}_3^+ \) groups of collagen and the \( \text{COO}^- \) groups of PAA.

In the 2.5–4.0 pH range, the complexes were obtained with an almost constant yield of about 77% by weight. PAA enhances the thermal stability of collagen, the best stabilizing effect occurring at pH = 4.0 and for a PAA content in the blend of about 20% by weight.

Thus, we conclude that the blending of collagen with a synthetic polymer like PAA allows us the possibility of producing bioartificial materials in which strong interactions occur between the synthetic and the biological component. The presence of these interactions allows the materials' good hydrolytic stability and avoids the necessity of crosslinking procedures that could compromise their biocompatibility. In addition, these materials would possess better physicochemical and mechanical properties with respect to bioartificial materials characterized by weak interactions. The study of the characteristics of these materials will be the object of future investigations.

REFERENCES