

Specific Interactions in Silk Fibroin–Poly(potassium acrylate) Blends

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(Received December 15, 1998)

ABSTRACT: FT-IR spectroscopy was utilized to study the specific interactions in silk fibroin (SF) and poly(potassium acrylate) (PAA-K) blends. Combining the study of model complexes, *e.g.*, mixtures of SF with KCl, KBr, and ZnCl₂, it is suggested that two kinds of specific interaction exist in the SF/PAA-K blends. One is hydrogen bonding between the C=O group of PAA-K and groups of electron acceptor in SF, and the other is the electrostatic interaction in K⁺-amide specific coordination complex. The final result is probably the coexistence of these two mechanisms of specific interaction in the SF/PAA-K blends. DSC results also show that SF and PAA-K could be mixed significantly.

KEY WORDS Silk Fibroin / Poly(potassium acrylate) / Hydrogen Bonding / Electrostatic Interaction / Blend /

Due to the excellent mechanical properties and dyeability in addition to surface luster or sheen, silk fibroin (SF) fibers from *Bombyx mori* silkworm have been utilized for thousands of years, primarily in textile industry. In the past few years, their use of the materials concerning with SF was also found in the areas of biomedical and biochemical. As a result, blends of SF with other natural and synthetic polymers, such as poly(vinyl alcohol),^{1,2} chitosan,³ sodium poly-glutamate,⁴ sodium alginate,⁵ and cellulose⁶ have attracted considerable academic and practical interests. It is anticipated that such materials will show new, desirable properties not to be expected in the conventional homopolymers. Clearly, understanding of the details of the miscibility of SF with these polymers may provide considerable insight into the design of new materials with selected functional performances. However, there have been only few detail studies dealing with the miscibility of SF with these polymers.

Quite recently we have shown a great deal of interests on acrylic polymer–silk fibroin blends. It was found in this laboratory that the silk fibroin is immiscible with polyacrylonitrile, but partially miscible with acrylonitrile/methyl acrylate copolymers because of the formation of hydrogen bonds between fibroin and copolymers.⁷ It is thus assumed that higher level of molecular mixing may exist in the blends of SF and stronger electron donors. The simplest candidate for confirming this hypothesis may be the copolymers of acrylonitrile and acrylic acid. To provide some fundamental information, poly(acrylic acid) (PAA) and its salts will be considered first. PAA and its salts are widely used as polyelectrolytes, and many investigations about the miscibility of these polymers with other macromolecules, for example, poly(ethylene glycol) (PEG),⁸ and Nylon 6⁹ can be found in the literature.

Our research showed that when one mixed dilute SF and PAA aqueous solutions together, transparent solutions, translucent solutions, hydrogels, and precipitates could be obtained in varied compositions. Further studies

suggested that the two polymers could form complexes (unpublished results in this laboratory).

In this paper, we report on the specific interactions in SF and poly(potassium acrylate) (PAA-K) blend films. It was found that SF/PAA-K blend films could be easily obtained over the entire composition range. The nature of the specific interactions in the polymer blend was probed with FT-IR spectroscopy.

EXPERIMENTAL

Materials

Degummed SF from *Bombyx mori* silkworm silk was dissolved in 9.5-mol L⁻¹ LiBr aqueous solution under constant stirring for several hours at room temperature.^{1,2} After dialysis against distilled water for 5 days, the solution was filtered and the concentration was made up to about 0.5 wt% by water.

PAA was synthesized according to the literature.¹⁰ Its molecular weight, as determined by intrinsic viscosity from the equation,¹¹ $[\eta] = 8.5 \times 10^{-4} M^{1/2}$, is about 35000. PAA-K was prepared by neutralization of 1.0 wt% PAA aqueous solution with an appropriate amount of dilute potassium hydroxide according to the method reported by Weiss *et al.*⁸ and probed by FT-IR spectroscopy. The solution was cast at room temperature and dried under vacuum at 50°C for a week.

Chosen volumes of SF solution were added dropwise into the required amount of 0.5 wt% PAA-K aqueous solution. The resultant solutions were carefully stirred at room temperature for at least 4 h. Films of the SF/PAA-K blends were cast from the above solution on a glass plate at room temperature for a few days and finally dried to constant mass under vacuum at 50°C for a week. The blend films thus produced have the compositions ranging from 1/9 to 8/2 (SF/PAA-K, w/w). 5/5 (w/w) SF/salts mixtures were obtained by adding appropriate amount of salts, *e.g.*, KCl, KBr, and ZnCl₂ to the aqueous SF solutions and then cast and dried to constant mass as above. Pure SF and PAA-K films were

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also prepared under the same conditions.

Measurements

To thoroughly remove absorbed moisture (PAA-K rich samples absorb moisture easily and unify their thermal history, the samples were annealed at 160°C under nitrogen for 10 min and quenched in liquid nitrogen just prior to all measurements.

FT-IR spectra of the films were recorded on a MAGNA IR-550 (Nicolet) spectrometer by the method of transmission. SF/PAA-K physical mixtures were obtained by mixing chosen amount of pure SF and PAA-K films together, and their FT-IR spectra were examined in KBr discs. For the purpose to obey the Beer-Lambert law, the films and KBr discs were made thin enough. For each spectrum, 32 scans were taken at a resolution of 4 cm⁻¹. All spectra presented here were expanded to full scale.

DSC measurement was performed on a SETARAM DSC 92 differential scanning calorimeter. A heating rate of 10°C min⁻¹ was employed using a sample of approximately 10–12 mg. The glass transition temperature, T_g , was defined as the midpoint of the transition in the heat capacity curve.

RESULTS AND DISCUSSION

FT-IR Study

Unlike SF/PAA blend solutions, which can form precipitates (unpublished results in this laboratory), all the SF/PAA-K blend solutions were optically clear to naked eye. Even after the blend solutions were allowed to stand for more than two weeks at room temperature, neither separation nor precipitation could be observed (it is interesting to note that pure SF aqueous solution on standing will form precipitate or gel in a few days).

In this paper, FT-IR was mainly employed to examine the possible specific interactions between the blend components. Figure 1 shows the spectra of SF (A), PAA-K (D), PAA (E), 4/6 SF and PAA-K physical mixture (B), and 4/6 blend (C). It can be seen that SF shows bands at 3291, 1655 (amide I), and 1544 cm⁻¹ (amide II), indicating random coil or silk I form.¹² PAA displays a typical band at 1719 cm⁻¹ (characteristic stretching, COO⁻),¹³ while in the spectrum of PAA-K, this band is centered at 1568 cm⁻¹. The 4/6 physical mixture of SF and PAA-K shows typical bands of the two polymers (the 1562 cm⁻¹ band might be the addition of the 1544 cm⁻¹ band of SF and the 1568 cm⁻¹ band of PAA-K). However, the 4/6 blend shows bands at 3304, 1647, 1585, and 1554 cm⁻¹, suggesting strong intermolecular interactions between the two polymers.

To view more details of the specific interactions in the blends, Figure 2 shows the 2950–3700 cm⁻¹ FT-IR spectra region for SF and its blends. The major band centered at 3291 cm⁻¹ of SF is the hydrogen-bonded N–H stretching vibration.¹⁴ Upon blending with PAA-K, this band is greatly broadened and gradually shifts to higher frequency. This broadening of the N–H stretching band at the high frequency side is due primarily to the breaking of hydrogen bonds and the increase of the “free” (unhydrogen-bonded) amide concentration of SF by the addition of PAA-K.¹⁵ According to the DSC

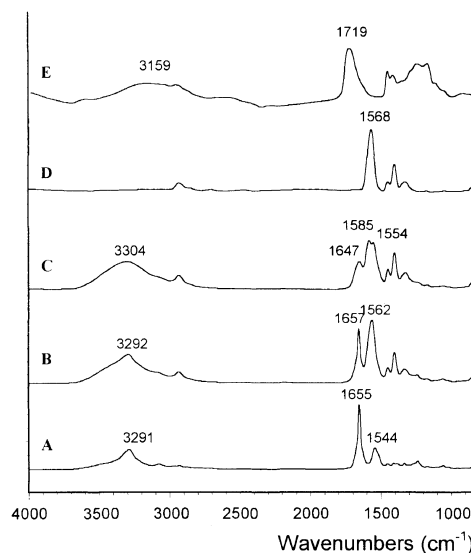


Figure 1. FT-IR spectra of (A) SF, (B) 4/6 SF and PAA-K physical mixture, (C) 4/6 SF and PAA-K blend, (D) PAA-K, and (E) PAA (see the text).

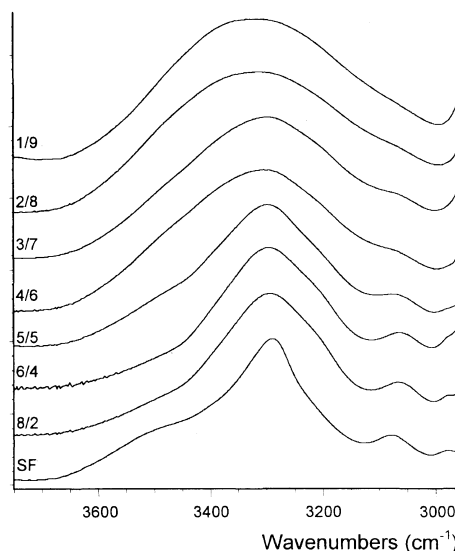


Figure 2. FT-IR spectra in the N–H stretching region for SF and the SF/PAA-K blends.

results shown below, the endothermic peaks of the SF-rich blend samples are broadened and appear at lower temperature than that of SF, indicating the hydrogen bonds in SF are weakened on blending with PAA-K, consistent with the FT-IR results. Besides, this broadening of the N–H stretching band suggests a broad distribution of local environment of the amide groups in the blends, including an appreciable concentration of “free” amide groups.

Difference spectra in the amide I region of SF are given in Figure 3 for the SF/PAA-K blends. The difference spectra were obtained by subtracting the spectrum of pure PAA-K from the spectra of the blends,¹⁵ and they represent the sum of the SF contributions to the blends as well as the changes due to interactions between the two components. The amide I band is primarily attributed to the C=O stretching vibration of the amide group of SF.¹⁶ Plain SF shows a broad band at 1655 cm⁻¹, attributed to random coil and/or silk I form.¹² In

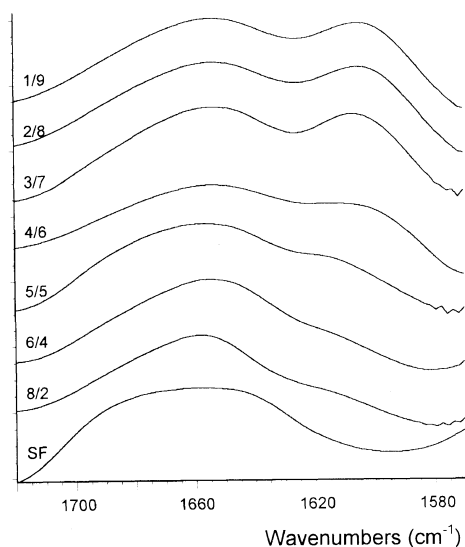


Figure 3. FT-IR spectra in the amide I region for SF and the SF/PAA-K blends. The spectra of the blends are difference spectra (see the text).

the blends, however, a new band centered at 1616 cm^{-1} appears, whose intensity increases with the increase of the PAA-K content in the blends, at the expense of the intensity of 1665 cm^{-1} band. This new band may be the result of specific interactions between the two polymers and these specific interactions may be stronger than the self-hydrogen bonding of the amide groups in SF. On the other hand, because of the relative size of the two repeat units and the restriction of conformations, not each amide group of SF would be expected to be involved in the interactions, and thus a doublet band could be detected in the IR study. It should be noted, however, in a previous paper,⁷ we proposed a "coexistence idea." That is to say, there be many groups of SF can form specific interactions with other polymers, and the restriction of conformations of SF may also play an important role in the formation of the specific interactions. Consequently, results from FT-IR spectra may be the coexistence of many kinds of possible specific interaction. It is assumed that the 1616 cm^{-1} band detected in present study might also be the result of "coexistence". We believe that the "coexistence" structure hypothesis is reasonable since the structure of SF is still somewhat in confusion. Because silk I/random coil and silk II conformations are dimorphic structure of SF and are known to easily interconvert depending on the conditions. Thus, they usually contain some extent of other form(s) (silk I/random coil or silk II) as minor component(s).¹²

Evidence for the specific interactions between the two polymers was also obtained from the IR regions characteristic of the amide II infrared vibrations. Figure 4 shows the difference spectra of the blends in the amide II region, *i.e.*, from 1480 to 1580 cm^{-1} , which were also obtained by subtracting the PAA-K spectrum from the spectra of the blends. The amide II band is a coupled mode vibration involving N-H stretching and $\text{C}\equiv\text{N}$ stretching. For SF, the amide II band appears at 1544 cm^{-1} . The spectra changes in the blends are somewhat in disorder: in the 8/2 blend, this band is centered at

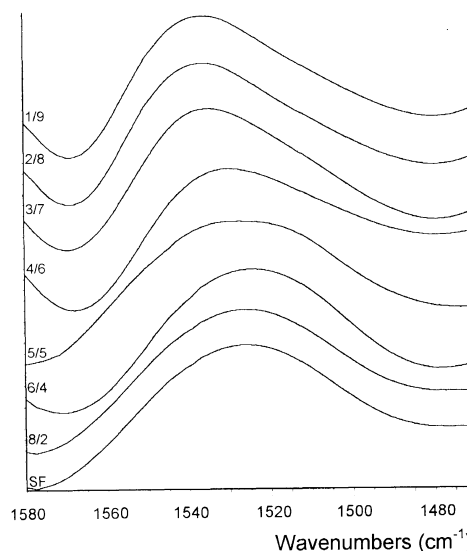


Figure 4. FT-IR spectra in the amide II region for SF and SF/PAA-K blends. The spectra of the blends are difference spectra (see the text).

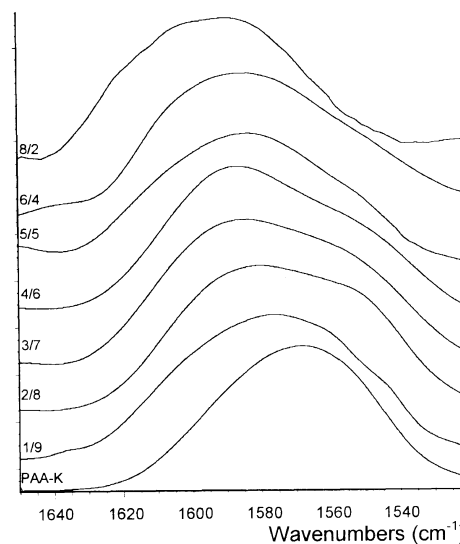


Figure 5. FT-IR spectra in the carboxyl region for PAA-K and the SF/PAA-K blends. The spectra of the blends are difference spectra (see the text).

1525 cm^{-1} , whereby, with the increase of the PAA-K amount in the blends, it gradually shifts to higher frequency. In the 1/9 blend, it appears at 1538 cm^{-1} . As this band is the combination of NH and CN stretching, this spectral change could be the result of the formation of specific interactions as well as the changes in the self-hydrogen bonding of SF.

To obtain further information about the specific interactions between the two polymers, we also examined the difference spectra of the blends in the carboxyl group vibration region of PAA-K. These difference spectra were obtained by subtracting the spectrum of SF from the blends. It can be seen from Figure 5 that PAA-K shows the carbonyl stretching vibration at 1568 cm^{-1} . On mixing with SF, the 1568 cm^{-1} band is broadened and shifts to higher frequency. In the 8/2 sample, this band appears at as high as 1587 cm^{-1} . Besides, some weak shoulders at the lower frequency side can be observed.

The broadening of the carbonyl group band at the

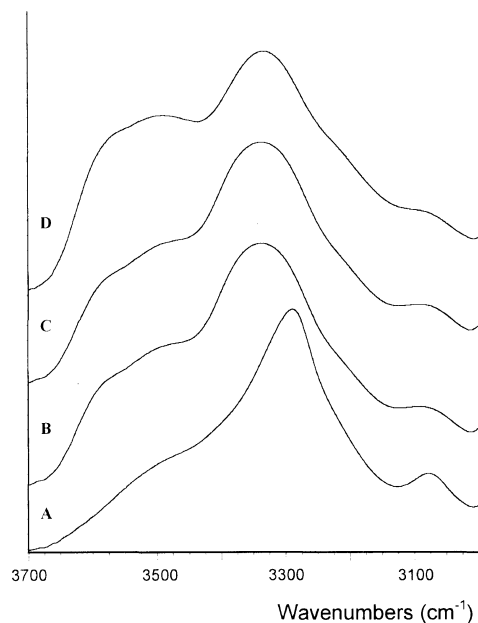


Figure 6. FT-IR spectra in the N-H stretching region for SF (A) and 5/5 (w/w) mixtures of SF with: (B) KCl, (C) KBr, and (D) ZnCl₂.

high frequency indicates that the major specific interactions between PAA-K and SF, *e.g.*, hydrogen-bonding etc. may be weaker than the self-interactions of PAA-K. Then, a question arises: if the specific interactions between SF and PAA-K are weaker than the self-associated PAA-K, why do these interactions occur? We believed it might be quite similar to the system of polyester/phenoxy, which has been pointed out by Coleman and Painter.¹⁷ It is reasonable to assert that the SF “gains” through association with the PAA-K relative to self-association (note particularly the 1616 cm⁻¹ band of SF in Figure 3). Conversely, the PAA-K “loses” through association with the SF compared to its original self-associated state. So long as the overall free energy of mixing is less than zero, the mixing and the formation of specific interactions are favored.¹⁷ Thus, two polymers could be mixed significantly.

The shoulders on the low frequency may be caused by (1) hydrogen bonds between SF and PAA-K. As mentioned above, there are many kinds of groups of SF, such as N-H, N-H₂, O-H, and COOH, etc. can form hydrogen bonds with PAA-K, and one cannot expect that the strengths of these possible hydrogen bonds are the same, and (2) K⁺-amide complex between the two polymers.

These findings indicate that significant specific interactions occurred in the blends. Specifically, in these blends there may mainly be two kinds of possible interaction between the two kinds of polymer: an interaction involving the C=O bond of PAA-K with N-H or O-H groups of SF, or the K⁺ of PAA-K with the nitrogen or oxygen atoms of SF. The former is believed to be a type of hydrogen bonding while the latter is electrostatic interaction.^{15,18-19} Because all these specific interactions will significantly perturb the free energy of mixing and conceivably influence the dispersion of the two polymers in the blends, the two kinds of specific interaction may not be contradictory in the final results of the mixing of the two polymers.

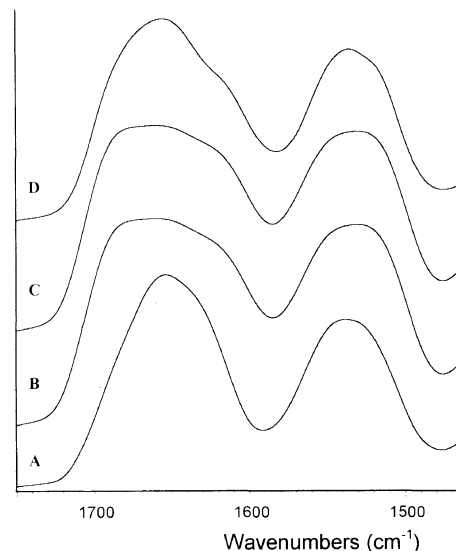


Figure 7. FT-IR spectra in the amide I and amide II region for SF (A), and 5/5 (w/w) mixtures of SF with: (B) KCl, (C) KBr, and (D) ZnCl₂.

However, we might be remiss if we do not at least make a qualitative discussion about the two mechanisms. Figure 6 compares the FT-IR spectra of SF and the mixtures formed from KCl, KBr, and ZnCl₂ with SF in the region of 3000–3700 cm⁻¹. The most striking feature of these spectra is that similar to the SF/PAA-K blend system, the N-H stretching vibration band of SF is broadened and shifts to higher frequency upon mixing with salts. Further, several shoulders at high frequency side of the N-H major band can also be detected in the SF/salt mixtures. It can be seen that the intensity of the shoulders only relates to the kinds of cation and those for ZnCl₂/SF mixtures are stronger than those for KCl or KBr/SF mixtures. This indicates that the specific interactions between Zn²⁺ and SF are stronger than those in K⁺-SF complexes. Another worthy noting about these spectra is that the band of SF centered at 3078 cm⁻¹ shifts to higher frequency in the mixtures. The 3078 cm⁻¹ band of SF can be ascribed to the amide II overtone band,¹⁸ and the shift to higher frequency of this band is the reasonable consequence of the formation of complex between the amide nitrogen or oxygen atoms of SF with the Zn²⁺ or K⁺ ions. All these findings suggest that the electrostatic interaction between PAA-K and SF may play an important role in the blends.

We also examined the IR spectra of the SF/salt mixtures in amide regions, as shown in Figure 7. Recall that in the SF/PAA-K blends, the carbonyl group stretching vibration of SF (amide I) shows a new band centered at 1616 cm⁻¹, whose intensity increases with the increase of the PAA-K content in the blends (Figure 3). In the SF/salt mixtures, however, the 1655 cm⁻¹ band of SF is broadened and shifts to higher frequency. Although some shoulders at lower frequency can also be observed, these shoulders are much weaker than that of the 1616 cm⁻¹ band in SF/PAA-K blends (note particularly the difference between Figures 3 and 7). It is thus reasonably concluded that the 1616 cm⁻¹ band observed in Figure 3 might be mainly caused by the specific interactions involving the C=O group of PAA-K

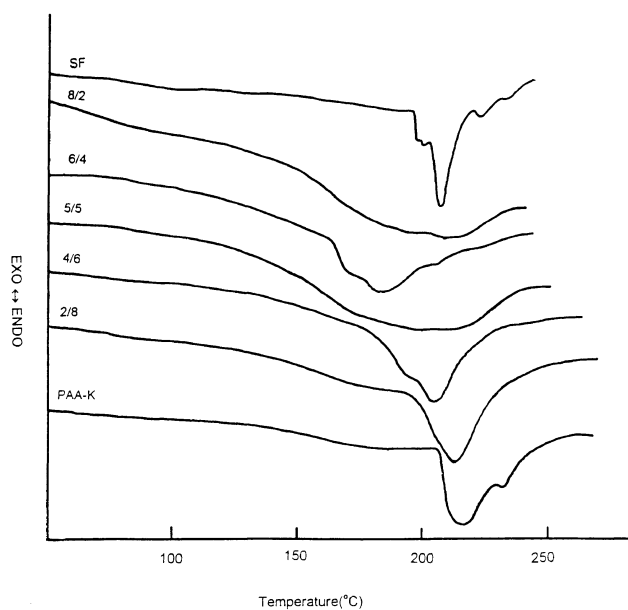


Figure 8. DSC heating thermograms of the homopolymers and the SF/PAA-K blends quenched from 160°C.

and the amide group (and/or other electron acceptors) of SF. In other words, there can exist two kinds of specific interactions in the SF/PAA-K blends, *i.e.*, hydrogen bonding and electrostatic interaction, and the final results observed in FT-IR study of the blends are the “addition” of these specific interactions. As to the amide II band of SF, broadness and slight shift can also be detected in the mixtures, which may also be the result of the electrostatic interaction.

DSC Study

It should be pointed out that all the above FT-IR results certainly suggest the significant molecular mixing of the two components, but not necessarily a single phase. Figure 8 shows the DSC thermograms of the homopolymers and their blends. The samples were annealed at 160°C for 10 min and quenched in liquid nitrogen prior to DSC scan. The T_g of PAA-K is estimated to be 150°C here. It has been reported that the T_g of pure PAA is 103°C¹⁰ or 106°C.²⁰ Clearly, the high T_g of PAA-K is caused by the strong electrostatic interaction between COO⁻ and K⁺. The characteristic endothermic peak of PAA is estimated to be 220°C.²¹ The T_g of pure SF is at about 170°C, which is the typical transition of SF in the random coil conformation.²² The attribution of the sharp endothermic peak at 203°C has not been defined yet, however, it could not be the decomposition of SF, as demonstrated under a thermogravimetric instrument (NETZSCH TG 209) confirming the results of Magoshi *et al.*²² and Tsukada *et al.*²³ Note that those polymers with self-association, such as PAA,²¹ poly(vinyl alcohol) (PVA)²⁴ and tussah silk²³ etc. also appeared the endothermic peak around 200°C on their DSC curves, we might assign this peak to the de-association of the specific interactions between the molecular chains of SF during the process of heating.

As to the blends, due to the disturbance of the endothermic peaks, it is difficult to identify their T_g s on the DSC curves. It should be pointed out that even if the

T_g s of the blends could be detected, a single composition-dependent T_g could not be the criterion of miscibility of the polymers, either. This is because that the T_g s of SF and PAA-K are very close, they separate, by *ca.* 20°C. As a result, the T_g of the blends detected by DSC would involve a high degree of uncertainty.²⁵ Nevertheless, we can get some useful information from these DSC curves. The main endothermic peak of PAA-K is centered at about 220°C, however, upon blending with SF, this peak is broadened and gradually shifts to lower temperature with the increase of SF content. It suggests that the endothermic peak of the blends is not a simple result of overlapping of the two peaks, *i.e.*, the endothermic peaks of PAA-K and SF. In the 6/4 sample, the endothermic peak is at around 180°C, about 40°C and 20°C lower than that of PAA-K and SF, respectively. As to the 8/2 sample, the endothermic peak is very broad, indicating a wider distribution of inter and intramolecular interactions in the blend. All these observations suggest a high level of molecular mixing in the blends, but whether this “endothermic peak shift phenomenon” is a criterion is still unknown. Last but not least, if the attribution of the endothermic peak of blends (and SF or PAA-K) is linked to the de-association of the specific interactions in the materials, this phenomenon might provide the evidence to confirm the previously result, *i.e.*, the specific interactions between SF and PAA-K are relatively weaker than those of in themselves.

CONCLUSION

FT-IR spectroscopy was utilized to study the specific interactions in SF/PAA-K blends. Strong specific interactions between the two polymers were confirmed. From the study of SF/salt mixtures, it is believed that two kinds of specific interaction may exist in the blends. One is the hydrogen bonding between the C=O group of PAA-K and the groups of electron acceptor in SF. The other is the electrostatic interaction between K⁺ and amide group. The final result probably is the coexistence of these two mechanisms of specific interactions. DSC results also suggest a high level of molecular mixing in the blends, but not necessarily a single phase.

It should be pointed out that there are at least two suggestions from the results of this investigation. (i), ionomers (or copolymers containing ionomers) may be suitable candidates to form miscible blends with SF. It is widely accepted that it is difficult to obtain miscible blends of SF with other polymers. However, it is shown in this study that metal cations may form specific coordination complexes with SF. As a result, one may control the miscibility between SF and other polymers by many methods, such as using copolymers with ionic polymers, judicious choice of the metal cations (note that the strength of metal ions–amide complexes is different from cation to cation, as shown in Figure 7), etc. (ii) the most common solvents for SF are concentrated aqueous solutions of inorganic salts, such as LiBr, NaSCN, ZnCl₂, CaCl₂, and [Cu(NH₃)₄](OH)₂, etc. It is thought that aqueous solutions of SF can be obtained by dialysis of the concentrated salt solutions against distilled water.^{2–6} However, we have to take great care in the process of dialysis, because any remain of salts may have great

influence on the final result for such study due to the formation of the complexes.

Acknowledgments. The authors thank for the financial support by the National Science Foundation of China.

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