Fluorescence and viscometry study of complexation of poly(acrylic acid) with poly(acrylamide) and hydrolysed poly(acrylamide)

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Abstract: Interpolymer complexation of poly(acrylic acid) with poly(acrylamide) and hydrolysed poly(acrylamide) was studied by fluorescence spectroscopy and viscometry in dilute aqueous solutions. Changes in chain conformation and flexibility due to the interpolymer association are reflected in the intramolecular excimer fluorescence of pyrene groups covalently attached to the polymer chain. Both poly(acrylamide) and hydrolysed poly(acrylamide) form stable complexes with poly(acrylic acid) at low pH. The molecular weight of poly(acrylic acid) and solution properties such as pH and ionic strength were found to influence the stability and the structure of the complexes. In addition, the polymer solutions mixing time showed an effect on the mean stoichiometry of the complex. The intrinsic viscosity of the solutions of mixed polymers at low pH suggested a compact polymer structure for the complex.

Key words: Interpolymer complexation; excimer fluorescence; cooperative binding; equilibration time; mean stoichiometry.

Introduction

Macromolecules in solutions often interact with each other, resulting in excluded volumes and ordered structures through the formation of inter-macromolecular complexes. It is well known that intermacromolecular complexes play an important role in many biological functions [1, 2]. An investigation of the mechanism and the factors affecting complexation using well-characterized synthetic polymers is of importance as these systems serve as models for complex biopolymers. In solutions containing mixtures of two chemically different macromolecules, the interactive forces responsible for complexation are usually secondary binding forces classified as electrostatic, hydrogen bonding, and hydrophobic. The structure, as well as the stability of the complexes, depends on the type of interac-

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tions, the solution conditions, and macromolecular characteristics such as pH, ionic strength, temperature, solvent, molecular weight, and conformation. For example, in systems containing polyelectrolytes the stability of the complexes depends on the pH of the medium since the interactions of the binding sites with the solvent and the conformation of the polyelectrolytes are affected greatly by pH.

Several studies have been reported [1–5] on interpolymer complexations between hydrogen bond donor and hydrogen bond acceptor polymers. Fluorescent methods [6–10] based on the photophysical and photochemical properties of covalently bonded fluorescent probes to the polymers have been found to be both sensitive and informative for investigation of interpolymer complexations. In mixed polymer systems, information on the behavior of polymer chains on a molecular level can be obtained using fluorescence methods, while the conventional methods (e.g., viscometry) provide information only on average properties representing the whole sys-

tem. In our own earlier investigations [11, 12] we had demonstrated the use of pyrene-labeled polymers to study polymer-surfactant interactions and interpolymer complexations. Oyama et al. also studied interpolymer complex formation by the reduction of excimer emission in pyrene-labeled polymers [13]. Complexation of poly(acrylic acid) with poly(acrylamide) and poly(acrylic acid-coacrylamide) using pyrene-labeled polymers is reported in this paper. Effects of complexation conditions such as pH, ionic strength, and the polymer molecular weight on the complexes are discussed. The extent of excimer formation is used as a parameter which measures the change in conformation and flexibility of the polymer chain.

Experimental

Materials

Poly(acrylamide) (PAAm), pyrene-labeled poly(acrylamide) (PyPAAm), and hydrolysed poly(acrylamide) (PyHPAAm) were prepared at the National Chemical Laboratory, Pune, India as part of an Indo-US joint research program. 2-[4-(1-pyrene)butanoyl]aminopropenoic acid used as the label was synthesized as described elsewhere [11]. Acrylamide and 2-[4-(1-pyrene)butanoyl] aminopropenoic acid were copolymerized by precipitation polymerization in DMF at 65 °C using azobis(isobutyronitrile) as initiator. Pyrene-labeled hydrolysed poly(acrylamide) was obtained by controlled hydrolysis [14] of labeled poly(acrylamide). Molecular weights and the pyrene content estimated from UV absorption are listed in Table 1 along with the manufacturer specified molecular weights of poly(acrylic acid) (PAA) samples purchased from Polysciences. Molecular weights of the unlabeled poly(acrylamide) and commercial samples are designated using a numerical index and the actual molecular weight is 1000 times this number.

Table 1. Molecular weight, pyrene content, and source of polymer samples used in the study

Polymer sample	Molecular weight, M_w	Pyrene content, weight %[c]	Source
PyPAAm	$7.3 \times 10^{4[b]}$	3.7	NCL[d]
PyHPAAm[a]	$6.8 \times 10^{4[b]}$	3.8	NCL[d]
PAAm-1000	$1.0 \times 10^{6[b]}$	—	NCL[d]
PAA-5	5.0×10^3	—	Polysciences
PAA-90	9.0×10^4	—	Polysciences
PAA-800	8.0×10^5	—	Polysciences
PAA-3000	3.0×10^6	—	Polysciences

[a]) extent of hydrolysis, 14.1 mole% by potentiometric titration; [b]) viscosity average molecular weight calculated from [13]; [c]) by UV absorption method, λ_{max} 342 nm [11], 4-(1-pyrene)butanoic acid as standard; [d]) National Chemical Laboratory, Pune, India

Polymer solutions were prepared in triple distilled water 24 h before the experiments to ensure complete dissolution of the polymers. The mixing of solutions and the adjustments of pH and ionic strength were carried out just before measurements of photoemission of the label.

Fluorescence measurements

The steady-state fluorescence emission spectra of the pyrene label were recorded on a SPEX Fluorolog Spectrofluorometer using excitation at 333 nm. The ratio of excimer (480–490 nm) to monomer (376 nm) intensities, I_e/I_m, was determined for various solution conditions. The concentration of the labeled polymer in these measurements was kept at low levels (5×10^{-4} monomer mol/L) to avoid intermolecular excimer formation. All the measurements were carried out at room temperature (25 ± 2 °C).

Viscosity measurements

Viscosity measurements of polymer solutions were carried out using an Ubbelohde suspension-type capillary viscometer at 30 ± 0.05 °C. The intrinsic viscosity $[\eta]$ and the Huggins constant K' were determined from Huggins equation. Shear rate corrections were not applied to these data.

Results and discussions

Poly(acrylamide) and partially hydrolysed poly(acrylamide) behave as random coils in dilute solutions in the presence of simple electrolytes. The randomly distributed pendant pyrene groups at low concentrations on the polymer chain did not affect its dissolution characteristics significantly. Pyrene groups are constantly in motion due to the high mobility of the polymer segments to which they are covalently attached. An excited pyrene group interacts with a ground state pyrene group to form an excimer when they approach each other within about 4–5 Å. The distance between the pyrene groups on the polymer chain is determined by its conformation and the mobility of the segments. Thus, the extent of intramolecular excimer formation (given by the parameter I_e/I_m) provides a measure of the statistical conformation of the labeled polymer chain. A large value of I_e/I_m suggests polymer chain contraction and/or segmental mobility, whereas a small value of I_e/I_m suggests polymer expansion and rigidity. The interaction of the functional groups is depicted in Fig. 1. Complexation increases the rigidity of the polymer coil and effectively separates the pyrene labels attached to it.

Figure 2 shows the plots of I_e/I_m ratios of pyrene-labeled poly(acrylamide) and hydrolysed poly(acrylamide) in the presence and the absence of
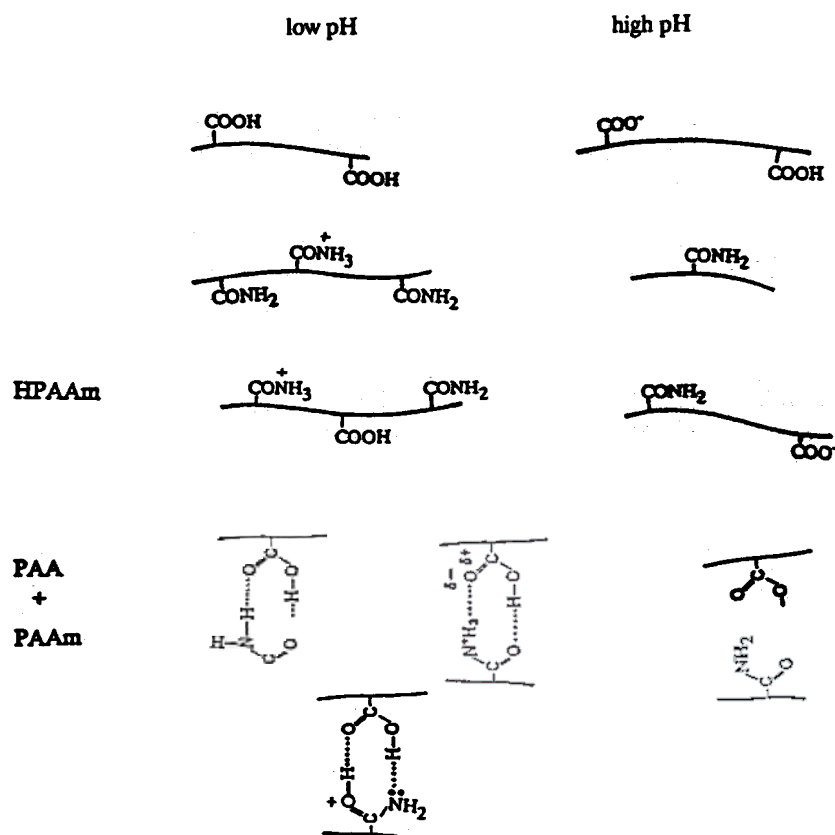
low pH   high pH

Fig. 1. The interacting functional groups of the polymers at low and high pH
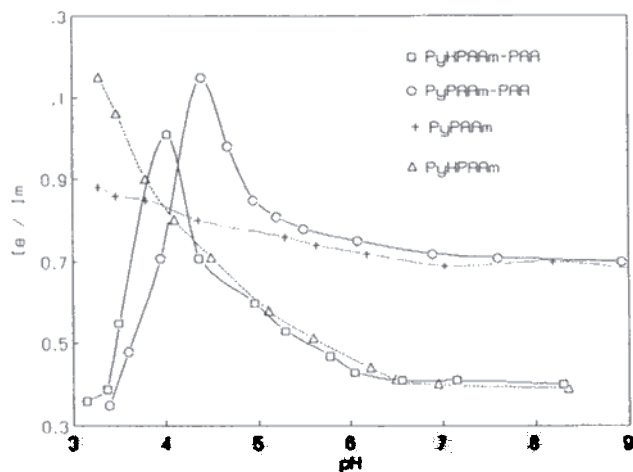

Fig. 2. Change in intramolecular excimer formation as a function of pH for pyrene-labeled poly(acrylamide) (PyPAAm) and pyrene-labeled hydrolysed poly(acrylamide) (PyHPAAm) in the presence and the absence of poly(acrylic acid) (PAA-90). (Concentrations of polymer: PyPAAm and PyHPAAm, 4×10^{-4} base mol/L; PAA-90, 4×10^{-3} base mol/L)

poly(acrylic acid) as a function of pH in 0.05 M sodium chloride solutions. Poly(acrylamide) being a nonionic polymer, pH variation is not expected to affect its coil size. In the low pH region, the slight increase in I_e/I_m with decreasing pH for PyPAAm may be due to a reduction in coil size that results from pH effects on polymer-solvent interactions or to an increase in ground state pyrene interactions. In the presence of poly(acrylic acid), I_e/I_m ratios remain unchanged at higher pH. As the pH is decreased below 5.0, the ratio increases sharply to reach a maximum at pH 4.3 and then decreases until phase separation is observed at pH 3.4. The analysis of the precipitate confirmed that PyPAAm and PAA formed an equimolar complex at the phase separation pH. The hydrolysed poly(acrylamide) (copolymer of acrylamide and acrylic acid) in solution contracts to a more coiled conformation as the pH is decreased. This is due to the decrease in the charge density on the polymer chain as a result of the reduction in number of ionized acid groups with pH. In the case of PyHPAAm–PAA mixture, the values fol-

low the same trend as that of PyHPAAm solution down to pH 4.2, below which a sharp increase and then a decrease similar to PyPAAm/PAA is observed.

The changes in I_e/I_m in the presence of poly(acrylic acid) can be attributed to the following changes in polymer conformation. At high pH conditions, interactions between the polymer chains are negligible. The number of undissociated acid groups available for complex formation is low and the charged carboxylate groups present on the polymer chain destabilize the complex through coulombic repulsions. As the pH is decreased the poly(acrylic acid) coil contracts and the unionized acid groups begin to interact with the amide groups of poly(-acrylamide) forcing its chain to adopt, along with poly(acrylic acid) chain, a more coiled conformation. The increase in I_e/I_m ratio below pH 5 is attributed to this initial coiling of PyPAAm . Further decrease in pH increases the contacts between the polymer chains producing a compact structure. Due to the decreased intramolecular mobility of the polymer molecule in this compact structure of the complex, the motion of the polymer segments and pyrene probes attached to them is restricted [10] and a reduction in excimer formation results.

Poly(acrylamide) (PAAm) has been reported [2,4,15] to form a complex with poly(methacrylic acid) (PMAA). The complex formation is through hydrogen bonds as well as ion-dipole interactions between some of the amide groups of poly(acrylamide) which are partially protonated, and $C = O$ dipoles of the carboxyl groups of poly(methacrylic acid) [2,15]. The hypercoiled structure of poly(methacrylic acid) also contributes to the stability of the complex. Recently, Wang and Morawetz [16] reported complexation of poly(acrylic acid) with poly(-N,N-dimethylacrylamide-co-acrylamide) based on the fluorescence properties of dansyl groups attached to poly(acrylic acid). They observed that copolymers with more than 45 mole% acrylamide did not form a complex with poly(acrylic acid) at pH 4, even though at pH 3 acrylamide contributed significantly to the stability. They assumed the acrylamide group was a much weaker hydrogen bond acceptor than the dimethylacrylamide group and the stability of the complex was entirely due to the latter. Since the probe used in this study reports only the hydrophobicity of the complex, the complex formed with the copolymer of higher acrylamide content is less hydrophobic and the emission char-

acteristics of the dansyl label may be only slightly affected by these complexes. Our results show that even a copolymer of acrylamide and acrylic acid interacts with poly(acrylic acid) to form a stable complex at pH below 4. Oyama and coworkers [17] have also reported pyrene label to be more sensitive to interpolymer complexation than dansyl label.

Figure 3 describes the effect of ionic strength on complexation between poly(acrylamide) and poly(acryl acid). As the ionic strength is increased the pH at which the interpolymer interaction begins decreases. Ionic strength affects both the dissociation of polyacid and the ion-dipole interactions which favor the complex formation. Reported pK_0 values [18] for poly(acrylic acid) in NaCl solutions and in water show that poly(acrylic acid) is more dissociated in NaCl solutions. Ion-dipole interactions are due to protonated amide groups of poly(-acrylamide) and the dissolved electrolytes are expected to screen these interactions. In the absence of the ion-dipole interactions more hydrogen bond linkages between the polymer chains are necessary for stabilizing the complexes. This can be achieved only by a decrease in the pH to increase the unionized acid groups on the polymer chain. Thus, the increase in ionic strength systematically shifted the onset of interpolymer association to lower pH values.

The molecular weight dependence of PAAm-PAA interactions is shown in Fig. 4. Fluorescence behavior of the PyPAAm in the presence of poly(acryl-
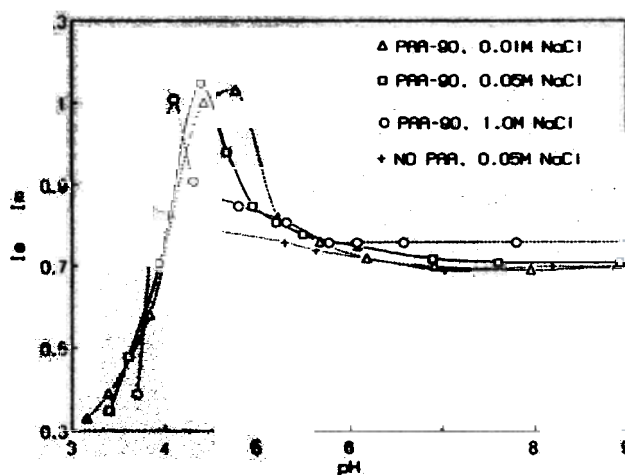

Fig. 3. Effect of ionic strength on pH dependence of intramolecular excimer formation. (Concentration of polymers: PyPAAm, 4×10^{-4} base mol/L; PAA, 4×10^{-3} base mol/L)
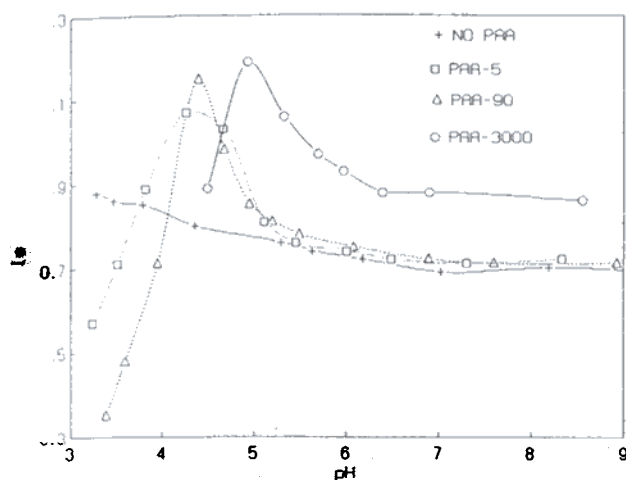
Fig. 4. Effect of molecular weight on intramolecular excimer formation. (Concentration of polymers: PyPAAm, 4×10^{-4} base mol/L; PAA, 4×10^{-3} base mol/L)

The effect of concentration of poly(acrylic acid) on PAAm/PAA complex structure is shown in Fig. 5 at three pH values. The I_e/I_m ratio of PyPAAm/PAA mixture relative to pure PyPAAm solution under identical pH and concentration, $(I_e/I_m)_0$, is plotted in the figure as a function of the concentration ratio of the mixture which is varied by increasing the poly(acrylic acid) concentration. At pH 7, the ratio is constant as the ionized polymer chains do not interact with each other. At pH 5, the $(I_e/I_m)/(I_e/I_m)_0$ ratio increases initially, then decreases as the concentration of poly(acrylic acid) in the mixture is increased. A sharper decrease in the excimer fluorescence ratio is observed at pH 3.5 at low concentration ratios.

ic acid) of different molecular weights is plotted. In the case of lower molecular weight poly(acrylic acids), the interpolymer interactions begin almost at the same pH, even though the structures of their complexes appear to be different. As evident from the I_e/I_m ratios at low pH, the complex formed with PAA-90 is more rigid (less excimer formation) than that of PAA-5. Moreover, the phase separation of PAAm/PAA-5 complex occurs at a lower pH (pH 3.2) than that of PAAm/PAA-90 complex (pH 3.4). For the highest molecular weight poly(acrylic acid) (PAA-3000), the interactions begin at pH 6.0 and the complex precipitates at pH 4.5. The relatively high I_e/I_m ratio above pH 6.0, where complex formation is not expected, is possibly due to the contraction of poly(acrylamide) coil in the presence of large interpenetrating poly(acrylic acid) chains. The hydrogen bonding forces and ion-dipole interactions are of low energies and the stability of complexes involving such interactions is believed to be due to the cooperative binding [1] between the complementary polymer molecules. Also, we observed that both acetic acid and succinic acid did not affect the I_e/I_m ratios of PyPAAm or induce any phase separation, even at very low pH values. Hence, cooperative interactions between many amide and carboxyl groups are essential for complex formation and the polymer chain length plays an important role in determining the stability and structure of the resulting complex.
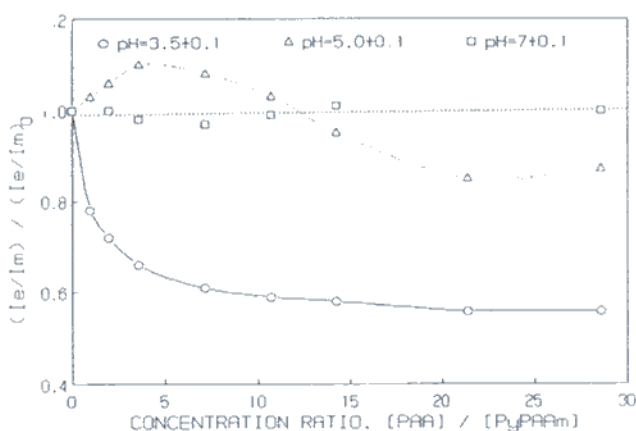

Fig. 5. Effect of poly(acrylic acid) (PAA) concentration on fluorescence intensity ratio at three pH conditions without equilibration. (Concentration of PyPAAm, 5×10^{-4} base mol/L)
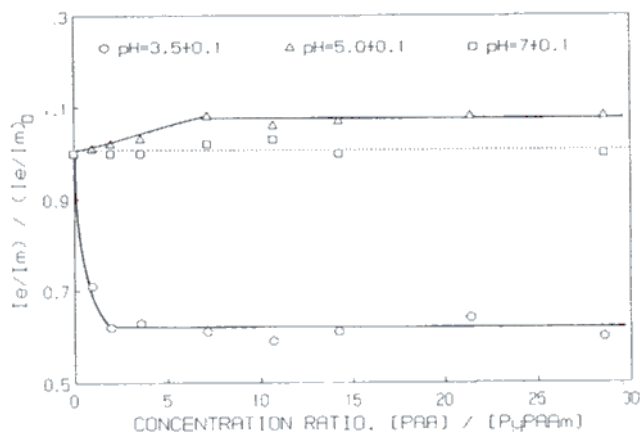

Fig. 6. The fluorescence intensity ratio after equilibration for 1 week. (The solutions and experimental conditions are the same as in Fig. 4)

It has been reported [1, 2] that when two polymers interact to form a stable complex, often it occurs with a definite composition of the interacting molecules. In the case of poly(ethylene oxide)/PAA system, a stable complex with a stoichiometric ratio of 1:1 is formed, irrespective of the polymer composition of the mixture in which it is formed. Our results indicate that the composition of the complex can very well depend on pH and equilibration time. The $(I_e/I_m)/(I_e/I_m)_0$ ratio, a measure of the segmental mobility of the polymer chain varies with poly(acrylic acid) concentration and becomes constant only at very high concentration of poly(acrylic acid). It appears that the time duration between the preparation of the mixture and the fluorescence intensity measurements is not adequate for the system to reach equilibrium and to rearrange the interpolymer contacts sufficiently to minimize the number of chains involved in the complexation. Hence, fluorescence intensities were measured for the polymer solutions equilibrated for 1 week and the result is presented in Fig. 6. It can be seen that the fluorescence intensity ratio remains unchanged at pH 7. The $(I_e/I_m)/(I_e/I_m)_0$ ratio becomes constant at pH 3.5 and 5 above polymer concentration ratios 2 and 6, respectively. For mixtures of high concentration ratios the local concentration of poly(acrylic acid) molecules around the poly(acrylamide) chains is high and possibly most of these molecules participate in hydrogen bonding with poly(acrylamide) molecules to form a rather crosslinked structure. As the system is equilibrated the poly(acrylamide) molecules apparently rearrange their contacts to accommodate only a limited number of poly(acrylic acid) molecules, though the number of hydrogen bond linkages will be same in both cases. This effect is more pronounced at pH 5. We also obtained the composition of the complex separated under the precipitation conditions (pH below 3.4) as follows. The supernatant solution after centrifugation was analyzed for total organic carbon to determine the total uncomplexed polymers in the solution. The pyrene-labeled polymer was estimated from the UV absorption. The composition of the precipitated complex thus calculated was very close to equimolar. These results suggest that the complexes formed have a fixed mean stoichiometry which depends only on pH when the system is equilibrated.

In Fig. 7, the intrinsic viscosity of PAA-800/PAAm-1000 mixtures at pH 4 and 7 is plotted as a function of its composition. The phase separation of
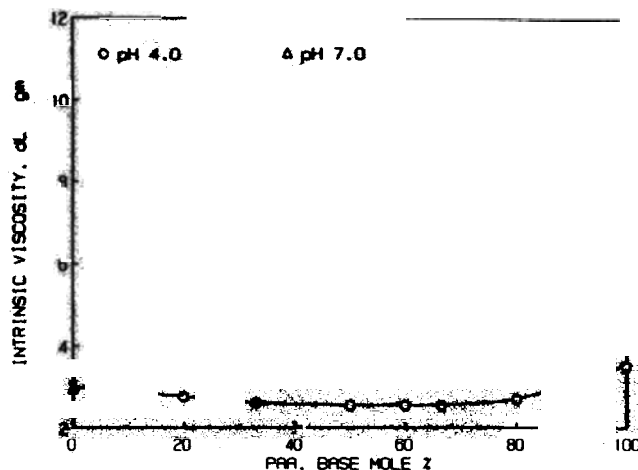

Fig. 7. Intrinsic viscosity of PAA-800/PAAm-1000 as a function of polymer composition at pH 4 and 7

this polymer couple was observed at pH 3.8. At pH 4, the intrinsic viscosity decreases with increasing concentration of poly(acrylic acid) in the mixture and reaches a minimum when the mixture is equimolar. The decreased intrinsic viscosity can be attributed to the compact structure of the polymer complex which has a smaller hydrodynamic volume compared to free molecules. The larger values of Huggins constant k' (given in Table 2) also indicate [19] strong interpolymer association. The intrinsic viscosity of the mixture at pH 7 shows a linear increase with PAA concentration. Furthermore, for a given mixture the intrinsic viscosity is equal to the sum of viscosities of the component polymer solutions as in the case of non interacting polymer mix-

Table 2. Intrinsic viscosity [η] and Huggins constant k' of PAA-800/PAAm-1000 mixture in 0.05 M NaCl solutions as a function of polymer composition

pH 4.0 PAA (base mole %)	[η] (dl/gm)	k'	pH 7.0 PAA (base mole %)	[η] (dl/gm)	k'
0	3.00	0.36	0	2.95	0.40
20	2.75	0.52	20	4.30	0.45
33.3	2.60	1.10	33.3	5.20	0.49
50	2.55	1.19	42.8	5.80	0.46
60	2.55	1.21	50	6.25	0.45
66.6	2.55	1.19	60	7.15	0.36
80	2.70	1.01	66.6	7.70	0.32
100	3.50	0.20	100	9.90	0.29

tures. Thus, the viscosity results are in agreement with the results obtained from fluorescence measurements.

Conclusion

The excimer fluorescence and the viscosity studies indicate that poly(acrylamide) forms a stable complex with poly(acrylic acid) at low pH values. The decrease in excimer fluorescence and intrinsic viscosity of the mixture of polymer solutions is attributed to the rigid compact structure of the complex. The fact that the hydrolysed poly(acrylamide) (copolymer of acrylamide and acrylic acid) also interacts with poly(acrylic acid) shows that the amide groups are strong hydrogen bond acceptors. The increase in ionic strength shifts the onset of interpolymer interaction to lower pH range as the dissolved simple electrolytes affect both the dissociation of polyacid and the interactive forces. The molecular weight dependence of the association behavior confirms the cooperative nature of the complexation. The complexes formed have fixed mean stoichiometry which depends only on pH if the system is equilibrated.

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