Colloid Polym Sci 275:1083–1087 (1997)

SHORT COMMUNICATION

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Viscosities of poly(N-vinylacetamide) solutions in the presence of salts, urea and guanidine hydrochloride

Received: 12 June 1997
Accepted: 4 August 1997

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Summary In the aqueous solutions of poly(N-vinylacetamide) in the presence of Na_2SO_4 or $(NH_4)_2SO_4$, the intrinsic viscosities were small and the Huggins constants were large compared with those in water. Similar but much less effects were observed for NaF. Opposite effects were found, however, for NaSCN. The poor solvent quality of aqueous sulfate solutions was significantly improved by the addition of guanidine hydrochloride to 3 M or higher. Urea was much less effective in improving the solvent quality. The different effects observed between urea and GdnHCl may be explained by their different binding affinities to amide group. Solubilization of PNVA flocculates induced by the presence of 0.8 M Na_2SO_4 took place by adding urea to 1 M, although only small changes were detected in the viscosity behavior accompanying the addition of 1 M urea.

Key words Poly(N-vinylacetamide) – viscosity – salting-out – urea – guanidine hydrochloride

Introduction

Recently, aqueous poly(N-vinylacetamide) solutions were found to exhibit the cloud point in the presence of salt [1]. This cloud point was shown to correspond to a liquid–liquid phase separation which was induced by the repulsive interaction between the salt and the polymer as characterized with the polymer-rich top phase and the salt-rich bottom phase. The specificity of salts in salting out the polymer resembled (except for Li_2SO_4) that in the salting-out of an oligopeptide [2] rather than that of nonpolar compounds like benzene [3]. The salting-out effect suggests that the solvent quality becomes poor on the addition of salts. To investigate the effect of the change of the solvent quality on the coil dimension of the polymer in relation to the phase separation, the viscosity of the polymer solution is examined in the present study. Protein denaturants such as urea and guanidine hydrochloride increase the solubility of amides in aqueous media. Their antagonistic action to the salting-out effect is also examined by viscometry.

Experimental

Poly(N-vinylacetamide) [PNVA; residue weight 85] was prepared by free radical polymerization of N-vinylacetamide which was obtained by the pyrolysis of N-(α-methoxyethyl acetamide) [4]. Sample C1 was the same sample as used previously (number-average molecular weights (Mn) was $(3.2 \pm 0.05) \times 10^5$) [1]. Sample D5 was a high molecular weight fraction obtained from sample D by fractionation with acetone as described previously [1]. The fractionation was carried out as follows. Acetone was successively added (in total 498 ml) to a 300 ml PNVA solution (5%) and the mixture was kept for one and half hour at 25 °C. Approximate molecular weight of D5 estimated by HPLC (the tandem columns of TSK gel G5000PW and G6000PW) at 35 °C using TSK

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standard poly(ethylene oxide) (PEO) was 7.9×10^5. Sodium phosphate buffer (0.01 M pH 7.0) was used as a mobile phase at the flow rate of 1.0 ml/min.

All the chemicals were of reagent grade and used without further purification. The chemicals used were sodium sulfate, ammonium sulfate, sodium thiocyanate, cesium chloride, sodium fluoride, urea and guanidine hydrochloride. Doubly distilled water was always used in the present study.

Viscosity was measured with Ubbelohde capillary viscometers with water flow times of 174 and 185 s at 25 ± 0.01 °C. From the measurements on water and acetone, it was found that the kinetic energy correction was below 0.1% for systems studied here and could be ignored.

Results

Viscosity of PNVA solutions in the presence of salts

Reduced viscosities of aqueous PNVA(C1) solutions in the presence of ammonium sulfate of different concentrations C_s are shown in Fig. 1 against the polymer concentration c_p (g/dl). The reduced viscosity η_{sp}/c_p is linearly proportional to the polymer concentration c_p, expressed as [5]

$$\eta_{sp}/c_p = [\eta] + k_H[\eta]^2 c_p \, . \tag{1}$$

Here $[\eta]$ and k_H denote the limiting viscosity number (the intrinsic viscosity) and the Huggins constant, respectively. The intrinsic viscosity and the Huggins constant were determined according to Eq. (1). When the concentration of ammonium sulfate, C_s, increased, reduced viscosity decreased significantly.

The effect of various salts on the intrinsic viscosity $[\eta]$ of PNVA is shown in Fig. 2. For the two salts consisting of SO_4^{2-} ion, $[\eta]$ values decreased with the increasing salt concentration. It is to be noted that these salts promoted the liquid–liquid phase separation [1]. In the case of NaSCN, which is located at the opposite side to SO_4^{2-} in the Hofmeister series and scarcely induced the phase separation, the limiting viscosity number rather increases with C_s. The salts locating between SO_4^{2-} and SCN^- in the anionic lyotropic series [6, 7], such as NaF, NaCl and CsCl, showed intermediate values of the intrinsic viscosity. It is to be noted that NaF showed a significant salting-out effect in Fig. 2. Because of the solubility limit of NaF, the phase separation of the polymer solutions could not be induced by the addition of NaF in the previous study [1]. We have the following salting-out order from the viscosity:

$$SO_4^{2-} \gg F^- > Cl^- > SCN^- \text{ (Na salt)} \, . \tag{2}$$

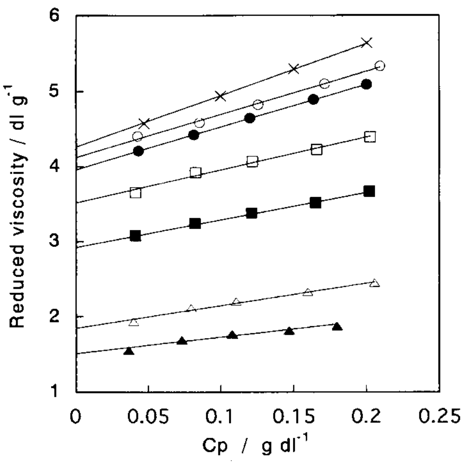

Fig. 1 Effects of $(NH_4)_2SO_4$ concentration (C_s) on the viscosity of PNVA. Sample C1. C_s (M); 0 (×); 0.1 (○); 0.2 (●); 0.5 (□); 0.75 (■); 1.0 (△); 1.1 (▲)

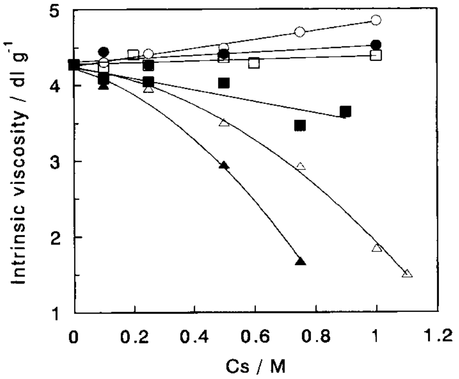

Fig. 2 Effects of various salts on the viscosity of PNVA. NaSCN (○); CsCl (●); NaCl (□); NaF (■); $(NH_4)_2SO_4$ (△); Na_2SO_4 (▲)

The Huggins constants as functions of salt concentration are shown in Fig. 3. The Huggins constant scarcely depended on C_s in most cases and their values fall within the range of 0.3–0.4 proposed for the normal cases [8, 9]. In the vicinity of the solubility limit for the two salts consisting of sulfate ion, however, the Huggins constant value increased remarkably up to about 1. The observed high k_H values suggest the presence of the polymer association in these salt solutions [10]. For thiocyanate, on the other hand, the Huggins constant tends to decrease with C_s.

A plot of the intrinsic viscosity vs. the Huggins constant of PNVA in Na_2SO_4 and $(NH_4)_2SO_4$ solutions is shown in Fig. 4. They show a definite correlation in contrast to the case of other salts. For the latter, k_H values fall in the range 0.3–0.4 independent of $[\eta]$ values varying

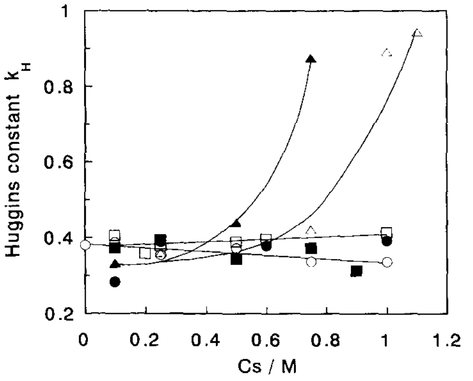

Fig. 3 Plots of Huggins constant as a function of salt concentration C_s. NaSCN (○); CsCl (●); NaCl (□); NaF (■); $(NH_4)_2SO_4$ (△); Na_2SO_4 (▲)

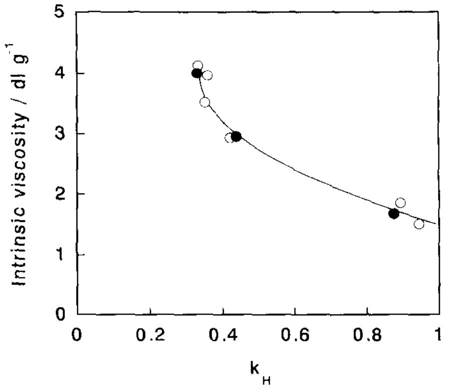

Fig. 4 Intrinsic viscosities vs. the Huggins constants of PNVA in salt solutions. $(NH_4)_2SO_4$ (○); Na_2SO_4 (●)

Table 1 The intrinsic viscosity and the Huggins constant of PNVA in urea or guanidine hydrochloride solutions

C_x [M]	Urea		Guanidine hydrochloride	
	$[\eta]^a$	k_H	$[\eta]^a$	k_H
0.0	3.88	0.34	3.88	0.34
0.1	3.90	0.39	4.14	0.32
0.5	3.87	0.39		
1.0	3.96	0.36	4.24	0.35
3.0	4.00	0.36	4.33	0.36
6.0	4.05	0.38	4.59	0.37

[a] Expressed in dl/g.

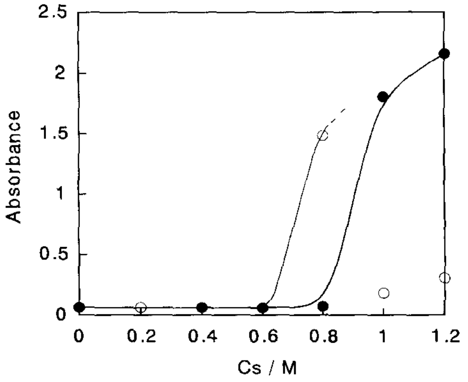

Fig. 5 Effects of urea on the phase separation of PNVA at various concentrations of sodium sulfate C_s. Na_2SO_4 (○); Na_2SO_4 + 1 M urea (●)

between 3 and 5 dl/g. Decreasing $[\eta]$ coupled with increasing k_H is a reflection of the poor solvent quality [10]. The polymer coils consequently shrink and the hydrogen bond will be formed between amide groups of the polymer. Intramolecular hydrogen bonds will be formed as a result of the shrinkage of the polymer coil, while intermolecular hydrogen bonds will be formed in the aggregates of the polymers at the C_s values corresponding to k_H values close to unity.

Effect of urea and guanidine hydrochloride on the viscosity

Urea and guanidine hydrochloride (GdnHCl) are well-known protein denaturants. Their denaturating action has been somehow related to their ability to form hydrogen bond to amide group. Urea and guanidine hydrochloride are expected to increase the solubility of the polymer in the sulfate salt solutions if they bind to amide group of the polymer. Therefore, they could dissolve the PNVA flocculates in the salt solution.

In the first place, we examined the effects of urea and GdnHCl on the viscosity of PNVA (D5) solutions without salt. As shown in Table 1, the increase of the intrinsic viscosities was more significant for GdnHCl than urea, while the Huggins constant remained essentially unaffected for both cases. The reason for different effects between urea and GdnHCl may be ascribed to different natures of their complexes with the polymer. The resulting complex with guanidinium ion becomes a polycation while that with urea remains uncharged.

As shown in Fig. 5, the addition of urea up to 1 M effectively dissolved the polymer flocculates in 0.8 M sodium sulfate. In other words, to induce the flocculation in 1 M urea, we have to add sodium sulfate to 1 M. In the absence of urea, at C_s = 1 and 1.2 M, the flocculates accumulated on the surface of the solution resulting in the very low absorbance.

Intrinsic viscosities of PNVA in solutions containing both sodium sulfate and urea or GdnHCl are shown in Fig. 6 and Table 2. In Table 2, values of the Huggins constant are also presented. In the case of urea, the intrinsic viscosity increased slightly and the Huggins constants were nearly unchanged, in spite of the dramatic dissolving power at 0.8 M sodium sulfate solution (Fig. 5). These results suggest that the effect of urea is more sensitively detected in the phase behavior than in viscosity behavior. In the case of GdnHCl at both 3 M and 6 M, on the contrary, we found that the intrinsic viscosities increased significantly. The Huggins constants decreased from the values in sodium sulfate solution to those in the normal range of 0.4. The correlation between $[\eta]$ and k_H remains essentially unaffected in the presence of urea as shown in Fig. 7.

Discussion

From the viscosity behavior, a poor solvent is characterized with small intrinsic viscosities and large Huggins constant which are in the range 0.3–0.4 for good solvents. According to this viscosity criterion, the aqueous solution becomes poor for PNVA at 0.75 and 1 M for Na_2SO_4 and $(NH_4)_2SO_4$, respectively. In the case of $(NH_4)_2SO_4$, the similar effect was observed on polyoxyethylene and polyvinylpyrrolidone while the opposite results were found on dextran and polyacrylamide (PAAm) [11]. The different effects between PAAm and the present polymer PNVA indicate the important role of methyl group of PNVA. The salting-in effect due to binding to amide is predominant for PAAm, while the superposed salting-out effect on the nonpolar methyl group makes the resultant effect to be salting-out.

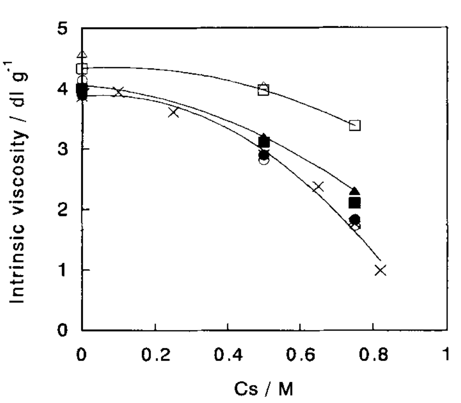

Fig. 6 Intrinsic viscosity of PNVA in sodium sulfate solutions with added urea or guanidine hydrochloride. C_s: Na_2SO_4 concentration. Concentration of urea or GdnHCl is denoted as C_x. C_x(M); 0 (×); urea 0.1 (●); 3 (■); 6 (▲); GdnHCl 10.1 (○); 3 (□); 6 (△)

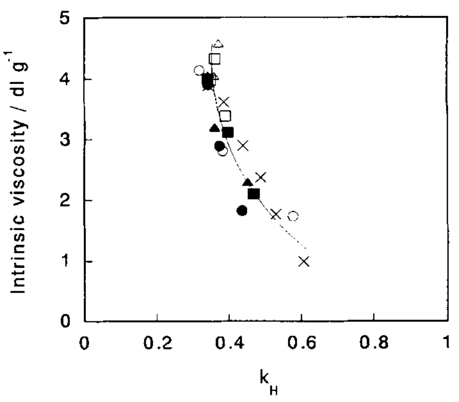

Fig. 7 The intrinsic viscosities vs. the Huggins constants of PNVA in sodium sulfate solutions with added urea or guanidine hydrochloride. Concentration of urea or GdnHCl is denoted as C_x. C_x(M); 0 (×); urea 0.1 (●); 3 (■); 6 (▲); GdnHCl 0.1 (○); 3 (□); 6 (△)

Table 2 The intrinsic viscosity and the Huggins constant of PNVA in solutions containing sodium sulfate and urea or guanidine hydrochloride

C_s [M]	$[\eta]^{a,b}$	$k_H{}^b$	Urea						Guanidine hydrochloride					
			0.1 M		3 M		6 M		0.1 M		3 M		6 M	
			$[\eta]^a$	k_H	$[\eta]^a$	k_H	$[\eta]^a$	k_H	$[\eta]^a$	k_H	$[\eta]^a$	k_H	$[\eta]^a$	k_H
0.00	3.88	0.34	3.90	0.34	4.00	0.34	4.05	0.34	4.14	0.32	4.33	0.36	4.59	0.37
0.10	3.95	0.35												
0.25	3.61	0.39												
0.50	2.89	0.44	2.90	0.37	3.11	0.40	3.19	0.36	2.81	0.38	3.97	0.35	4.05	0.36
0.65	2.37	0.49												
0.75	1.76	0.53	1.83	0.44	2.10	0.47	2.29	0.45	1.73	0.58	3.38	0.39		
0.82	0.987	0.61												

[a] Expressed in dl/g.

[b] Referred to Na_2SO_4 solutions without urea and guanidine hydrochloride.

The salt solution becomes good or moderate in the presence of GdnHCl at 3 and 6 M. But the presence of urea is much less effective to improve the solvent quality than GdnHCl. It is well known that a lower GdnHCl concentration is sufficient to induce the same effect in unfolding globular proteins than the urea concentration [12, 13]. In the present study 3 M GdnHCl is much more effective than 6 M urea. A simple quantitative estimate can be done as follows. Robinson and Jencks have proposed a complex formation hypothesis between a peptide and a denaturant (D) which is either urea or GdnHCl [14]. The proposed formation constants K (M^{-1}) at 25 °C are 0.29 and 0.90 for urea and GdnHCl, respectively. For 3 M GdnHCl, $[D] = 3$ and $K[D] = 2.7$. This is larger than the value $K[D] = 1.7$ of 6 M urea. The different effects observed between urea and GdnHCl in the present study may be explained by the "quantitative" difference between either their denaturating powers. Another possible mechanism in terms of the different natures of the complexes should be examined: the complex with guanidinium ion is a polycation while that with urea is a nonionic polymer. This mechanism is introduced to account for the different viscosity behaviors in the absence of salt. In the presence of salt, however, this mechanism is not likely if the high ionic strength of the media (0.8 M Na_2SO_4) is taken into account. Another possibility that the activity of urea is decreased by Na_2SO_4 is briefly discussed. As far as the classical electrostatic salting-out mechanism [15] is concerned, the salting-in rather than the salting-out of urea is expected, since the dielectric constant of the aqueous urea solution increases with urea concentration [16].

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