Intermolecular Interactions Between Bovine Serum Albumin and Certain Water-Soluble Polymers at Various Temperatures

Serap Kavlak, Ali Güner

Chemistry Department, Faculty of Science, Hacettepe University, Beytepe, TR-06532, Ankara, Turkey

ABSTRACT: Viscometric behaviors of dextran (Dx), poly(N-vinyl-2-pyrrolidone) (PVP), and poly(ethylene oxide) (PEO) with bovine serum albumin (BSA) in aqueous solutions have been studied at 25, 30, and 35°C. The reduced viscosity and intrinsic viscosity have been experimentally measured for the polymer/water and polymer/BSA/water systems by classical Huggins equation. Measurements of reduced viscosities of the Dx, PVP, and PEO in water have been calculated and all intrinsic viscosities of PEO($[\eta]_{PEO}$) are larger than that of Dx($[\eta]_{Dx}$), and PVP($[\eta]_{PVP}$) in aqueous solutions, at all temperatures. The intrinsic viscosities of PVP, PEO, and Dx were found to be dependent on the concentration of BSA. The presence of BSA (0.05, 0.10, and 0.30 wt %) led to a decrease in the intrinsic viscosities of polymers, at 25, 30, and 35°C. The concentration difference of BSA ($\Delta[BSA]$) is most effective in decreasing the intrinsic viscosities of Dx at 25°C and PEO at 30 and 35°C. In other words, $\Delta[\eta]$ (%) order followed as Dx > PEO > PVP at 25°C and PEO > Dx > PVP at 30 and 35°C. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 100: 1554–1560, 2006

Key words: water-soluble polymers; bovine serum albumin; viscosity

INTRODUCTION

Polymer–protein interaction plays an essential role in many kinds of food systems, health care, and pharmaceutical products.[1,2] Many studies have been conducted to investigate the protein–polymer interactions, especially protein–polysaccharide interactions in aqueous solutions.[3–7]

Bovine serum albumin (BSA) is one of the most important globular proteins because its structure and physicochemical properties are well characterized. It is a relatively large globular protein. It consists of 607 amino acids with 17 disulfide bonds and one free cysteine group. It has relatively high water solubility because it contains a large number of ionizable amino acids.[8–10]

Dextran (Dx), poly(N-vinyl-2-pyrrolidone) (PVP), and poly(ethylene oxide) (PEO) are common water-soluble polymers. Dx is the collective name of a large class of polysaccharide compounds consisting of D-glucose units. Moderately concentrated Dx solutions are usually used to maintain physiological osmotic pressure.[11] PVP is one of the most important synthetic polymers employed in biological systems and in medicine. The existing similarities between the chemical structure of PVP and proteins have led to the proposal of PVP as a synthetic polymeric model for proteins, and PEO is another important water-soluble polymer is used as pharmaceutical and cosmetic bases. Thermodynamic properties and interactions of these polymers in aqueous solutions have been investigated in previous studies.[12,13]

In this report, we have performed a study on the viscometric behaviors of Dx, PVP, and PEO with BSA, in aqueous solutions. Interactions in binary (polymer/water) and in ternary (polymer/BSA/water) systems are discussed at three different temperatures.
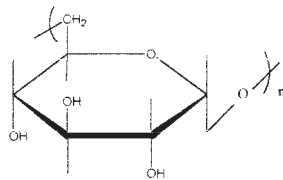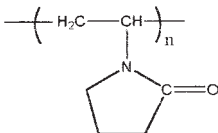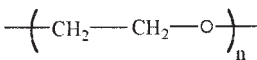
EXPERIMENTAL

Materials

Three different water-soluble polymers have been used in this work and the properties of these polymers are given in Table I. BSA was from Sigma (Sweden), with molecular weight of 70,000. The water used as solvent was deionized and bidistilled.

Experimental method

The viscometric behaviors of polymer/H_2O and polymer/BSA/H_2O, solutions were performed by using Ubbelohde type capillary viscometer, at 25, 30, and 35°C. The temperature of the thermostat was controlled within the range of ±0.1°C, and the flow times

TABLE I
Details of Materials Used

Polymer	Structure	M_n	Manufacturer
Dextran (Dx)		70000	Pharmacia Fine Chemicals
Poly(N-vinyl-2-pyrrolidone) (PVP)		44000	BDH
Poly(ethylene oxide) (PEO)		35000	Hoecht

were measured with a digital electronic reader with an accuracy of ± 0.01 s. The stock solution of each binary and ternary system was made by dissolving polymer in water and polymer in BSA/water, with a polymer and BSA concentrations of 0.400–0.193 and 1.2 $\times 10^{-3}$–2 $\times 10^{-4}$ g dL^{-1}, respectively. Measurements started after an equilibrium time of 10 min. The average elution times of solutions were determined after several measurements.

RESULTS AND DISCUSSION

On mixing of polymer and protein in solution, either one of the following possibilities are displayed; incompatibility, cosolubility, and complexation. The interaction of polymer and protein may result in segregation and association depending on functional groups, type of interactions, and properties of the medium.

Interaction in polymer systems can be analyzed accurately by studying the viscosity behavior. To this end, a different theoretical approaches have been formulated with reference to an ideal polymer system. One of the classical equations was developed by Huggins.[14] This equation expresses the specific viscosity η_{sp} of a single polymer solution as a function of the concentration C,

$$\eta_{sp}/C = [\eta] + k'[\eta]^2 C \qquad (1)$$

where η_{sp}/C, $[\eta]$, and k' etc. is the reduced specific viscosity (viscosity number) (η_{red}), intrinsic viscosity

(limiting viscosity number), and a constant, respectively. The term k' $[\eta]^2$ is termed as the specific interaction coefficient b, arising from the polymer interactions at finite concentrations. A Huggins plot of η_{sp}/C versus C of the polymer solution should yield a straight line with intercept and gradient equal to $[\eta]$ and b, respectively.

Before the discussion of data on polymers in BSA/ H$_2$O, it is necessary to characterize the viscometric behavior of each polymer separately in water. Measurements of reduced viscosities of the Dx, PVP, and PEO in water have been conducted, and their respective plots fitted through eq. (1), at 25, 30, and 35°C are shown in Figure 1. It can be seen that all the plots are linear in the whole range of concentration. On extrapolating to zero concentration, it is important to notice that all intrinsic viscosities of PEO($[\eta]_{PEO}$), are larger than that of Dx($[\eta]_{Dx}$), and PVP($[\eta]_{PVP}$) in water, at all temperatures. We come to the conclusion that, interactions between PEO–water are stronger than PVP– water and Dx–water interactions, which lead to swelling of the coils in solution and thus increase the intrinsic viscosity. For all polymers, reduced viscosities decreased with increased temperature. All these polymers form H-bond with water molecules and exhibit a lower critical solution temperature (LCST) in aqueous solutions. The H-bond formation and/or molecular association between polymer and polar water molecules is disturbed with increments of temperature, and these H-bonds will break among the water molecules
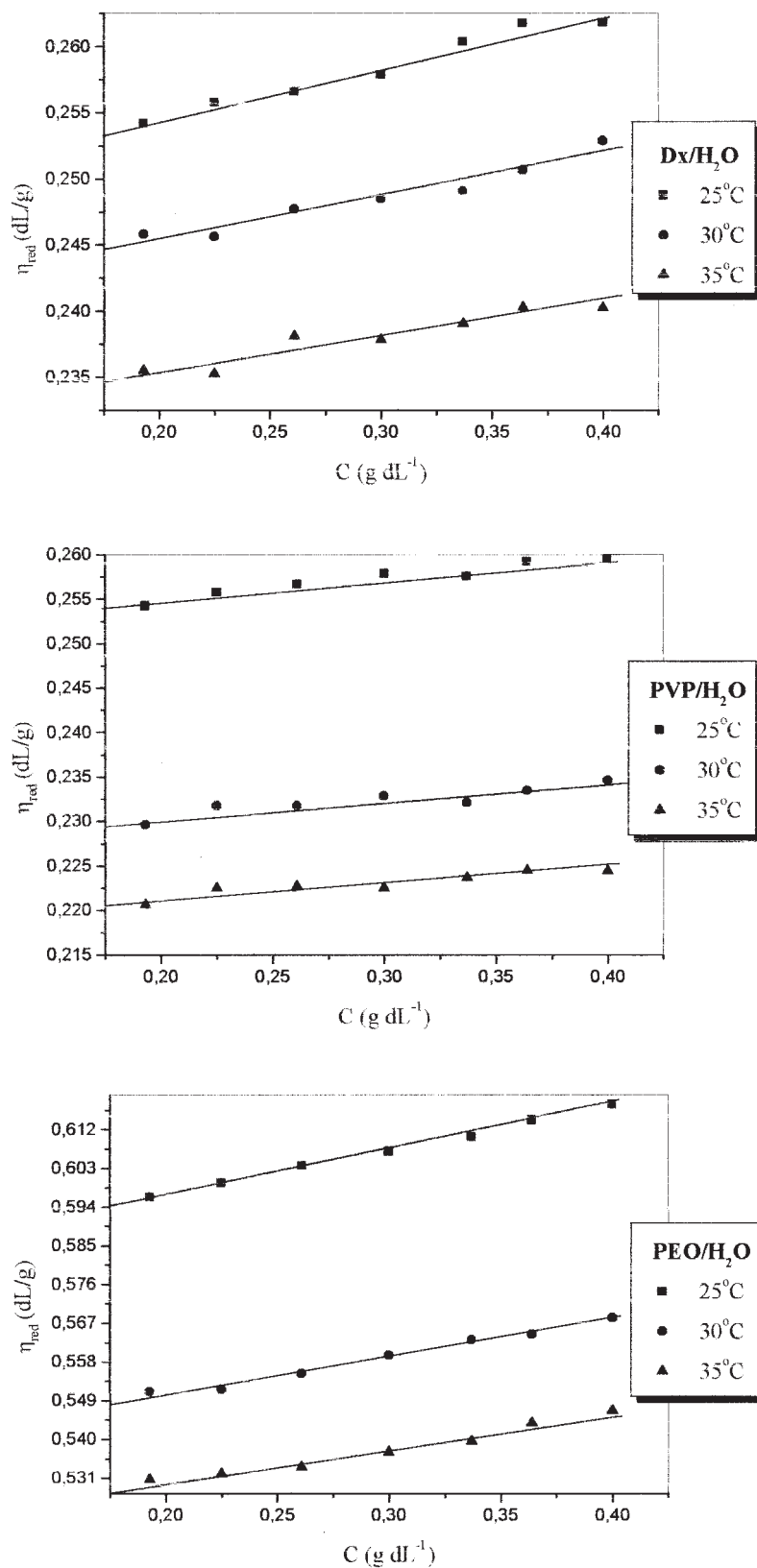
Figure 1 Huggins plots for Dx, PVP, and PEO in aqueous solutions at 25, 30, and 35°C.

and so water–polymer at increased temperature and the hydrophobic interactions between polymer segments will be more dominant. Because of these inter-

actions, a contraction will be observed in the polymer coil. Notably, the difference in the intrinsic viscosities ($\Delta[\eta]$) at consecutive temperatures decreased to 12.8,

TABLE II
Intrinsic Viscosities ([η]) for Dx, PVP, PEO in Water and in BSA Solutions at 25°C

Sample	$[\eta]_{Dx}$ (dL/g^{-1})			$[\eta]_{PVP}$ (dL/g^{-1})			$[\eta]_{PEO}$ (dL/g^{-1})		
	25°C	30°C	35°C	25°C	30°C	35°C	25°C	30°C	35°C
0.1 g Polymer/BSA (0.05 wt%)/water	0.212	0.208	0.206	0.227	0.218	0.185	0.540	0.515	0.499
0.1 g Polymer/BSA (0.1 wt%)/water	0.195	0.191	0.183	0.207	0.186	0.171	0.517	0.499	0.490
0.1 g Polymer/BSA (0.3 wt%)/water	0.168	0.165	0.163	0.185	0.160	0.138	0.496	0.485	0.479

10.8, and 6.8% for PVP, PEO, and Dx, respectively. This behavior can be explained by the strong intramolecular H-bonding of Dx in accordance with its chemical structure.

The interaction of the biopolymers in solution may be segregative (biopolymers repel each other and are denoted as incompatible) or associative (biopolymers attract one another). For very dilute solutions, the system is stable since the mixing entropy dominates and proteins and polysaccharides are cosoluble. Upon increasing the concentration of the biopolymers, the system may become unstable, depending on the type of interaction.[4] Segregative phase separation occurs only at high ionic strength and under specific pH conditions, with respect to the isoelectronic point. Under other conditions, compatibility or complex formation would be expected.[1] In our system, with the addition of PVP, PEO, and Dx to BSA/water solution all respective plots are again linear and the calculated intrinsic viscosities ([η]) are given in Table II, at 25, 30, and 35°C. The intrinsic viscosities of PVP, PEO, and Dx depend on the concentration of BSA and temperature. These relations are also shown in Figures 2–4. According to these figures, the presence of BSA (0.05, 0.10, and 0.30 wt %) led to a decrease in the intrinsic

viscosities of polymers, at chosen temperatures. The dimensions of [η] are a measure of the effective hydrodynamic volume of the polymer in solution, and also represent the interactions between the polymer and the additive systems. The H-bond formation and/or molecular association between polymer and
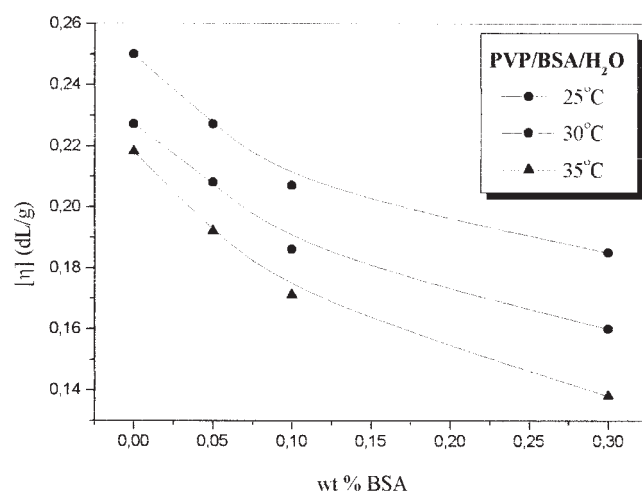
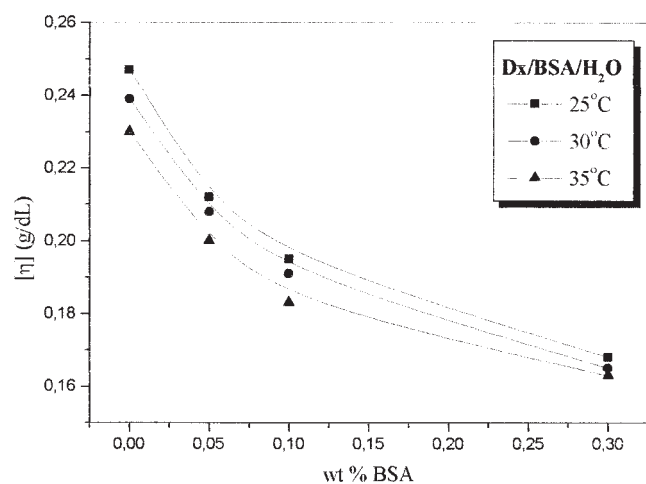**Figure 3** Effect of BSA concentration (wt %) and temperature on the [η] of PVP.

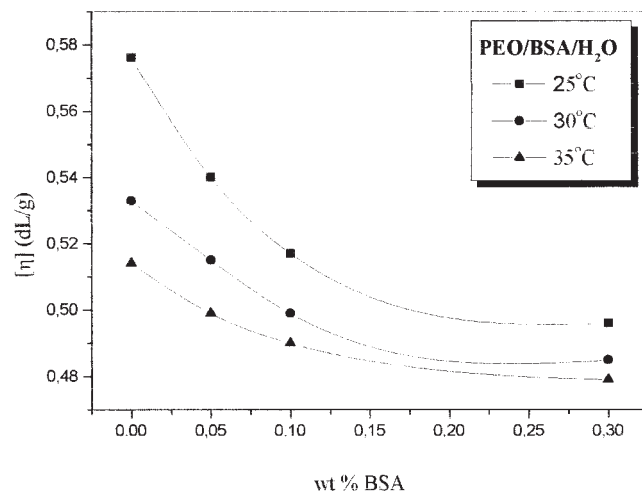**Figure 2** Effect of BSA concentration (wt %) and temperature on the [η] of Dx.

Figure 4 Effect of BSA concentration (wt %) and temperature on the [η] of PEO.

TABLE III
The Percentage Difference in Intrinsic Viscosities {Δ [η] (%)} of Dx, PEO, and PVP
Solutions by Increment of BSA Concentration at 25°C, 30°C, and 35°C.

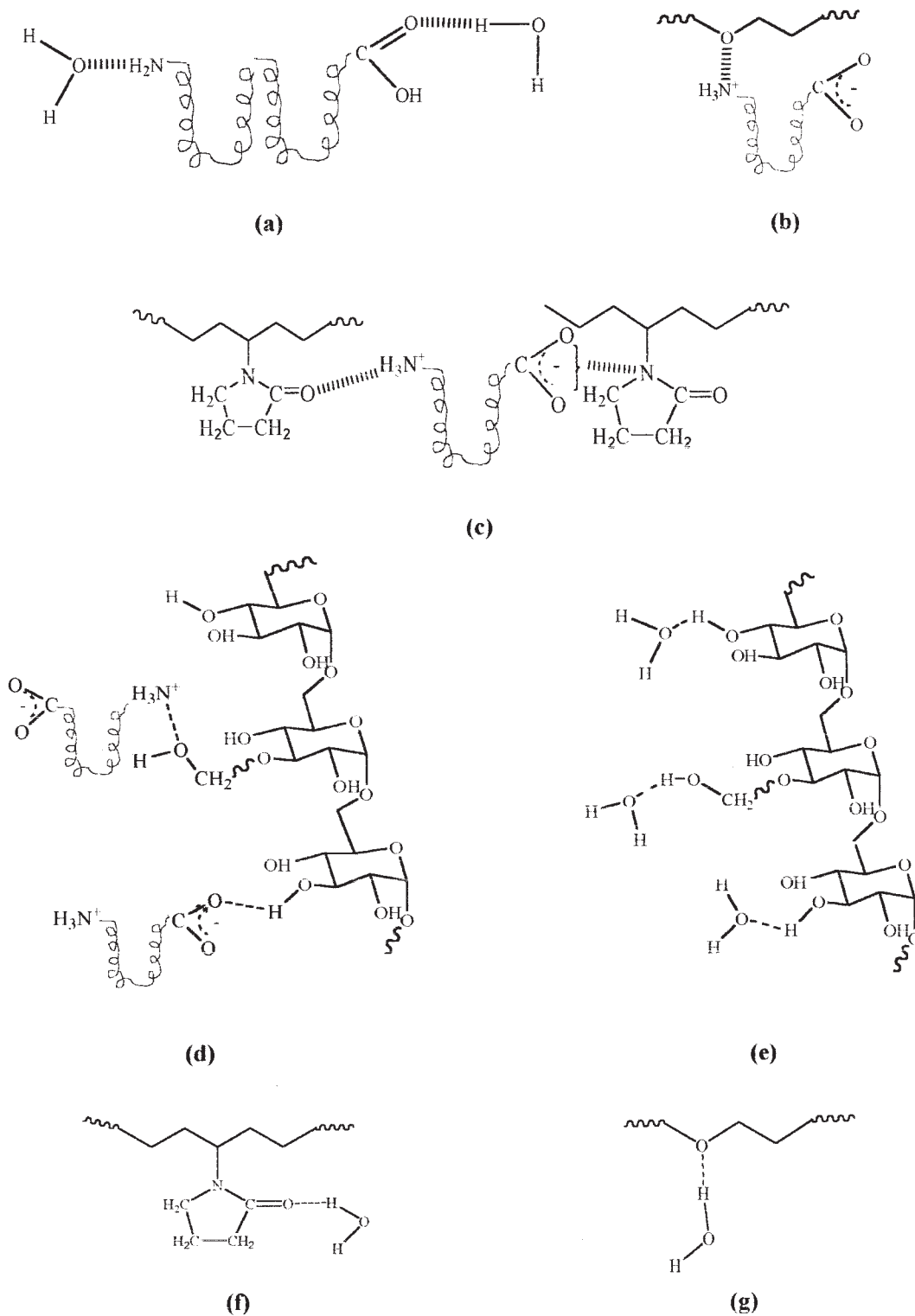|	$\Delta\ [\eta]\ (dL/g^{-1})\ (\%)$		
Sample	25°C	30°C	35°C
Dx/BSA (0.05 wt%–0.3 wt%)/water	20.8	20.7	20.8
PEO/BSA (0.05 wt%–0.3 wt%)/water	18.5	26.6	25.4
PVP/BSA (0.05 wt%–0.3 wt%)/water	8.2	5.8	4.0

Figure 5 Possible interactions between the functional groups of the (a) BSA/H$_2$O, (b) PEO/BSA, (c) PVP/BSA, (d) Dx/BSA, (e) Dx/H$_2$O, (f) PVP/H$_2$O, and (g) PEO/H$_2$O.

TABLE IV
The Percentage Difference in Huggins Constants {$\Delta k'$ (%)} of Dx, PEO, and PVP
Solutions by Increment of BSA Concentration at 25°C, 30°C, and 35°C

	$\Delta k'$ (%)		
Sample	25°C	30°C	35°C
Dx/BSA (0.05 wt%–0.3 wt%)/water	71.2	71.3	80.9
PEO/BSA (0.05 wt%–0.3 wt%)/water	46.6	46.9	31.5
PVP/BSA (0.05 wt%–0.3 wt%)/water	79.4	89.4	75.6

water molecules are disturbed in the presence of BSA with increments of temperature. Intrinsic viscosities decreased in polymer solution with the addition of 0.05, 0.10, and 0.30 wt % BSA. The smaller the values of the intrinsic viscosities, the smaller the size of the macromolecule in the solution. This behavior further supports more interactions occurring at lower concentrations and lower temperatures for PVP, PEO, and Dx in BSA/water system. The concentration of BSA increased from 0.05 wt % to 0.30 wt %, and the percentage difference in intrinsic viscosities, $\Delta[\eta]$ (%) at consecutive temperatures are given in Table III. As can be seen from this table, the concentration difference of BSA (Δ[BSA]) is the most effective in decreasing the intrinsic viscosity of Dx at 25°C and PEO at 30 and 35°C. In other words, $\Delta[\eta]$ (%) order is as follows: Dx > PEO > PVP at 25°C and PEO > Dx > PVP at 30 and 35°C. These orders can be explained by thermodynamic behaviors of ternary systems (polymer/BSA/water) and interactions of functional groups. Dx and PEO are less interested in BSA compared with PVP. Because of their chemical structure, Dx and PEO form intramolecular H-bonding and association, effectively. Consequently, the interaction of the polymer with BSA molecules is more effective for PVP/BSA system in aqueous solutions and at all definite temperatures. This can be attributed to the hydrophilic and hydrophobic interactions of the hydrocarbon helical chain, —COO$^-$, and N$^+$H$_3$ of BSA, and the vinyl chain and ketonic group of PVP molecules.[3] Possible interactions between polymer/water, BSA/water, and polymer/BSA are shown in Figure 5.

Another approach may be given by the denaturating property of additive. As the weight percentage of BSA is increased in the polymer solution intrinsic viscosity values of polymer solutions is decreased, indicating a reduction in the size of the polymer coils, and this can be explained by the breaking of the interactions (especially hydrogen bonds) between polymer molecules.

Huggins constant (k') is another important criteria for the measurement of the polymer–solvent interaction, and it is about 0.35 in good solvents for flexible polymer molecules. The difficulty of interpreting the Huggins constant for polymer molecules is more involved because of the interpenetration of polymer coil.

The extent of this penetration is a function of the polymer–polymer (or segment–segment) and polymer–solvent interaction, which affects the intramolecular hydrodynamic interaction and molecular dimensions.[15,16] The percentage difference in Huggins constants, $\Delta k'$ (%) at 25, 30, and 35°C are given in Table IV. According to this table, concentration difference of BSA (Δ[BSA]) is most effective in increasing the Huggins constants of PVP especially at 30°C and it is less effective for PEO at higher temperature, as expected. Higher values of Huggins constants for PVP are interpreted to be due to molecular association. These high values may be a result of the interactions of lactam groups of the polymer with water molecules/ionic species or H-bond acceptor–donor denaturating agent in solution by hydrogen bonding. k' may also be influenced by the aggregation of the polymer segments in a solution, and aggregation of polymer molecules may be responsible for the high k' values.[17,18]

CONCLUSIONS

In the present study, viscometric investigations of PVP/water, PEO/water, Dx/water, PVP/BSA/water, PEO/BSA/water, and DX/BSA/water systems have been investigated. On extrapolating reduces viscosities to zero concentration, all intrinsic viscosities of PEO($[\eta]_{PEO}$) are larger than that of Dx($[\eta]_{Dx}$), and PVP($[\eta]_{PVP}$) in water, at 25, 30, and 35°C. A reasonable interpretation can be assigned to the coils of PEO; because of strong interactions between PEO and water, molecular excluded volume of PEO in solution tends to rise. Finally, due to these interactions, polymer coil extended and so viscosity is higher. The intrinsic viscosities of polymers in BSA/water decrease with an increasing BSA concentration from 0.05 wt % to 0.3 wt %. This difference is most effective in decreasing the intrinsic viscosity of Dx at 25°C while PEO at 30 and 35°C. $\Delta[\eta]$ (%) order followed as Dx > PEO > PVP at 25°C and PEO > Dx > PVP at 30 and 35°C. A reasonable interpretation may be assigned to the interaction of the polymer with BSA molecules and this interaction is more effective for PVP/BSA system in aqueous solutions at all definite temperatures.

References

1. Doublier, J. L.; Garnier, C.; Renard, D.; Sanchez, C. Curr Opin Colloid Interface Sci 2000, 5, 202.
2. Kinsella, J. E.; Whitehead, D. M. Adv in Food Nutr Res 1989, 33, 343.
3. Singh, M.; Kumar, S. J Appl Polym Sci 2003, 87, 1001.
4. Kruif, C. G.; Tuinier, R. Food Hydrocolloids 2001, 15, 555.
5. Hemar, Y.; Tamehana, M.; Munro, P. A.; Singh, H. Food Hydrocolloids 2001, 15, 565.
6. Galazka, V. B.; Smith, D.; Ledward, D. A.; Dickinson, E. Food Chem 1999, 64, 303.
7. Schaink, H. M.; Smith, J. A. M. J Chem Phys 1997, 107, 1004.
8. Kelley, D.; McClements, D. J. Food Hydrocolloids 2003, 17, 73.
9. Hilger, C.; Grigionia, F.; De Beaufort, C.; Michael, G.; Hentges, F. Bovine Serum Albumin; National Center for Biochemical Information, National Institutes of Health, 1998. Available at http://www.ncbi.nlm.nin.gov.
10. Giancola, C.; De Sena, C.; Fessas, D.; Graziano, G.; Barone, G. Int J Biol Macromol 1997, 20, 193.
11. Hirata, Y.; Sano, Y.; Aoki, M.; Shohji, H.; Katoh, S.; Abe, J.; Hitsukuri, S.; Yamamoto, H. Carbohydr Polym 2003, 53, 331.
12. Kavlak, S.; Güner, A. J Appl Polym Sci 2000, 78, 507.
13. Uraz, İ.; Güner, A. Carbohydr Polym 1997, 34, 127.
14. Huggins, M. L. J Am Chem Soc 1942, 64, 2716.
15. Güner, A. J Appl Polym Sci 1996, 62, 785.
16. Bohdanecky, M.; Kovar, J. Viscosity of Polymer Solutions; Elsevier: Amsterdam, 1982; p 178.
17. Güner, A. J Appl Polym Sci 1998, 67, 1615.
18. Güner, A.; Kibarer, G. Eur Polym J 2001, 37, 619.