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Research Article

Study of Bovine Serum Albumin Solubility in Aqueous Solutions by Intrinsic Viscosity Measurements

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The behavior of bovine serum albumin (BSA) in water is scarcely studied, and the thermodynamic properties arising from the experimental measurements have not been reported. Intrinsic viscosity measurements are very useful in assessing the interaction between the solute and solvent. This work discussed in a simple determination of the enthalpy of BSA in aqueous solution when the concentration ranges from 0.2 to 36.71% wt. and the temperature from 35 to 40°C. The relationship between the concentration and intrinsic viscosity is determined according to the method of Huggins. The temperature increase reduces the ratio between inherent viscosity and concentration (η_i/c). This is reflected in the Van't Hoff curve. Furthermore, this work proposes hydrodynamic cohesion value as an indicator of the degree of affinity of protein with water and thermodynamic implications in conformational changes.

1. Introduction

Solubility is the ability of a substance to dissolve into another; it is given by the solubility constant which is in equilibrium with the solute excess or ions excess. Basic studies on proteins have focused on protein concentration, pH, ionic strength, polymeric additives, the dielectric properties of solvent and solvent mixtures, and effect temperature. In the case of proteins and polysaccharides, solubility studies are closely related to studies of gelation that try to determine the temperature and the concentration of gelation (T_{gel} and C_g) (Djabourov) [1].

The conformational stability of a protein is determined by intramolecular factors and solvent interactions (hydration). Solubility is determined primarily by intermolecular effects (protein/protein), but, as proteins are solvated, the hydration effects are also involved in changes in solubility. Conformational changes (changes in functional activity) can be induced by changes in temperature, pressure, and the solvent medium. The technological performance of proteins depends critically on conformation, hydration (water-holding capacity), and solubility. Like other polymers, proteins can be characterized by their chain conformations. However, methods of polymer statistics cannot be applied to most proteins because they adopt specific (native) conformations under different physiological conditions. Since proteins are polyelectrolytes, their solubility behavior is governed largely by electrostatic (ionic) interactions. In determining charge/charge interactions, pK_a and pK_b values of individual amino acids play an important role (Franks) [2].

Barton [3] worked with solubility parameters, trying to explain their nature and to extend solubility theory to liquid mixtures. Cohesion parameters (solubility parameters) provide one of the simplest methods for correlating and predicting the cohesive and adhesive properties of polymers and solvents based on the knowledge of the properties of the individual components isolated.

The nature of solubility may be understood in terms of molecular interactions broadly classified as either "reactive" (involving relatively strong "chemical" forces: complex formation, etc.) or "nonreactive" (involving relatively weak "physical" or "van der Waals" forces). Solution nonideality can of course be best explained when both "chemical" and "physical" forces are considered; the truth lies between these two extremes. The solubility parameter approach is basically "physical," but the introduction of specific interaction

components has taken it some way toward a reasonably balanced position, except when "solvation" is considered. This restriction usually limits this approach to nonelectrolyte solutions, but an extension of it to ionic systems is possible. General theories of the liquid state and of solutions involve complex expressions linking molecular interaction potential energy, thermal energy, and volume; for many practical purposes it is convenient to use simple or semiempirical methods. It has been found that a good solvent for a certain (nonelectrolyte) solute such as a polymer has a "solubility parameter" (δ, defined below) value close to that of the solute. Often, a mixture of two solvents, one with a higher δ value and the other with a lower δ value than that of the solute, is a better solvent than each of the two components of the mixture (Barton [4]).

Hansen's [5] study of solubility parameter concepts can be used to interpret relations involved in liquid miscibility, polymer solubility, polymer compatibility, adsorption on solid surfaces, dispersion phenomena, solubility of inorganic and organic materials in organic liquids, and "salting in" phenomena in biological molecules. Mangarj [6] was the first author to work with intrinsic viscosities relating it the Hildebrand and Hansen solubility parameters.

Bozdogan [7] applied the intrinsic viscosity-temperature data of polystyrene (PS) fractions in decalin, cyclohexane, dioctyl phthalate and toluene solutions in theta temperatures, and obtained solubility parameters. This author proposed an equation using the critical volume fraction and segment number of the polymer (PS) for calculating the partial molar entropy of a polymer in dilute solution.

Shulgin and Ruckenstein [8] studied the local composition around protein molecules in aqueous mixtures containing polyethylene glycol (PEG) and solubility of proteins in water-PEG solutions. They concluded that their theory predicts that PEG acts as a salting-out agent for lysozyme, β-lactoglobulin, and bovine serum albumin.

Guner [9] determined the solubility parameters for dextrans/solvents systems using algorithms by group contribution and intrinsic viscosity measurements. Ravindra et al. [10] reported the solubility parameters for chitin and chitosan using group contribution methods. The solubility parameters of chitin and chitosan, as determined by these methods, are more or less equal to the experimental value of 41 $J^{1/2}$ $cm^{-3/2}$. They proposed a method for estimating the overall solubility parameter of chitosan with any deacetylation degree. Kong et al. [11] studied the basicity, water solubility, intrinsic viscosity, and molecular weight of carboxymethyl chitosan.

Naskar et al. [12] studied the viscosity and solubility behaviors of inulin in water solutions. This polysaccharide contains β $(2 \rightarrow 1)$ fructosyl fructose units and exhibits a compact (globular) in aqueous medium, and its solubility is a weak endothermic process.

Brodersen [13] studied the solubility of bilirubin in water and its interaction with phospholipids and albumin at pH 7.1–7.4 to 37°C, confirming that the logarithm of solubility changes linearly with pH. These authors conclude that the deviation of the slope from the theoretical value can be explained by the heterogeneity of bilirubin in the solid phase.

Minghetti et al. [14] investigated the solubility parameters using capillary viscometry (intrinsic viscosity), that may be useful in studies of patch formulation, as this parameter is predictive of the thermodynamic activity of a drug in a matrix.

Bustamante et al. [15] investigated partial solubility parameters from intrinsic viscosity measurements and suggested that this is a versatile method suitable to be used for the study of drugs and both nonpolymeric and polymeric excipients.

Adamska et al. [16] determined the solubility parameters from intrinsic viscosity measurements for a series of pharmaceutical excipients, this data can be used for predicting their behavior in a multicomponent system.

Cohn [17, 18] studied the ionic strength and the isoelectric point which are related to the solubility of globular proteins, concluding that the solubility of proteins in the neighborhood of their isoelectric points depends in a large part upon the degree of their dissociation, the more soluble proteins being the more dissociated.

Shaw et al. [19] studied the effect of net charge on the solubility of ribonuclease Sa, while Schmittschmitt and Scholtz [20] performed similar work in amyloid fibril.

Ferreira Machado et al. [21] performed studies of egg white protein solubility-density. Solubility increased with increases in pH, with the highest solubility at pH 9.0 and the lowest solubility at pH 4.6. This behavior was verified for all the salts analyzed. With an acid pH 3.0, a tendency of increasing solubility linked to the increase of the saline concentration was observed, due to the salting-in effect, and density increased with increases of the salt concentration.

Monkos [22, 23] in various works has made a detailed study of the viscosity and intrinsic viscosity of different globular proteins in terms of concentration and temperature in order to find a mathematical relationship that describes this phenomenon.

Olivares et al. [24] studied gelatin chain aggregation in dilute aqueous solutions at temperatures below the gel point ($T < T_g$) which were subject to different maturation temperatures T_m and maturation times t_m.

Curvale et al. [25] determined the intrinsic viscosity of BSA in aqueous solutions and found an abnormal behavior, according to measurements with the Huggins method; $[\eta]$ is an expression of the interaction between a biopolymer and solvent which reflects the solvent's ability to swell the macromolecule. Thus, we can see that BSA has a very low $[\eta]$ value at pH 7.4, which explains the assigned globular shape.

Sousa et al. [26] performed studies on the hydrodynamics of the Lupin protein, evaluating its intrinsic viscosity, and solubility. Shen [27] studied soy protein by solubility, intrinsic viscosity and optical rotation measurements.

Arakawa and Timasheff [28] worked on protein solubility. Their review said that protein concentration at equilibrium was a complex function of a number of factors such as the physical and chemical natures of the proteins themselves and of environmental parameters such as pH, temperature, nature of the salt, and the kind of organic solvent and its concentration.

Peters [29] wrote that the solubility of albumins was related to their high total electric charge, with corresponding strong hydrophilicity and attraction for water molecules; near neutrality, albumins were extremely soluble in water, 35% wt. in dilute salt solutions, and 50% wt. in water pure solutions. A similar analysis of protein solubility was proposed by Haworth [30], which highlights the hydrophobicity of the composition and the type of amino acids that compose it.

In concordance with the modified treatment of Bozdogan [7], in this work we studied the thermodynamic properties of BSA in aqueous solutions within a temperature range of 35 to 40°C; the increase in relative viscosity (η_i) was calculated for each case. In addition, this work proposes the hydrodynamic value of cohesion as an indicator of the degree of affinity of macromolecules with water and the thermodynamic implications of the conformational changes that water molecules present in association with BSA.

2. Materials and Methods

BSA (lyophilized and deionized powder, purity grade >98%) was obtained from Fedesa S.A.-UNSL; the BSA molecular weight is 66,500 g mol^{-1}, and $\nu_{(a/b)}$ universal shape function is 4.27 for physiological BSA (Curvale et al. [25]). Measurements were taken from fresh BSA solutions of 0.2 and 36.71% wt. in volume with pH 6.5. Solutions and dissolutions were prepared with deionized water. The different temperatures were maintained using a HAAKE C thermostatic bath (±0.1°C). Determinations were done using an Ubbelohde "suspended level" viscometer (IVA 1), with a water draining time of 34.25 s. Even though this viscometer works independently of the volume of the solution, it was here used for performing at least three measurements for each concentration and was later washed until the draining time of the solvent was obtained (Masuelli [31]). These solutions were previously prepared from a mother solution with a concentration determined by UV-absorbance at 278 nm with a Shimadzu UV-160A spectrophotometer. Density of each solution was measured using an Anton Paar DMA35N densimeter.

3. Theory

The solution equilibrium between the mixing solvent and the biopolymer, in concordance with the treatment of Bozdogan [7], is written as

$$S + B \overset{K}{\longleftrightarrow} S \ldots B. \tag{1}$$

The equilibrium constant is

$$K = \frac{[S \ldots B]}{[S]\,[B]}, \tag{2}$$

where $[S]$ is the solvent, and $[S \ldots B]$ and $[B]$ are the mixed and unmixed biopolymer concentrations, respectively. However, solvent concentration is greater than the concentration of biopolymer in solution. Therefore, (2) can be rewritten as

$$K_s = \frac{[S \ldots B]}{[B]}. \tag{3}$$

In the mixing process, the intrinsic viscosity is proportional to the mixed biopolymer concentration (Segarceanua and Leca [32]):

$$[\eta] = f\,[S \ldots B], \tag{4}$$

and unmixed biopolymer concentration is expressed by the following equation:

$$[\eta]_{\text{lim}} - [\eta] = f\,[B], \tag{5}$$

where $[\eta]_{\text{lim}}$ is the limiting intrinsic viscosity, which corresponds to the maximum swelling of the biopolymer, while f is the proportionality constant [7].

Equation (3) can be rewritten as

$$K_s^* = \frac{[\eta]}{[\eta]_{\text{lim}} - [\eta]}. \tag{6}$$

In Huggins' method [25, 31], intrinsic viscosity $[\eta]$ is defined as the ratio of the increase in relative viscosity (η_i) to concentration (c in g cm^{-3}) when the latter tends to zero:

$$\frac{\eta_i}{c} = [\eta] + K_H[\eta]^2 c. \tag{7}$$

IUPAC recommends the term "increment of relative viscosity (η_i)," instead of "specific viscosity," because it has no attributions of specific quantity, meaning:

$$\eta_i = \eta_r - 1. \tag{8}$$

And let us remember that

$$\eta_r = \frac{\eta_s}{\eta_0} = \frac{\rho_s \cdot t_s}{\rho_0 \cdot t_0}, \tag{9}$$

where the subindex "s" indicates "solution" and "0" indicates "solvent."

Since intrinsic viscosity is relevant for dilute solutions of BSA in the range of 0.1 to 4% wt., when high concentrations are used, it is better to start with the first term of the Huggins equation "$\eta_i/c = \eta_{\text{red}}$."

The second law of thermodynamics is,

$$\begin{aligned} \overline{\Delta G}_2 &= -RT \ln K_{si}^*, \\ \overline{\Delta G}_2 &= \overline{\Delta H}_2 - T\overline{\Delta S}_2. \end{aligned} \tag{10}$$

Combining (6) and (7), according to a modified treatment of Bozdogan [7], the following equation is obtained:

$$\ln \frac{\eta_i/c}{(\eta_i/c)_{\text{lim}} - (\eta_i/c)} = -\frac{\overline{\Delta H}_2}{RT} + \frac{\overline{\Delta S}_2}{R}, \tag{11}$$

where $\overline{\Delta G}_2$, $\overline{\Delta H}_2$, and $\overline{\Delta S}_2$ are Gibbs free energy, enthalpy, and entropy changes when mixing a biopolymer fraction, respectively. Plotting $\ln K_{si}^*$ as a function of T^{-1} will give a straight line with a slope of $-\overline{\Delta H}_2/R$ and intercept of $\overline{\Delta S}_2/R$.

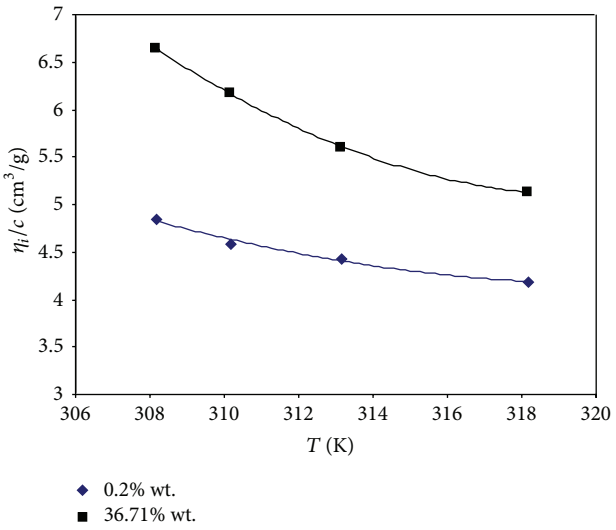

FIGURE 1: Plot of η_i/c versus T, where standard deviations are 0.9599 and 0.9729 for 0.2% and 36.71% wt.

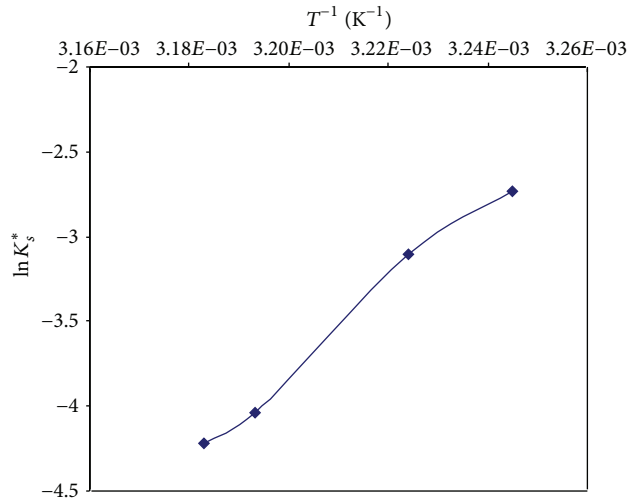

FIGURE 2: Plot ln K_{si}^* in function T^{-1} for water-BSA system.

The cohesion or Hildebrand solubility parameter "δ" of a polymer can be written as the following equation:

$$\delta = \left(\frac{\overline{\Delta H}_2}{V_M}\right)^{1/2}, \tag{12}$$

where V_M is the polymer molar volume (cm^3 mol^{-1}).

The hydrodynamic cohesion parameter "δ_{Hy}" for hydrosoluble biopolymers can be written as the following equation:

$$\delta_H = \left(\frac{\overline{\Delta H}_2}{V_H}\right)^{1/2}, \tag{13}$$

where V_H, is the hydrodynamic molar volume of biopolymer (cm^3 mol^{-1}), and can be written as the following equation:

$$V_H = \frac{M[\eta]}{\nu_{a/b}}. \tag{14}$$

Equation (13) gives an account of the thermodynamic changes and the degree of solvation of the macromolecule in a polar solvent and also shows the phase changes for different temperatures (Masuelli [31]).

4. Results and Discussion

The shape of the curve reduced viscosity ($\eta_i/c = \eta_{\mathrm{red}}$) as a function of temperature, shown in Figure 1, indicates a decrease of η_{red} with temperature, in agreement with other authors as Monkos [33, 34].

When studying the case of BSA in water it is very difficult to find works on solubility as a function of temperature and concentration determined by intrinsic viscosity measurements. Therefore in this work we propose a better alternative to working with ($\eta_i/c = \eta_{\mathrm{red}}$). We see in Figure 2 that for working concentrations the curve has a positive slope from 308 to 313 K.

Agostini et al. [35] investigated the relationship between the thermodynamics and the kinetics of protein aggregation, comparing the solubility of proteins with their aggregation rates. They found a significant correlation between these two quantities when considering a database of protein solubility values that were measured using an in vitro reconstituted translation system containing about 70% of *Escherichia coli* proteins. The existence of such correlation suggests that the thermodynamic stability of the native states of proteins with respect to the aggregate states is closely linked with the kinetic barriers that separate them. A similar study is released by Jiang et al. [36] in pH and thermal function.

Boye et al. [37] found that maximum thermal stability for denatured BSA was observed at pH 5 and 63°C. The denaturation of BSA, observed by IR, resulted in the loss of the 1,654 cm^{-1} band attributed to an α-helical structure and to the rise of two bands at 1,616 and 1,684 cm^{-1} attributed to the formation of an ordered nonnative β-sheet structure associated with aggregation.

Clark et al. [38] performed an extensive study on whey protein mixtures, where structure-property relationships are very difficult to establish. Furthermore, although presumably all globular proteins above the critical gelation concentration C_g can gelify given appropriate conditions, these authors worked on BSA and β-lactoglobulin gels. These authors failed to perform rheological studies of these proteins due to the difficulty of accurately measuring the thickness of the gel.

A similar study was realised by Pelegrine and Gasparetto [39] for whey proteins at a pH of 5.65, where protein solubility increased with temperature, indicating that there was neither coagulation or aggregation between protein molecules possibly because at this pH value the β-lactoglobulin is a dimmer that is dissociated in monomers at 50°C, and only above 60°C the proteins unfold and the hydrophobic groups react.

Analyzing Figure 2, the values of thermodynamic properties $\overline{\Delta H}_2$ and $\overline{\Delta S}_2$ are calculated for A 211 kJ mol^{-1}

and $-0.76\,\mathrm{kJ\,mol^{-1}\,K^{-1}}$ and for B $-31.3\,\mathrm{kJ\,mol^{-1}}$ and $0.078\,\mathrm{kJ\,mol^{-1}\,K^{-1}}$, respectively. The cutoff point between segments A and B is 308.16 K. This tells us that rank A is for endothermic, while rank B is for exothermic and spontaneous solubilization. This phenomenon of a change in the slope between segments A and B can be reasonably explained by taking into account the behavior of concentrate BSA in the water system because BSA-water is a complex solution that consumes energy in this system related with nonideality; read below.

The chemical potential for protein-water system can be written as

$$\mu - \mu_0 = -\frac{RT\nu_0 c}{M}\left\{1 + BMc + \cdots\right\}, \tag{15}$$

where ν_0 is the molar volume of pure solvent ($\mathrm{mol\,cm^{-3}}$), and B is virial expansion of the solvent chemical potential. The quantity B, the second virial coefficient, serves as a convenient measure of solution nonideality (van Holde [40]).

The biopolymers in which there are strong side-chain interactions tend to coil up into dense globular conformation; these are called globular proteins (BSA). For a discussion of nonideal behavior of BSA solutions (because BSA interacts strongly with water) may be due to two effects: either $\Delta H_2 \neq 0$ or ΔS_2 may contain contributions from ordering or disordering of the solvent. A negative heat of mixing corresponds to favorable BSA-water interaction. This should lead to an even greater decrease in the solvent chemical potential than expected for ideal solutions and thus a positive value of B.

The derivation of that law involved the assumption that the solute molecules are of the same order of size as solvent molecules so that the solvent and solute might be interchanged at random in a hypothetical lattice. But this is by no means a solvent molecule; in fact, the monomer units of the protein are more comparable to the water molecules in size. In other words, a BSA solution more nearly resembles one in which the solute particles are required to move together in clumps. Put another way, this says that the distribution of solute molecules in a macromolecular solution can never be entirely random. The center of each molecule is excluded from a volume determined by the volumes occupied by all of the other molecules. It is not surprising, then, to find that the nonideality and hence the second virial coefficient depend on the excluded volume (ν_{exc}) in $\mathrm{cm^3\,g^{-1}}$ as a fellowship equation

$$B = \frac{N\nu_{\mathrm{exc}}}{2M^2}. \tag{16}$$

The second virial coefficient, that defines nonideality, is determined by,

$$B = \frac{4\nu_s}{M}, \tag{17}$$

where ν_s is the specific volume in $\mathrm{cm^3\,g^{-1}}$.

The conclusion to be drawn is that while this contribution to nonideality is small for spherical molecules, it can become large for very asymmetric rod or random coils

Table 1: Data of specific volume and second virial coefficient for different temperatures and concentrations.

T/K^{-1}	0.2% wt.		36.71% wt.	
	$\nu_s/\mathrm{cm^{-3}\,g}$	$B \times 10^5$	$\nu_s/\mathrm{cm^{-3}\,g}$	$B \times 10^5$
314.16	0.4151	2.4969	0.5089	3.0612
313.16	0.4396	2.6440	0.5562	3.3458
310.16	0.4558	2.7414	0.6135	3.6901
308.16	0.4809	2.8927	0.6596	3.9681

(see Table 1). This is understandable for the number of ways in which such particles can be packed into the solution is quite limited. The macromolecular solutions can exhibit very nearly ideal behavior under some conditions. For example, for the random coil polymers there is often a particular temperature (called the θ temperature) at which $B = 0$. The excluded volume effect is always such as to lower the chemical potential of solvent. It is an entropic effect. On the other hand, in poor solvents the solute-solute interaction may be such as to increase the solvent chemical potential. This is an enthalpic term. There may exist a temperature (θ temperature) at which these enthalpic and entropic terms exactly are canceled, and the solution is ideal. Putting this mechanistic term, we regard the excluded volume effect as one that pushes molecules apart (they cannot interpenetrate). This may be compensated for by an interaction of the sort that makes solute molecules clump together. The work of a θ temperature for a particular macromolecule-solvent system is a prize indeed for it greatly simplifies all physical studies for ideal solutions. This θ temperature is very difficult to find in BSA since in our case this protein behaves globularly in the temperature range used, and the value of B is in the range between the spherical and rod-like form (van Holde [40] and Curvale [25]).

Furthermore, observing Table 1, it can be ensured that the effect of temperature on the estrangement of the ideality is higher at temperatures below 314 K, and the nonideality also increases with increasing the concentration of BSA in aqueous solution.

Fan et al. [41] studied BSA solutions (2.5%, pH 7.2) which under heat treatment can undergo structural changes. They found that α-helices are transformed to random coils at 67°C, resulting in an increased rotation angle. With subsequent heating, the transformed random coils may once again transform to nonnative β-sheets and restore the optical rotation angle. These two states are reversible. However, when the heating temperature goes up to 69°C, the denatured BSA starts to transform into a rigid network and becomes an irreversible state.

Wetzel et al. [42] studied the structural alterations of albumin, their dependence on concentration, and the role of free-SH groups in thermal denaturation, as well as the reversibility of thermally induced structural changes. The helix content changes with heat treatment, giving rise to beta structures which are amplified when cooled and which are correlated with the aggregation of albumin. With rising temperature and concentration the proportion of beta structures and aggregates increases. At denaturation degrees of

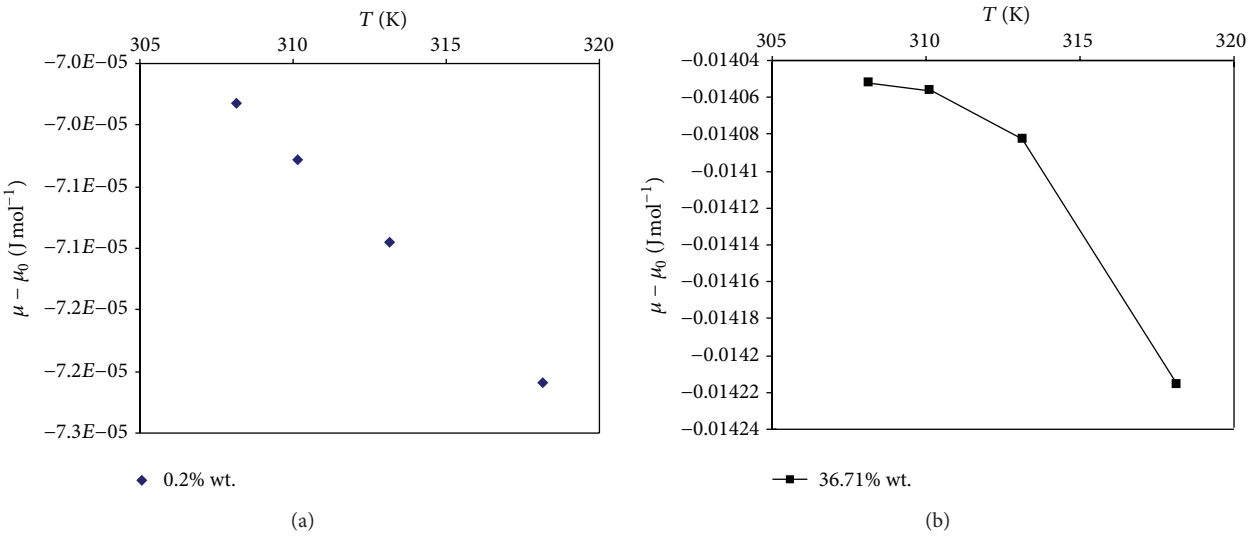

Figure 3: Plot of chemical potential in function of T: (a) 0.2% wt., (b) 36.71% wt.

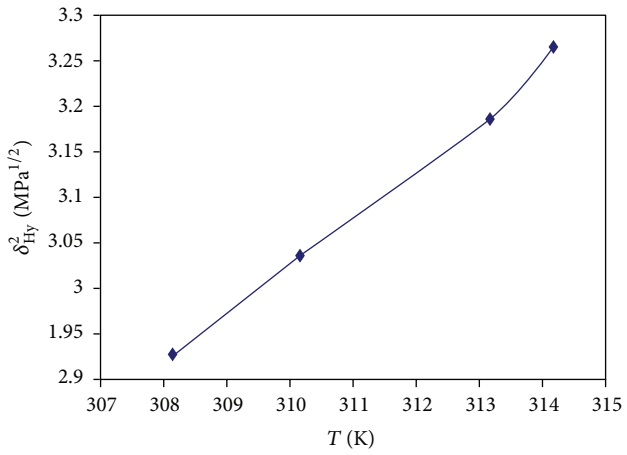

Figure 4: Plot of δ_{Hy} in function of T.

up to 20% complete renaturation is possibly in every case. The structure content is concentration dependent even at room temperature. It may be that intermolecular interactions induce additional alpha-helix structures, which are less stable than the ones stabilized by intramolecular interactions. Unfolding of the pocket containing the free -SH group of cysteine-34 enables disulphide bridges to be formed leading to stable aggregates and irreversible structural alterations. At temperatures below 65–70°C, oligomers are formed mainly via intermolecular beta structures.

Takeda et al. [43] studied the thermal denaturation of bovine serum albumin (BSA) at pH of 2.8 and 7.0 in the range of 2–65°C. They found, by the curve-fitting method of circular dichroism spectra, that the relative proportions of α-helix, β-structures, and disordered structures in the protein's conformation were determined as a function of temperature. With the rise of temperature at pH 7.0, the proportion of α-helix decreased above 30°C, and those of β-structures and disordered structures increased in the same temperature range. The structural change was reversible in the temperature range below 45°C. However, the structural change was partially reversible upon cooling to room temperature subsequent to heating to 65°C. On the other hand, the structural change of BSA at pH 2.3 was completely reversible in the temperature range of 2–65°C probably because the interactions between domains and subdomains disappear due to the acid expansion. The secondary structure of disulfide bridges-cleaved BSA remained unchanged during the heat treatment up to 65°C at pH of 2.8 and 7.0.

The Figure 3(a) is linear (i.e., an ideal system), and Figure 3(b) can be seen a nonideal system, which may be due to two effects: firstly a thermal effect and secondly a concentration effect, this is due to increase in temperature and increase in the BSA concentration in the system which causes departure from ideality.

As for the Hildebrand parameter, it remains constant from 309 to 313 K with a value of 1.37 MPa$^{1/2}$ because the gyration radius of BSA does not change with temperature, and the denaturation of the protein begins at 314 K. The principal utility of the Hildebrand parameter is that it provides simple predictions of phase equilibrium based on a single parameter that is readily obtained for most materials. These predictions are often useful for nonpolar and slightly polar (dipole moment <2 debyes) systems without hydrogen bonding. It has found a particular use in predicting solubility and swelling of polymers by solvents. The principal limitation of the solubility parameter approach is that it applies only to associated solutions ("like dissolves like" or positive deviations from Raoult's law): it cannot account for negative deviations from Raoult's law that result from effects such as solvation or the formation of electron donor-acceptor complexes. Like any simple, predictive theory it can inspire overconfidence: it is best used for screening with data used to verify the predictions.

BSA is a water-soluble protein that is widely used in the biochemical, pharmaceutical, and food industries. The molecule possesses two amino acids, one between repeating units and the other in the ring besides the many hydroxyl groups. Due to this interesting structure, BSA has the ability to form hydrogen bonds both within its own structure and with polar solvents, providing BSA significant solubility or a tendency to form molecular associations. The fact that BSA has hydrogen bonding within its own structure, it interacts with the solvent through hydrogen bonds and is an important fact to be taken into account in the BSA-water system. The solubility parameter may easily be determined for liquids from their heats of vaporization; however, for polymers this process is inapplicable due to their nonvolatility. Yet, polymer solubility parameter values may be evaluated by indirect methods, such as by finding the liquids that cause maximum swelling of a slightly crosslinked network of the polymer or that yield a maximum limiting viscosity number from which the δ value of this solvent may be lower that of the polymer. The alternative method is calculating δ from the group molar attraction constants.

Analyzing the hydrodynamic cohesion parameter "δ_{Hy}" (Figure 4), a light increase can be observed from 309 to 313 K (2.93–3.19 $MPa^{1/2}$); this is because the hydrodynamic radius of BSA changes with temperature. The hydrodynamic cohesion parameter is used for polar molecules and macromolecules with dipole moment >2 debyes (BSA dipole moment is ≈400 debyes, obtained by Curvale, unpublished data); this system may have hydrogen bonding. It has been particularly useful for predicting solubility and swelling of biopolymers in aqueous solvents. It can account for negative deviations from Raoult's law that result from effects such as solvation or the formation of electron donor-acceptor complexes. The value of δ_{Hy} is about twice larger than that of δ due to the degree of solvation of BSA.

Solubility in water also increased significantly when the temperature increased (Pace et al. [44]). These excellent water solubility properties are due to the exposure of treated samples to the strong mechanical forces which cause particles to collide over short-time intervals, changing their size and shape. This behavior was previously confirmed by a significant decrease in the particle size of proteins accompanied by an increase in the number of charged groups $-NH_3^+$; $-COO^-$. Consequently, enhanced protein-water interactions occurred since electrostatic forces are higher, and more water interacts with protein molecules.

5. Conclusions

The viscosity of BSA solutions at temperatures of up to 308 K, in a wide range of concentrations at pH values near the isoelectric point, may be quantitatively described by the generalized Van't Hoff equation. The generalized Van't Hoff equation can be used for dilute concentrations of polymers (a condition for using intrinsic viscosity). Given the difficulty of measuring the intrinsic viscosity at high concentrations, this work proposes the use of the η_i/c ratio and molar concentration for solving this difficulty. The η_i/c decreases with increases in temperature. Both quantities depend on temperature, and at the high temperature limit, the η_i/c agrees well with the theoretical values obtained for the random coil conformation. In the hydrodynamic cohesion parameter "δ_{Hy}" (Figure 4), a slight increase can be observed from 308 to 313 K (2.93–3.19 $MPa^{1/2}$); this is because the hydrodynamic radius of BSA changes with temperature.

Acknowledgments

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