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Salting-out effect in polypropylene glycol-amino acid aqueous solutions revealed by vapor pressure osmometry

Nosaibah Ebrahimi, Rahmat Sadeghi*

Department of Chemistry, University of Kurdistan, Sanandaj, Iran

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ABSTRACT

Precise vapor pressure osmometry measurements at $T = 303.15$, 308.15 and 313.15 K have been performed for low concentrations of different binary and ternary aqueous solutions including: amino acids (glycine, alanine, serine and proline) in pure water, PPG725 in pure water and amino acids in aqueous solutions of 0.01 w/w PPG725. In order to study the salting effects occurring in polymer-amino acid aqueous systems, deviations from the semi-ideal behavior have been evaluated. The results indicate that because of unfavorable interactions between PPG and amino acids, they are preferentially hydrated in aqueous media. Therefore, the amount of free water molecules in the PPG + amino acid + water solutions is less than that expected based on the semi-ideal behavior. Hence, under condition that solutes molalities in the ternary solutions are the same as those in the corresponding binary solutions, the values of $a_w + 1 - \left(a^{\circ}_{wp} + a^{\circ}_{wa}\right)$ and $\Delta p - (\Delta p^{\circ}_p + \Delta p^{\circ}_a)$ are negative and become more negative by increasing temperature and hydrophilicity of amino acids side chain.

1. Introduction

Polymer–amino acid–water systems are the simplest model for studying the effect of polymers on the very complex tertiary structure of proteins in aqueous media. The polymer influence on amino acid aqueous solutions covers various properties and allows for elaborating molecular interactions underling protein stability, denaturation and enzyme activity. Further, distinct pairs of polymers and amino acids in aqueous solutions enable of forming aqueous biphasic systems (ABS) above the certain concentrations [1], this fact deserves accurate thermodynamic investigations of these mixtures. Since both equilibrium phases in ABS are mainly composed of water, liquid-liquid extraction utilizing ABS presents a powerful technique for purification and enrichment of biomolecules, cells, cell particles, anions, cations and et cetera [2–4]. Thermodynamic properties of polymer–amino acid aqueous systems (specially their water activity which is a pivotal solution property and closely related with the other thermodynamic properties) are necessary for a fundamental understanding of phase-forming behavior of these systems and also for suitable design of industrial separation process dealing with them. In this regard, several publications have only focused on volumetric and compressibility properties of polymer–amino acid aqueous solutions [5–8]. In contrast, despite its importance, no attention has been devoted to the water activity of these systems; except our previous publication [1] which reports water activities for ternary {polymer (polyethylene glycols (PEG400, PEG2000, PEG6000 and PEG10000) and polypropylene glycol 400 (PPG400)) + amino acid (alanine and glycine) + water} systems at 298.15 K via the isopiestic method at high concentrations. Vapor pressure osmometry (VPO) is a very precise, reliable and cheap (low material and low time consuming) technique for measuring water activity of aqueous solutions containing non-volatile solutes over wide concentration and temperature ranges [9,10]. However, until now VPO has not been applied to polymer – amino acid aqueous mixtures.

The comparison study of water activity data for a given ternary A + B + water system and its corresponding binary A + water and B + water solutions, can provide some valuable information about salting effect produced by addition of one solute to aqueous solution of another solute, and thus stipulate phase-forming ability of these systems. In continuation of our previous studies on the polymer-amino acid aqueous systems [1,8] herein, the VPO method has been used to determine water activities of ternary PPG725 + amino acid (alanine, glycine, serine, proline) + water and corresponding binary PPG725 + water and amino acid + water systems at different temperatures and low solute concentrations.

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* Corresponding author.
E-mail addresses: rahsadeghi@yahoo.com, rsadeghi@uok.ac.ir (R. Sadeghi).

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The results have been discussed based on salting-out phenomenon occurring in these systems.

2. Experimental section

2.1. Material

The properties of chemicals used in this work along with their purification method were listed in Table 1. Double distilled and deionized water was used. Chemical structures of the amino acids and PPG are sketched in Scheme 1.

2.2. Methods

Solutions were prepared by mass with the use of a Sartorius CP124S balance precisely within $\pm 1.10^{-7}$ kg. The measurements of water activity were performed at 303.15, 308.15 and 313.15 K by means of an Osmomat K-7000 (Knauer Inc.). The cornerstone of instrument is two thermistors which are part of a Wheatstone bridge and measure resistance changes caused by changes in temperature. The two thermistors are located in a glass beaker enriched with water vapor; this collection forms the measuring cell. The cell temperature is electronically controlled and maintained with an accuracy of $\pm 1.10^{-3}$ K. First, with the help of a micro-syringe one drop of pure water is located on either thermistor, after receiving equilibration the measurement value is adjusted to zero. Afterward, the pure water on one thermistor is replaced by the under study aqueous solution (special care should be taken to keep the drop size and shape equal on both thermistors). This replacement leads to a vapor pressure difference between the two droplets, hence some vapor of pure water that saturated the gas phase condenses on the solution droplet until the vapor pressures are balanced. This process warms the thermistor containing aqueous solution due to the heat of condensation. Differential measurements of resistances arising from temperature differences at the thermistors are accomplished. Once equilibrium is reached a constant measurement value is achieved (generally a time of 4–8 min suffices to reach it). Initially, the instrument was calibrated using aqueous sodium chloride solutions as reference with known osmotic coefficients, and from which a correlation between the panel readings and respective concentrations of sodium chloride solutions was determined at different temperatures. Then in the same conditions, the panel readings were specified for the studied aqueous solution including binary (polymer + water) and (amino acid + water) and ternary (polymer + amino acid + water) systems. For each solution, 10 determinations (zero point adjustment after 5 determinations and repeating) were performed and the mean value was calculated. Generally, the relative deviations from the mean value were less than 1%, otherwise the measurements were repeated. At the end of measurements, the calibration was controlled again to exclude any base-line drifts of the apparatus. For a certain aqueous solution which has the same instrument reading as a sodium chloride solution with molality m_{NaCl}, the water activity, a_w, was obtained through the following equation:

$$a_w = \exp(-0.001\nu_{\mathrm{NaCl}} m_{\mathrm{NaCl}} \Phi_{\mathrm{NaCl}} M_w) \quad (1)$$

where ν_{NaCl} is the stoichiometric number of reference electrolyte and M_w is the molar mass of water. Φ_{NaCl} is the osmotic coefficient for aqueous solution of NaCl with molality m_{NaCl} calculated from the correlation of Clarke and Glew [11]. The uncertainty in the measurement of water activity was found to be better than $\pm 3.10^{-5}$. From the water activity values, the vapor pressures of aqueous solutions, p, were determined according to:

$$\ln(a_w) = \ln\left(\frac{p}{p^*}\right) + \frac{\left(B - V_w^*\right)(p - p^*)}{RT} \quad (2)$$

where B is the second virial coefficient of water vapor that calculated using the equation provided by Rard and Platford [12]. p^* is the vapor pressure of pure water which calculated using the state equation of Saul and Wagner [13]. V_w^*, R and T are the molar volume of liquid water, gas constant and absolute temperature, respectively.

3. Result and discussion

This work follows two main objectives: (i) the presentation of new, reliable and so far unavailable experimental water activity data at different temperatures for dilute solutions including PPG725 in water, amino acid (alanine, glycine, serine and proline) in water and amino acid in aqueous solution of 0.01 w/w PPG725 (mass fraction of PPG725 in solvent is 0.01, means that monomer molality of polymer in solvent is 0.1739 mol kg^{-1}), (ii) the study of salting effects occurring in PPG-amino acid aqueous solutions according to deviations from the semi-ideally behavior. It is necessary to note that the control of pH of the PPG-amino acid aqueous systems investigated in this work, indicates that the pH value of these solutions is about 5.5–6.5, which is close to isoelectric points of the amino acids. Therefore in these systems amino acids are in their zwitterionic form. The reason for selection dilute concentration region in this study is that PPG725 has low water solubility and besides ternary mixtures of PPG725 + amino acid + water become biphasic by increasing concentration of solutes. Therefore based on our previous data about phase diagrams of PPG-amino acid aqueous systems [1], dilute concentration region which corresponds to homogenous systems has been selected for study.

Tables 2 and 3 present the experimental water activity and vapor pressure values of the investigated binary and ternary systems at 303.15, 308.15 and 313.15 K. According to our survey, vapor-liquid equilibria properties of ternary PPG725-amino acid aqueous solutions are being reported for the first time. Although water activities of binary amino acid + water solutions have been extensively investigated [14–19], there are so scarce water activity data for dilute regions that we studied here (0.004 mol kg^{-1} < molality of amino acid (m_a) < 0.19 mol kg^{-1}). As shown in Fig. 1, there is a good

Table 1
Specification of chemicals used in this work.

Chemical	10^3 molar mass/kg mol^{-1}	Source	Purification method	Mass fraction purity
Glycine	75.07	Merck	None	0.997
S (+)-alanine	89.09	Merck	None	0.990
L-serine	105.09	Merck	None	0.990
L-proline	115.13	BDH	None	0.990
PPG	725	Aldrich	None	
Sodium chloride	58.44	Merck	Was dried in an electrical oven at about 110 °C for 24 h prior to use	≥0.995