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Liquid–liquid equilibria, electrical conductivity, and refractive indices of Poly(ethylene glycol) + sodium sulfate + guanidine hydrochloride aqueous two-phase systems: Correlation and thermodynamic modeling

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ABSTRACT

The initial recovery steps for recombinant protein were carried out using aqueous two-phase systems in the presence of guanidine hydrochloride. To investigate the phase behavior of such systems, equilibrium data was obtained for molecular weight fractions of PEG (3000 and 6000) + sodium sulfate (pH 7) + guanidine hydrochloride (5% and 10% mass) + water at 25 °C. Displacement of the binodal toward higher concentrations through an increase in guanidine hydrochloride concentration was clearly observed. A slightly larger two-phase was obtained as the PEG molecular weight increased from PEG 3000 to 6000. The experimental binodal data of the system was successfully correlated with Bleasdale equations (high R^2 and low SE). The effects of molecular weight, guanidine hydrochloride concentration, tie-line length, and slope of tie-line on the partition behaviors of guanidine hydrochloride were examined at constant pH. The compositions of each aqueous phase were calculated using two thermodynamic models for the activity coefficient. The calculated results showed that the extended Pitzer and extended UNIQUAC models can predict the data in the quaternary system well. The fitted binary interaction parameters of the models were reported.

1. Introduction

Solubilization of the inclusion bodies and refolding of the aggregated proteins into bioactive form is difficult. The proteins purification from inclusion bodies consists of four major steps including isolation of purified inclusion bodies, solubilization of inclusion bodies, refolding of solubilized proteins and purification of refolded proteins. The stages 2 and 3 are the most crucial steps in the recovery of bioactive protein from inclusion bodies. Guanidine hydrochloride (GuHCl) and urea are examples of such denaturants [1]. Urea is a powerful protein denaturant that disrupts non-covalent bonds in proteins. GuHCl is a strong denaturant that can coat the exterior of the protein and which, above certain protein-specific concentrations, can fully denature a protein [2]. It is preferred to urea because urea solution can contain and spontaneously produce cyanate [3], which can carbamylate the amino groups of the protein [4].

The aqueous two-phase system (ATPS) is an efficient procedure for refolding proteins to produce recombinant protein in the presence of denaturants [5,6]. Studies have applied GuHCl and urea in ATPSs as the initial recovery step [6–8], but few have focused on the complex problem of how denaturants affect phase diagram behavior or determined their partition coefficients and the thermodynamics behavior for an ATPS. Determining the phase equilibria composition in an ATPS is necessary for separation of biomolecules in industrial processes. The use of proper mathematic and thermodynamic models helps prevent expensive and time-consuming experimentation under different operational conditions.

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The theoretical models regarding ATPS can be divided to three groups. The first group of models is based on lattice theory. These molecules are set in a regular lattice and the enthalpy of mixing is calculated from their interaction with near-neighbor molecules [9]. The second group contains the models based on local composition of some models of this group, including NRTL [10], UNIQUAC [11], and Wilson [12]. More developed models of this group are MNRTL-NRF [13], UNIQUAC-NRF [14], and the modified Wilson [15,16]; these have been extended in recent years. The third group consists of models that calculate the phase behavior of ATPS based on the virial osmotic theory proposed by McMillan and Mayer [17], the Hill theory [18], the solution the VERS model [19], and extension of the Pitzer model [20].

One reason for the rare use of ATPSs in industry is the lack of knowledge about phase diagrams, thermodynamic properties, and partition coefficients. This lack of knowledge has attracted the attention of researchers in recent years [21,22]. The current study obtained phase equilibrium data for PEG (3000, 6000) + sodium sulfate + GuHCl (5% and 10% mass) + water at 25 °C and pH 7. The effect of PEG/salt ratio, GuHCl concentration, tie-line length, and slope of tie-line on the partition behaviors of GuHCl, were examined at constant pH.

The extended Pitzer and extended UNIQUAC models were applied to model the liquid–liquid equilibrium (LLE). Modeling involved the use of activity coefficients, but, as this LLE system is strongly non-ideal, the use of appropriate binary interaction parameters appeared necessary. These models were applied successfully to correlate the experimental LLE data. It is hoped that the results increase useful knowledge of aqueous two-phase separation and improve the yield of protein refolding.

2. Experimental

2.1. Materials

Poly(ethylene glycol) with a mass average of 3000 and 6000, sodium sulfate (anhydrous GR for analysis >99%), sodium hydroxide (NaOH; mass purity >0.99) and sulfuric acid (95%–97% H_2SO_4, GR for analysis >95.0%) were obtained from Merck (Germany) and used without further purification. GuHCl was purchased from Sigma–Aldrich. Distilled deionized water was used for the preparation of solutions. All other materials were of analytical grade. Additional details, such as final mole fraction purity or purification methods given in Table 1.

2.2. Apparatus and procedure

The biphasic systems were prepared by mixing PEG, sodium sulfate, and GuHCl at the optimal pH from data reported by Rämesh et al.(1999) [8], after which the composition of the mixture (mass) was determined. Feed samples (10 g) were prepared by mixing appropriate amounts of polymer, salt, GuHCl, and water in 15 ml graduated cylinders utilizing an analytical balance (A&D GF 300; Japan) with a precision of $\pm 10^{-4}$ g at 25 °C. To achieve a constant temperature (25 °C) with an uncertainty of 0.05 °C, the tubes were placed in a thermostatic bath (Memert INE 400; Germany). The pH of the salt solutions was adjusted by mixing the appropriate ratio of sodium sulfate, sodium hydroxide, and sulfuric acid. The pH of the solutions was measured precisely using a Metrohm 827 pH lab meter (Switzerland).

For each system, 5% and 10% (w/w) samples of GuHCl were prepared. The contents of the test tubes were then rigorously vortexed for 10 min before being placed in a 25 °C thermostatic bath for 2 h. To separate the resulting phases, the test tubes were centrifuged (Hermle Z206A; Germany) at 6000 rpm for 5 min. The phases showed no turbidity and the top and bottom samples could be easily separated.

The electrical conductivity and refractive index of each sample were measured at 25 °C using a Jenway 4510 with a precision of 0.01 mS to 1 mS and a refractometer (CETI; Belgium) with a precision of 0.0001 nD, respectively. All data measurements were conducted in duplicate and the average values were reported. The salt concentration (Na_2SO_4) was determined by atomic absorption spectroscopy (Shimatsu AA-6300; Japan). The calibration plots for refractive index and conductivity were prepared for the known polymer and denaturant compositions for the individual salt concentrations at 25 °C and the measured values interpolated. The average relative deviation of denaturants and polymer concentration by this method was about 0.1% (wt).

3. Thermodynamic framework

In the present study, the excess Gibbs energy of studied quaternary system consisted of water + polymer + salt + GuHCl, which is calculated as:

$$G^E = G^{E,LR} + G^{E,SR} \tag{1}$$

where $G^{E,LR}$ denotes the long-range and $G^{E,SR}$ denotes the short-range interaction contribution of the excess Gibbs free energy. The activity coefficient of each component in the mixture in Eq. (1) can be calculated as:

$$\ln \gamma_i = \ln \gamma_i^{LR} + \ln \gamma_i^{SR} \tag{2}$$

where the extended Debye–Hűckle [23] equation is used to calculate the long-range activity coefficient. The extended UNIQUAC model [24] and extended Pitzer model were applied separately to obtain the short range activity coefficient of each component.

3.1. Long-range interaction contribution

The long range coefficient of non-ionic components such as the water–polymer is calculated as:

Table 1
Sample provenance table.

Chemical name	Source	Initial mole fraction purity	Purification method	Final mole fraction purity	Analysis method
PEG[a] 3000 and 6000	Merck	0.95	Two phase separation	0.999	Calibration curve
sodium sulfate	Merck	0.99	Two phase separation	0.9997	AAS[c]
sodium hydroxide	Merck	0.99	none	–	Mass balance
sulfuric acid	Merck	0.95–0.97	none	–	Mass balance
GuHCl[b]	Sigma–Aldrich	0.98	Two phase separation	0.999	Calibration curve

[a] Poly(ethylene glycol).
[b] Guanidine Hydrochloride.
[c] Atomic absorption spectroscopy.