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Amino acids partitioning in aqueous two-phase system of polypropylene glycol and magnesium sulfate

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Abstract

The counter-current chromatography method using aqueous two-phase systems, which is a form of liquid–liquid partition chromatography, could be applied for separation of the amino acids. This method needs some information about the partition coefficient of the amino acids in such systems. In this work, partitioning of amino acids D-alanine, L-valine and L-leucine was investigated in aqueous two-phase system of polypropylene glycol (PPG425) + $MgSO_4$ + H_2O at 298.15 K. The results showed that increasing the amino acid hydrophobicity lead to a corresponding increase in the partition coefficients and increasing tie line length lead to decreasing partition coefficients. The effect of the pH on amino acids partitioning was also determined. The experimental data are correlated using a modified virial-type model. The comparisons between the correlation and the experimental data reveal a good agreement.

Keywords: Aqueous two-phase system; Polypropylene glycol; Amino acids; Partition coefficient; Magnesium sulfate

1. Introduction

Aqueous two-phase systems (ATPS) are discussed for the separation and extraction of biomolecules, like, amino acids, peptides and enzymes from aqueous phases [1,2]. These two-phase systems can be made from aqueous solutions of two water-soluble polymers or a polymer and a salt. Poly(ethylene glycol) (PEG) is often used in this technique. The most widely studied partitioning of amino acids are those systems formed by PEG and salts [3–5]. Poly(propylene glycol) (PPG) is a polymer that is structurally closely related to PEG. In our previous works, the liquid–liquid equilibrium (LLE) data for the systems composed of PPG have been determined [6]. Using these solvent systems, several different approaches have been made for performing purification of biological samples, such as single step partitioning, repetitive batch extraction, counter-current distribution (CCD) and counter-current chromatography (CCC) [7]. CCC is a form of liquid–liquid partition chromatography. The unique feature of CCC among other chromatographic systems is derived from the fact that the method uses no solid support and the stationary phase is retained in the column by an Archimedes screw effect and the centrifugal force. Consequently, the system eliminates various complications arising from the use of solid supports. The cross-axis coil planet centrifuge (CPC) allows satisfactory levels of stationary phase retention for the aqueous–aqueous polymer phase systems so that it can be effectively used for the preparative separation of proteins [8]. With some PEG-salt systems, this method has been used successfully for the separations of a variety of protein samples [9–11]. The CCC method using aqueous two-phase systems could also be applied for separation of the amino acids. For achieving efficient purification, it is essential to obtain the partition coefficient of the amino acids in two-phase solvent systems by selecting a proper condition for the system. In continuation of our investigations on thermodynamic properties of aqueous PPG425 + salt mixtures [12–14], the present contribution reports experimental results for the partitioning of amino acids L-valine, D-alanine and L-leucine in aqueous two-phase system of PPG425 and $MgSO_4$ at 298.15 K. The effect of tie line length and pH on the partition coefficients of amino acids has been studied in detail. The experimental data on the phase behavior and amino acids partitioning were correlated using an excess Gibbs energy

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virial-type model modified in order to account for coulombic interactions.

2. Experimental

2.1. Chemicals

The poly(propylene glycol), of molecular weight 425 g mol^{-1}, was obtained from the Aldrich. Magnesium sulfate was obtained from Merck (GR. Min 99.5%). D-Alanine, L-valine and L-isoleucine were also purchased from Merck.

2.2. Preparation of phase systems

The experiments were performed in 40 ml glass vessel. Aqueous two-phase systems were prepared from stock solutions of the single solutes, polypropylene glycol, magnesium sulfate and amino acid. The pH of the samples has been increased with a 1 M aqueous sodium hydroxide solution and measured with a precision pH meter (744 Metrohm). The total weight of these components was about 30 g. The concentration of each amino acid in each vessel was about 1 g/L. The mixture was shaken for about 30 min and then placed in a thermostated water bath for at least 12 h to ensure complete equilibration, as indicated by the absence of turbidity in each phase. A thermostat maintained the temperature of the bath at 298.15 K. After equilibration of the systems, the samples of approximately 10 ml from the upper and lower phases were taken out carefully for analysis using syringes.

2.3. Analysis of samples

The concentration of $MgSO_4$ was determined by magnesium analysis using atomic absorption spectroscopy (AAS). The AAS measurements were carried out with Shimadzu Atomic Absorption Spectrophotometer AA-670 G. The average relative deviation of the weight percent of salt by this method is about 0.2%. The concentration of PPG was determined from refractive index measurement performed at 298.15 K using Mettler TOLEDO refractometer with temperature control (RE50). The precision of the refractive index determination was 0.00001 refractive index units. Since the refractive index of the phase samples depends on the PPG and salt concentrations, calibration plots of refractive index versus polymer concentration were prepared for different concentrations of salts. The average relative deviation of the weight percent of PPG by this method is about 0.4%.

The amino acid concentrations were determined by HPLC-method with a HPLC system KNAUER, a k-1001 HPLC pump, a k-1500 degasser-solvent organizer, a TRIATLON autosampler, a RF-10 AXL fluorescence detector, a Eurospher-100 column.

3. Thermodynamic framework

The excess Gibbs energy model used to correlate the experimental data obtained was based on the model proposed by others, in which the correlation of ATPS and insulin partition coefficient were employed [15,16]. In this model short-range term of the excess Gibbs energy is given by

$$\frac{1000}{n_w M_w}\frac{G^{E,SR}}{RT} = \sum_i \sum_j A_{ij} m_i m_j \quad (1)$$

where M_w is the molecular mass of water (in g mol^{-1}), n_w is number of moles of water, A_{ij} is the second virial coefficient or interaction parameter related to species i and j and m is the molality of species i or j.

The long-range term is a modified Debye-Hückel term written as:

$$\frac{1000}{n_w M_w}\frac{G^{E,LR}}{RT} = -\frac{4A_\gamma I}{b}\ln(1 + b\sqrt{I}) \quad (2)$$

where b is a constant equal to 1.2 kg$^{1/2}$ mol$^{-1/2}$ and A_γ for solvent water at 298.15 K is 0.3914 kg$^{1/2}$ mol$^{-1/2}$. The ionic strength I is calculated as:

$$I = \frac{1}{2}\sum_i m_i Z_i^2 \quad (3)$$

where Z_i is the charge of ion i.

The partition coefficient is calculated for the infinite dilution limit. Defining the partition coefficient as:

$$K = \frac{C_a^{top}}{C_a^{bottom}} \quad (4)$$

where the subscript a stands for amino acid. Using the equilibrium criteria, this relation can be written as

$$K = \frac{\gamma_a^{bottom}}{\gamma_a^{top}} \quad (5)$$

where γ is the activity coefficient. With the infinite dilution limit:

$$K = \frac{\gamma_a^{\infty,bottom}}{\gamma_a^{\infty,top}} \quad (6)$$

The activity coefficient of amino acid can be straightforwardly obtained from the excess Gibbs energy model through the activity coefficients evaluated from the thermodynamic expression:

$$\ln\ \gamma_a = \frac{1}{RT}\left(\frac{\partial G^E}{\partial n_a}\right)_{T,P,n_{i\neq a}} \quad (7)$$

Using the above expression, the activity coefficient of salt (γ_s) and polymer (γ_p) can also be obtained.

4. Results and discussion

First, it has to be noted that the influence of the pH and the concentration of dissolved amino acids on the phase behavior of the aqueous two-phase systems can be neglected. The analysis of polymer and salt for the phase system of PPG + $MgSO_4$ + D-alanine + water confirm this subject. Because this, most polymer and salt concentrations were not measured for these systems, and all were extrapolated from the corresponding systems without

Table 1
Compositions of PPG425 (1) + $MgSO_4$ (2) + H_2O (3) two-phase system at 298.15 K

Total system			Top phase			Bottom phase		
$100w_1$	$100w_2$	$100w_3$	$100w_1$	$100w_2$	$100w_3$	$100w_1$	$100w_2$	$100w_3$
22.7	4.6	72.7	31.0	2.2	66.8	11.6	8.4	80.0
26.3	6.3	67.4	49.3	0.2	50.5	6.1	12.6	81.3
31.2	7.7	61.1	55.7	0.1	44.2	3.0	15.5	81.5
40.9	11.0	48.1	70.3	0.1	29.6	2.5	20.3	77.2

Table 2
Partition coefficients of D-alanine in aqueous two-phase system of PPG425 + $MgSO_4$ + H_2O for various pH at 298.15 K

TLL	*K* (pH 3.15)	*K* (pH 6.01)	*K* (pH 9)
20.29	0.39	0.43	0.47
45.02	0.18	0.17	0.22
54.81	0.09	0.07	0.17
70.78	0.02	0.03	0.04

Table 3
Partition coefficients of L-valine in aqueous two-phase system of PPG425 + $MgSO_4$ + H_2O for various pH at 298.15 K

TLL	*K* (pH 3.15)	*K* (pH 5.97)	*K* (pH 9)
20.29	0.53	0.48	0.37
45.02	0.14	0.23	0.26
54.81	0.17	0.12	0.23
70.78	0.06	0.11	0.12

amino acids. Table 1 gives four experimental equilibrium compositions for the PPG425 + $MgSO_4$ + H_2O system at 298.15 K, which determined in this work. This system has been studied previously in detail [6].

The experimental results for the partition coefficients, K, of D-alanine, L-leucine and L-valine at various pH are listed in Tables 2–4 and shown schematically in Figs. 1–3. They are expressed as a function of the tie line length (TLL), calculated by the following equation:

$$\mathrm{TLL} = \sqrt{(w_s^{top} - w_s^{bottom})^2 + (w_p^{top} - w_p^{bottom})^2} \quad (8)$$

where w_s and w_p are weight percent of the salt and polymer in the top or bottom phases. Figs. 1–3 show the dependence of partition coefficients for all amino acids on the TLL for various pH. The fluctuation in data could be partly due to specific properties of the phase systems, such as the very small density difference, and experimental errors that result from these properties. These

Table 4
Partition coefficients of L-leucine in aqueous two-phase system of PPG425 + $MgSO_4$ + H_2O for various pH at 298.15 K

TLL	*K* (pH 3.15)	*K* (pH 5.98)	*K* (pH 9)
20.29	1.16	0.53	0.46
45.02	0.84	0.29	0.34
54.81	0.50	0.21	0.27
70.78	0.11	0.15	0.05

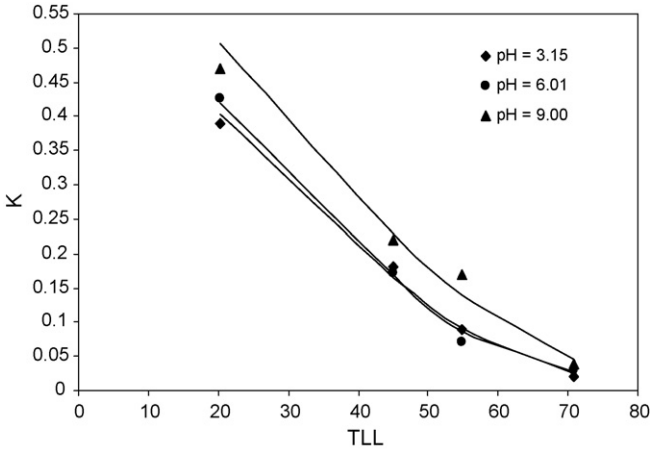

Fig. 1. Comparison of experimental results (♦, ●, ▲) and calculated (solid lines) partition coefficient for D-alanine, as a function of the tie line length for various pH at 298.15 K.

systems with extremely small physical phase differences make taking and handling samples very difficult. Small fluctuations in temperature and pressure and also minor shaking of the vessel that is inevitable when taking it out of the water bath or when inserting a syringe, can result in minor remixing of the phases or entrainment of droplets of one phase into another.

An important property influencing partition coefficients of amino acids is hydrophobicities of the amino acids. Nozaki and Tanford [17] calculated a hydrophobicity scale for amino acids based on the free energy of transfer of amino acid side chains

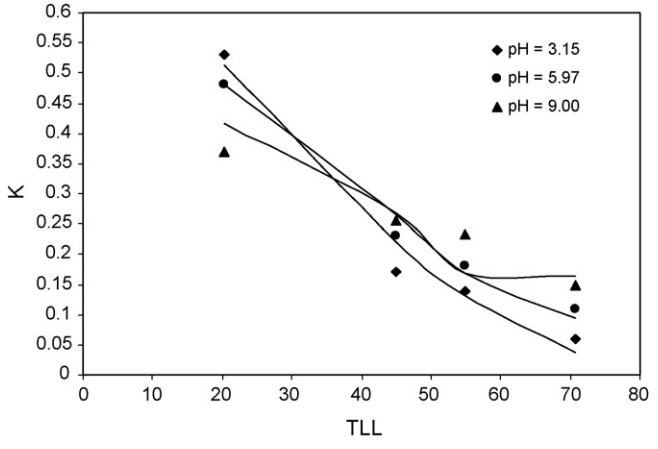

Fig. 2. Comparison of experimental results (♦, ●, ▲) and calculated (solid lines) partition coefficient for L-valine, as a function of the tie line length for various pH at 298.15 K.

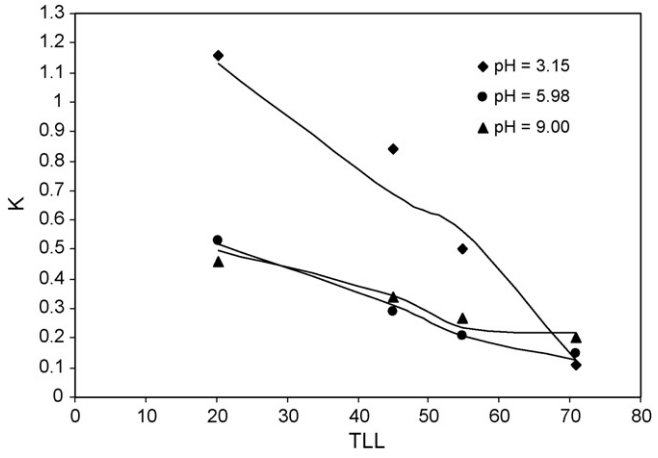

Fig. 3. Comparison of experimental results (♦, ●, ▲) and calculated (solid lines) partition coefficient for L-leucine, as a function of the tie line length for various pH at 298.15 K.

from an organic solvent to water. They concluded that larger is the hydrophobicity of an amino acid, larger is the affinity for the more hydrophobic PPG-rich phase, and consequently, larger is the partition coefficient. Experimental studies on partitioning of amino acids in polymer-salt aqueous two-phase systems have proven the validity of such a statement [18]. Amino acid partitioning studies in other phase systems, such as water–butanol mixtures show similar results [19].

The results in the present work also show the same dependence of amino acid partition coefficients on hydrophobicity. The hydrophobicity of the amino acids decreases as follows: L-leucine > L-valine > D-alanine. As expected, the longer a tie line, the more extreme the partition coefficients. Also, the larger the hydrophobicity, the larger partition coefficient (Tables 2–4 and Figs. 1–3). As we can see from these figures, the effect of pH has not a regular trend on the partition coefficients.

The optimal values of the second virial coefficient between amino acids and other compounds (polymer and salt), $A_{p\text{-}a}$ and $A_{s\text{-}a}$, were obtained by minimizing the following objective function:

$$\mathrm{OF} = \sqrt{\frac{\sum_{n}(K_i^{\mathrm{exp}} - K_i^{\mathrm{calc}})^2}{n}} \tag{9}$$

The partition coefficients (K_i^{calc}) were calculated according to Eq. (6) and the results for optimised virial coefficients are presented in Table 5. The calculated partition coefficients for different systems and different pH at 298.15 K are shown in Figs. 1–3. The calculated partition coefficients are reasonable and agree with the experimental values to within the experimental uncertainty of the measurement.

The model interaction parameters of polymer–polymer ($A_{p\text{–}p}$), polymer–salt ($A_{p\text{–}s}$) and salt–salt ($A_{s\text{–}s}$) were estimated by the minimizing the following objective function:

$$\mathrm{OF} = \sum_{P}\sum_{l}\sum_{i}(m_{P,l,i}^{\mathrm{Calc}} - m_{P,l,i}^{\mathrm{Exp}})^2 \tag{10}$$

Table 5
Amino acid interaction parameters in PPG + $MgSO_4$ + H_2O systems at 298.15 K

	pH	$A_{p\text{–}a}$ (kg mol^{-1})	$A_{s\text{–}a}$ (kg mol^{-1})
L-Leucine	3.15	0.892	1.328
	5.98	−0.104	−1.228
	9.00	−0.353	−1.610
L-Valine	3.15	0.323	−0.717
	5.97	−0.097	−1.349
	9.00	−0.484	−2.076
D-Alanine	3.15	0.180	−1.224
	6.01	0.110	−1.381
	9.00	0.230	−0.853

where $m_{p,l,i}$ is the molality of the component i in the phase p for lth tie line. LLE data of Table 1 were correlated using Eq. (10) and the equilibrium condition:

$$(m_i\gamma_i)^{\mathrm{top}} = (m_i\gamma_i)^{\mathrm{bot}} \tag{11}$$

The fitting parameters of the model were obtained as: $A_{p\text{–}p} = -0.785$, $A_{p\text{–}s} = -0.465$ and $A_{s\text{–}s} = 0.433$ kg mol^{-1}.

5. Conclusion

The partition coefficients of three amino acids L-leucine, L-valine and D-alanine were obtained in aqueous two-phase system of PPG425 + $MgSO_4$ + H_2O at 298.15 K. The effect of pH and tie line length on amino acids partitioning was also determined. It was verified that larger is the hydrophobicity of an amino acid, larger is the affinity for the more hydrophobic PPG-rich phase, and consequently, larger is the partition coefficient. Consequently, the longer a tie line length, the more decrease in the partition coefficients. These results are useful for selecting the best condition for CCC purification. The experimental data on the phase behavior and amino acids partitioning were correlated reasonably well by a modified excess Gibbs energy virial-type model.

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