VI Congresso Brasileiro de Termodinâmica Aplicada (CBTermo 2011)
22 a 25 de novembro de 2011
Salvador - Bahia - Brasil

Programação CBTermo 2011

22/11 - Noite

Horário	Evento
18:30	Cerimônia de abertura
19:00	Mesa redonda: Perspectivas de Inovação na Indústria Brasileira e o Papel das Universidades. Participantes: Paulo Coutinho (Braskem-IDEOM); Rogério Espósito (Petrobrás); Fernando P. Pessoa (UFRJ); Mediador: Frederico W. Tavares (UFRJ).
21:00	Coquetel

23/11 - Manhã

Horário	Evento
8:30-9:30	**Palestra Prof. Rafiqul Gani**: Property Modelling for Applications in Chemical Product and Process Design Sessão orientada para estimação e determinação de propriedades.
9:30-9:50	066 A new two stage recombination process for crude oil. Simulation and experiments. Papa Matar Ndiaye, Mohamed Nakoua, Marcelo Castier, Abdulrazag Y. Zekri, Reyadh A. Almehaideb
9:50-10:10	157 Novo modelo de coeficiente de atividade que mescla uma teoria de superfícies de contato e contribuição de grupos. Renan Pereira Gerber, Rafael de Pelegrini Soares
10:10-10:30	228 Modelagem de temperatura de ebulição, densidade, índice de refração e viscosidade de séries de n-alquil-hidrocarbonetos por propriedades assintóticas. Márcio L.L. Paredes, Josinira A. Amorim

Horário	Evento
8:30-9:30	**Palestra Prof. João A.P. Coutinho**: Ionic Liquids Aqueous Two Phase Systems for the Extraction and Purification of Biomolecules Sessão orientada para biomoléculas e biotecnologia
9:30-9:50	125 Fluidic and thermodynamic insights on the formation of polymeric microcapsules by using micromixers. Luizildo Pitol-Filho, Carles Torras, Josep Bonet-Avalos, Ricard García-Valls
9:50-10:10	102 Equilíbrio sólido-líquido da amoxicilina e da p-hidróxifenilglicina em água pelo método analítico. Italla Medeiros Bezerra, Osvaldo Chiavone Filho, Silvana Mattedi
10:10-10:30	035 Estudo sobre a relação entre o segundo coeficiente virial, a concentração do reagente precipitante e a solubilidade de proteínas em solução aquosa. Luís Fernando Mercier Franco, Pedro de Alcântara Pessôa Filho
10:30-11:00	Coffee-break
11:00-11:20	140 Extração de compostos com ação antifúngica de folhas de Senna Reticulata com CO2 supercrítico. Max Adilson Lima Costa, Fernanda Pereira Barbosa, Naimy Farias de Castro, Ademir Castro e Silva, Paulo de Tarso Vieira e Rosa, Everson Alves Miranda
11:20-11:40	195 Análise da Influência da presença de frutose no equilíbrio líquido-vapor da solução água e etanol. Carlos Eduardo Crestani, André Bernardo, Caliane Bastos Borba Costa, Marco Giulietti
11:40-12:00	120 Biosorção de azo corantes utilizando Spirulina platensis: Equilíbrio e termodinâmica. Guilherme L. Dotto, Mery Luiza G. Vieira, Vanessa M. Esquerdo, Luiz A.A. Pinto
12:00-12:20	012 Liquid-liquid equilibrium of water + 1-butanol + amino acid (glycine or dl-alanine or l-leucine). Geormenny Rocha dos Santos, Danilo C. Souza, Martín Aznar, Simão P. Pinho, Eugénia A. Macedo

12:20-14:20 Almoço

24/11 - Tarde

Horário	Evento
14:20-15:00	**Palestra Prof. Ernesto Pinheiro Borges**: Termodinâmica não extensiva - Alguns conceitos fundamentais e evidências observacionais Sessão orientada para modelagem molecular
15:00-	049 Produção de hidrogênio a partir de GLP: avaliação do uso da modelagem molecular na obtenção de dados termodinâmicos. Priscila Pereira Silva, José

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LIQUID-LIQUID EQUILIBRIUM OF WATER + 1-BUTANOL + AMINO ACID (GLYCINE OR DL-ALANINE OR L-LEUCINE)

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Amino acids play an important role both in animal metabolism and in industrial processes. Since they are rarely found in nature in a free form, they must be obtained from hydrolysis of protein-containing materials, or by fermentation. These production methods often result in aqueous mixtures containing various solutes including several types of amino acids. As a consequence, the cost of the separation processes and concentration of biomolecules from the media can be as high as 90 per cent of their total manufacturing cost. In this way, the design of such processes requires the knowledge of the partitioning behaviour in two-phase systems and thermodynamic models should support this optimization procedure. However, both the complexity of biomolecules, due to their multiple functional groups, and the crucial role of water, make the standard available thermodynamic models unattractive for the computation of phase behaviour. Therefore, new thermodynamic models for the description of the phase behaviour of systems containing biomolecules are required.
In this work, liquid–liquid equilibria (LLE) of the ternary systems 1-butanol/water/amino acid (glycine, DL-alanine or L-leucine) were measured at 40 ^{o}C. The experimental results were correlated and interaction parameters for the modified-NRTL, UNIFAC and UNIFAC-Campinas models were estimated.

Keywords: liquid-liquid equilibrium, amino acid, alcohol, experimental, modeling

Introduction

Biotechnology has progressed much in recent years, representing the most advantageous method for obtaining some very important products for human activity. In this context, the production of amino acids, which act as building blocks for proteins and as precursors for hormones, neurotransmitters, antioxidants, nucleic acids and other complex body constituents, has been significantly increased. The amino acids can be obtained by biosynthesis or from protein hydrolysis, but their separation from fermentation broths or protein hydrolysates is rather difficult (Cascaval et al., 2001). In an industrial process, the cost of separation and concentration of biomolecules from aqueous media, in which they are usually produced, can be as high as 90% of their total cost of manufacturing (Eyal and Bressler, 1993) and so, for

the design of equilibrium-based separation processes, the accurate prediction of their activity coefficients in solution is essential.
In this way, the design of such processes requires the knowledge of the partitioning behaviour in solvent systems and thermodynamic models should support this optimization procedure. However, both the complexity of biomolecules, due to their multiple functional groups, and the crucial role of water make the standard available thermodynamic models unattractive for the computation of phase behaviour. Therefore, new thermodynamic models for the description of the phase behaviour of systems containing biomolecules are required (Rudolph et al., 2001).
To evaluate the capabilities of a thermodynamic model, reliable experimental phase equilibrium data are necessary, such as solubilities in mixed and single solvent systems as well as partition coefficients. In recent years, several research groups investigated the phase behaviour of systems containing amino acids and proteins. Amino acids have been used especially because the influence of the increasing complexity of the biomolecule is easily investigated by varying the functional side-chains of the amino acids (Rudolph et al. 2001). However, just few data of systems in liquid-liquid equilibrium have been reported in the literature (Gude et al., 1996b; van Berlo et al., 1997).
In this work, liquid–liquid equilibria (LLE) of the ternary systems 1-butanol/water/amino acid (glycine, DL-alanine or L-leucine) were measured at 40 ^{o}C. Additionally, the modified NRTL model, developed by Vetere (2000) and extended to multicomponent systems, was applied to the studied systems and compared with the results obtained with the UNIFAC-Campinas (Santos, 2005) and the UNIFAC group contribution method (Fredenslund et al., 1975; Fredenslund et al., 1977) using the LLE interaction parameters reported by Magnussen and co-workers (1981) and the new interaction parameters estimated in this work.
Amino acids are zwitterionic (dipolar) substances, and depending on the pH of the solution, they can exist as neutral or charged species. An unbuffered aqueous solution of a single amino acid at all but extremely low concentrations attains a characteristic pH, referred to as the isoelectric point, at which amino acid molecules in solution are almost entirely (>99.9%) present in the form of overall neutral species carrying two discrete charges at fixed positions (Gude et al., 1996a) In the present work, only unbuffered solutions of amino acids were studied, and thus, the amount of ionic amino acid species (<0.1%) could be neglected in the modelling.

Materials and Methods

Chemicals

In all experiments double-ionized water was used. Glycine 99,7% purity, 1-butanol 99,5% purity, ethanol 99,7% purity, acetone 99,8% purity were supplied by Merck. DL-alanine 99% purity and L-leucine 99% purity were supplied by Fluka. The purities of solvents were verified by gas chromatography and the chemicals were used without further purification.

Experimental Procedure

For each experiment, mixtures were prepared inside the immiscibility region, covering the whole composition range, by weighting known amounts of each substance using an analytical balance (Adam equipment, AAA 250) with a precision of 0.0001 mg. The liquid-liquid equilibrium was accomplished by using a jacketed glass cell connected to thermostatic baths models (Tempunit® TU-16D and TE-8D, Techne) whose temperature stability was 0.1 ^{o}C. The temperature in the cell was measured with a mercury thermometer with a precision of 0.1

^{o}C. To promote the contact among the phases inside the equilibrium cells, magnetic stirrers (Agimatic-N, Selecta) and Teflon-covered magnetic bars were used. Each mixture was stirred for 3 hours and then left to settle for at least 16 hours. After, four samples of approximately 2 ml were taken from the upper and lower phase with a syringe. One of the samples was used to measure the solvent composition in the equilibrium phases, and the other three samples to quantify the amino acid content in each phase.

Chromatographic analyses

The amounts of alcohol and water in the samples were determined by gas chromatography using a Varian CP-3380GC-1041 chromatograph equipped with a thermal conductivity detector. The separation of the components was made using 25 m x 0.53 mm I.D. WCOT fused-silica capillary column. The carrier gas used was helium ultra pure and the flow rate was 30 ml/min and the injection volume was 0.2µl. Each one of the samples was analyzed in triplicate; the final result was the average among those three values. In this work the external standard calibration method was used.
Before injecting the samples in the chromatograph they were diluted with acetone. The addition of acetone prevents phase separation effects when changing the temperature after the separation of the phases and promotes the amino acid precipitation in the sample. These samples were then submitted to centrifugation (Mini Spin - Eppendorf) at 10 000 rpm for 30 seconds and only the supernatant solution was analyzed.

Gravimetric analyses

The concentrations of amino acid in the equilibrium phases were determined gravimetrically. Three samples of approximately 2 ml of each phase were withdrawn, weighed, and completely dried in an oven (Scientific, 9000 series), and the remaining solids weighed again. The oven temperature was set at 70 °C, in order to prevent degradation of the amino acid. This degradation is easily perceived by the change of color of the amino acid during the drying. At the defined drying conditions this degradation did not occur. The weights were measured on the same analytical balance (Adam equipment, AAA 250) with a resolution of 0.0001 g.

To verify that the methodology used in this work was capable to correctly quantify the composition of each phase in equilibrium, reference data previously published by several authors in the literature were reproduced experimentally in this study. This goal was overtaken analyzing ternary systems with and without amino acids. The data obtained in the present work are in very good agreement with the data published in the literature.

Thermodynamic modeling

Local Composition Model: Modified NRTL - A simple modification of the NRTL equation was proposed by Vetere (2000), which consists of inserting the ratio of the molar volumes of the pure compounds as a multiplying factor of the binary parameters G_{ij} and G_{ji}. The extension of this model to multicomponent mixtures is straightforward, being the expression for the excess Gibbs energy as follows:

$$\frac{g^E}{RT} = \sum_{i=1}^{n} x_i \frac{\sum_{j=1}^{n} \tau_{ji} V_j / V_i G_{ji} x_j}{\sum_{l=1}^{n} G_{li} V_l / V_i x_l} \tag{1}$$

Using standard thermodynamics (Santos et al, 2008), the activity coefficient can be easily obtained:

$$\ln \gamma_i = \frac{\sum_{j=1}^{n} \tau_{ji} V_i / V_j G_{ji} x_j}{\sum_{l=1}^{n} V_i / V_l G_{li} x_l} + \sum_{j=1}^{n} \frac{V_j / V_i G_{ij} x_j}{\sum_{l=1}^{n} V_j / V_l G_{lj} x_l} \left[\tau_{ij} - \frac{\sum_{r=1}^{n} \tau_{rj} V_j / V_r G_{rj} x_r}{\sum_{l=1}^{n} \tau_{lj} V_j / V_l x_l} \right] \tag{2}$$

where the binary NRTL parameters G_{ij} and G_{ji} are linked to the energy parameters τ_{ij} and τ_{ji} according to

$$G_{ij} = \exp\left(-\alpha_{ij} \cdot \tau_{ij}\right) \tag{3}$$

where α_{ij} is the non-randomness parameters and

$$\tau_{ij} = \frac{a_{ij}}{RT} \qquad \tau_{ji} = \frac{a_{ji}}{RT} \tag{4}$$

where a_{ij} is treated as an adjustable parameter estimated from correlation of experimental liquid-liquid equilibrium data.

In this work, the solvent/solvent and solvent/solute parameters were estimated from correlation of experimental liquid-liquid equilibrium data. Due the modification proposed by Vetere (2000), the molar volumes of pure substances are necessary. For the solvents and amino acids they were obtained from density of liquid and solid data, respectively (O'Neil, 2001 and Lide, 1997). These values are given in Table 1.

Table 1. Molar volumes of amino acids and solvents at 40ºC

Components	Molar volume (cm^3mol^{-1})
water	18.178[a]
glycine	44.018[b]
DL-alanine	61.200[b]
L-leucine	108.340[c]
1-butanol	93.432[a]

[a] Perry et al, 1997, [b] Cibulka et al, 2010, [c] Rajagopal and Gladson, (2011)

Group contribution method – UNIFAC-Campinas - The UNIFAC-Campinas model (Santos, 2005), shows modifications in the combinatorial and residual terms on the basic form of the UNIFAC model,

$$\ln \gamma_i = \ln \gamma_i^{com} + \ln \gamma_i^{res} \tag{5}$$

In this model, the functional groups are determined by ab initio calculations of quantum mechanics, according to the proposal of Wu and Sandler (1991). The resulting functional groups have structures whose electric charges are roughly neutral, thus ensuring the stability

of the molecule. The values of the parameters of volume and area of the van der Waals groups are calculated by Polarizable Continuum Model – PCM (Miertuš et al., 1981). The parameters of volume and area of the van der Waals groups used in this work are shown in Table 2.

Table 2. Parameters of volume and area of the van der Waals groups (Santos, 2005).

Group	R_k	Q_k
H_2O	0.7710	0.8495
CH_3	0.8418	0.7570
CH_2	0.5914	0.3843
CH	0.3203	0.0000
CH_2OH	1.1710	0.9603
NH_2CH_2COOH	2.4672	2.0482
$NH_2CH(CH_3)COOH$	3.0712	2.3963
$NH_2CH(CH_2)COOH$	2.8195	1.8985

The combinatorial term was changed and has a structure similar to the Flory-Huggins combinatorial.

$$\ln\gamma_i^C = \ln\frac{\phi_i'}{x_i} + 1 - \frac{\phi_i'}{x_i} \tag{6}$$

The molecular volume fraction (Φ') was also slightly modified using the volume parameter (r_i) raised to an exponent p = 1/2.

$$\phi_i' = \frac{x_i r_i^p}{\sum_j x_j r_i^p} \tag{7}$$

The expression for the residual term adopted for this model has the same shape as the residual term of UNIFAC-Dortmund.

$$\psi_{nm} = \exp\left[-\frac{\left(a_{nm} + b_{nm}T + c_{nm}T^2\right)}{T}\right] \tag{8}$$

In this work, the partition coefficient is used to properly analyse the quality of the experimental results measured. In general, the partition coefficient is defined as:

$$K_i = x_i(organic \quad phase)/x_i(aqueous \quad phase) \tag{9}$$

where, x_i is the mole fraction of the solute.

Parameter estimation and correlation of data

In the modified-NRTL model, the experimental liquid-liquid equilibrium data are used to determine the interaction parameters between solvent and amino acid; these in turn are used to determine the activity coefficients by the model. For the UNIFAC model, the activity coefficients are calculated using the estimated interaction parameters between the groups H_2O, CH_n, COOH, aCH, aCOH, OH and the new groups CH_nNH_2 and NH used to build the molecules of amino acids. All the other parameters were obtained from literature (Magnussen et al., 1981). For the UNIFAC-Campinas model, the activity coefficients are calculated using the estimated interaction parameters between the groups H_2O, CH_n, CH_2OH and the groups

NH_2CH_2COOH and $NH_2CH(CH_3)COOH$ used to build the molecules of amino acids. All the other parameters were obtained from literature (Santos, 2005).
Molecular interaction parameters for the pairs solvent-solvent and solvent-solute in modified-NRTL model and the group interaction parameters in the UNIFAC and UNIFAC-Campinas models were estimated by minimization of the objective function, F, applying the Simplex procedure, using part of the experimental liquid-liquid equilibrium data.

$$F = \sum_{l}^{ns} \sum_{i}^{np} \sum_{i}^{nc} \left[\frac{K_{ijl}^{\exp} - K_{jil}^{cal}}{K_{ijl}^{\exp}} \right]^2 \qquad (10)$$

where *nc*, *np* and *ns* mean the component, number of tie-lines and number of systems, respectively. This objective function was minimized using the isoactivity criterion in all components for the calculation of the distribution coefficients. With these estimated parameters, the liquid-liquid equilibrium flash calculation (García-Sánchez et al., 1996) was applied to evaluate the compositions of the tie line by using the experimental total compositions as input data. The other part of the data was used for prediction.

Results and Discussion

In this work, the experimental determination of the liquid-liquid data of the following systems was accomplished: 1-butanol/water/amino acid (glycine, DL-alanine or L-leucine), at 40 ºC. These data are reported in Table 3 along with the partition coefficients of each experimental point.

Table 3. Liquid-liquid equilibrium (molar fraction) for the system amino acid (i) in water (1)/1-butanol (2) at 40 ºC.

Organic Phase		Aqueous Phase		
x_1	x_i	x_1	x_i	K_i
glycine				
0.4917	0.0000	0.9778	0.0000	-
0.4771	0.0004	0.9727	0.0143	0.0280
0.4618	0.0007	0.9598	0.0290	0.0241
0.4409	0.0007	0.9567	0.0323	0.0217
0.3905	0.0010	0.9373	0.0527	0.0190
DL-alanine				
0.4917	0.0000	0.9778	0.0000	-
0.4736	0.0003	0.9762	0.0060	0.0500
0.4439	0.0006	0.9716	0.0114	0.0526
0.4485	0.0008	0.9668	0.0167	0.0479
0.4463	0.0013	0.9607	0.0260	0.0500
0.4339	0.0015	0.9565	0.0308	0.0487
0.4315	0.0017	0.9545	0.0327	0.0520
L-leucine				
0.4917	0.0000	0.9778	0.0000	-
0.5086	0.0008	0.9758	0.0012	0.6667
0.5134	0.0011	0.9749	0.0018	0.6111
0.5148	0.0017	0.9730	0.0025	0.6800
0.5163	0.0022	0.9727	0.0032	0.6875
0.5180	0.0021	0.9696	0.0032	0.6563
0.5195	0.0022	0.9657	0.0032	0.6875

The estimated parameters in this work for the modified-NRTL model are presented in Table 4, while the estimated interaction parameters between groups for the UNIFAC and UNIFAC-Campinas models are presented in the Table 5 and 6, respectively. In order to decrease the number of parameters, the non-randomness parameter, α_{ij}, was kept constant in the modified-NRTL model; for the interactions solvent/solvent, $\alpha_{ij,}$ was considered equal to 0.3, and for interactions between the solvent and the amino acid, 0.2.

Table 4. Modified NRTL parameters estimated in this work.

Component i	Component j	a_{ij}	a_{ji}	α_{ij}
water	1-butanol	4442.8	-2886.7	0.3
water	glycine	3128.0	-2162.1	0.2
water	DL-alanine	888.70	-2805.7	0.2
water	L-leucine	599.23	6448.8	0.2
1-butanol	glycine	9999.0	9255.7	0.2
1-butanol	DL-alanine	969.96	9929.8	0.2
1-butanol	L-leucina	9983.0	1750.7	0.2

Figure 1 shows the experimental distribution coefficients of glycine for the 1-butanol + water system at 40ºC, the correlated and predicted values obtained with modified-NRTL, UNIFAC and UNIFAC-Campinas. In this figure, it can be observed that: i) modified-NRTL model show a good agreement between the calculated and experimental values with a ARD of 20.25%, ii) the UNIFAC predictions differs significantly from the experimental values with an ARD of 68.15%; and iii) the UNIFAC-Campinas model provides good results for the analyzed system, with an average relative deviation (ARD) of 25%. The average relative deviation (ARD) was calculated according to:

$$ARD = \frac{1}{n}\sum_{n}\left|\frac{\left(K_i^{\exp} - K_i^{calc}\right)}{K_i^{\exp}}\right| \qquad (11)$$

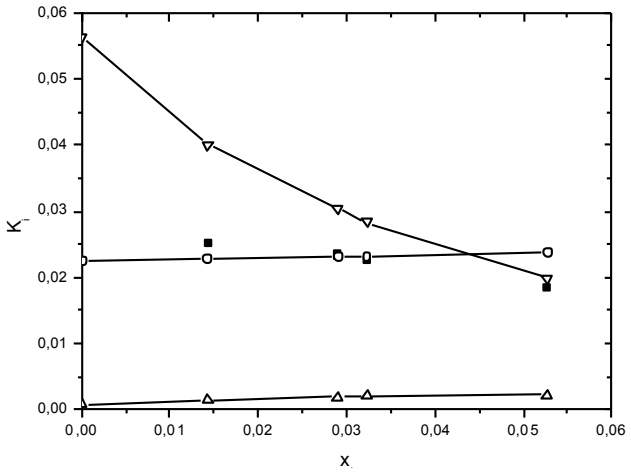

Figure 1. Partition coefficient of (■) glycine versus mole fraction of glycine in the aqueous phase and correlated and predicted values by (○) modified NRTL (ARD=3.06%), (Δ) UNIFAC (ARD=91.3%), and (∇) UNIFAC-Campinas (ARD=30.5%).