Biochemical Engineering Journal 24 (2005) 147–155

Biochemical Engineering Journal

www.elsevier.com/locate/bej

Prediction of protein partition in polymer/salt aqueous two-phase systems using the modified Wilson model

Pedro P. Madeira [a,b], Xin Xu [a], José A. Teixeira [b], Eugénia A. Macedo [a,*]

[a] *Laboratory of Separation and Reaction Engineering, Departamento de Engenharia Química, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal*

[b] *Centro de Engenharia Biológica, Universidade do Minho, 4710-057 Braga, Portugal*

Received 20 October 2004; received in revised form 24 January 2005; accepted 4 February 2005

Abstract

The extension of the modified Wilson model to multicomponent mixtures, presented in a previous publication, is applied to predict the partition of the following proteins: bovine serum albumin (BSA), lysozyme, glucosidase and catalase, in the Na_2SO_4/PEG6000 and K_2HPO_4/PEG6000 aqueous two-phase systems at 298.15 K. The results obtained with the model are, in general, in fair agreement with the experimental data.

In the modelling methodology adopted here, special emphasis on the so-called "charge effects" to the protein partition was given. To our knowledge, no experimental information is available in the literature that allows to estimate the interaction parameters between these macromolecules and the components present in the aqueous two-phase systems (water, salts and polymer). Thus, the deviations observed between calculated and experimental protein partition are mainly due to some assumptions made in the predictive methodology.

Keywords: Protein recovery; Aqueous two-phase systems; Modelling; Excess gibbs energy; Modified Wilson model; Activity coefficients

1. Introduction

In the last decades considerable advances concerning theoretical aspects of aqueous two-phase systems (ATPS), as a mean to separate aqueous mixtures of proteins, have been reported [1–13]. As Albertsson [14] pointed out, an aqueous two-phase system will occur when we add, to an aqueous medium, and above some minimum concentrations, two thermodynamically incompatible substances. These can be either two polymers or a polymer and a salt. When we introduce proteins to an ATPS they will "prefer" one or another phase, and therefore separation can be achieved.

Among the major factors leading to the success of the ATPS as an extraction technique, are the fact that they provide an innocuous environment for the biomolecules, their ability in conferring good resolution factors as well as high activities yields, the easy direct use of available chemical engineering equipment and the possibility to directly apply the ATPS extraction technique to a fermentation broth, where proteins are usually produced [11,12].

The major factors governing protein partition, when affinity ligands are not incorporated, are well known and include the so called environmental conditions, such as pH, type of buffer, ionic strength, temperature and the phase-forming polymer or salts used, and the characteristics of the proteins, i.e., hydrophobicity, molecular size, conformation and charge.

It is common practice, when predicting the partition of proteins in an ATPS, to use the expression derived by Albertsson [1]:

$$\ln K_p = \ln K_0 + \frac{z_p F}{RT}\Delta\varphi \quad (1)$$

where K_p denotes the partition coefficient of a protein, of net surface charge z_p. $\Delta\varphi$ is the electrical potential difference between both phases, while K_0 is the partition coefficient of

* Corresponding author. Tel.: +351 22 5081653; fax: +351 22 5081674.
E-mail addresses: ppalma@fe.up.pt (P.P. Madeira), eamacedo@fe.up.pt (E.A. Macedo).

1369-703X/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bej.2005.02.004

Nomenclature

a	interaction parameter defined in Eq. (21), or activity
A	Debye–Hückel parameter
B	parameter
exp.	experimental
F	Faraday's constant
G, G^E	binary parameter, excess Gibbs free energy
I	ionic strength
K	partition coefficient
K_0	partition coefficient in the absence of an electrical potential difference
m	molality
n	mole number of segment–segment pairs, or polymerization degree
q	effective segment number of polymer
r	number of segments per molecule
R	universal gas constant
T	absolute temperature
T_0	reference temperature, 298.15 K
x	mole fraction of polymer solutions
X	effective mole fraction of segments
z	charge number

Greek letters

α	non-random factor in the Wilson model
Δ	difference
Φ	volume fraction
φ	electrical potential
γ	activity coefficient
μ	electrochemical potential
θ	surface/area fraction
τ	binary interaction parameter

Subscripts

a, a', a"	anion
c, c ,c"	cation
comb	combinatorial
i, j, m	any species or segments
ii, ij, jj	segment–segment pairs
k	reference ion
LR	long-range
p	protein
SR	short-range
1, 2, 3	water, polymer and salt, respectively

Superscripts

b	bottom phase
E	notation of excess quality
t	top phase
ref	reference state

the same protein in the absence of either a net charge or an electrical potential difference. F, R and T stand for Faraday constant, universal gas constant and absolute temperature, respectively. Thus, to predict the partition coefficient of a given protein in an ATPS, according to this method, we need not only a mean to obtain $\Delta\varphi$, but also a way to calculate the net charge of the protein as well as an established model to calculate K_0.

The electrostatic potential difference between the aqueous phases is attributed to the unevenly distribution of the ionic species and seems to play an important role in the partition of proteins in ATPS [9,10,15]. Despite the predominant influence of the electrical contribution on the partition of charged proteins, some doubt remains in the state of art about the experimental assessment as well as about the theoretical explanation of the electrical potential difference between phases [9]. Some authors use an indirect approach to calculate $\Delta\varphi$ [16–18]. They obtain the electrical potential difference between phases from experimental results of the partition coefficient of proteins. According to Eq. (1) a linear relationship between $\ln K_p$ and z_p is expected, as long as K_0 remains constant. Although simple in nature, some premises are doubtful. For instance, K_0 may vary with changing conditions such as pH, ionic strength, type and concentration of phase forming polymers/salt. Besides, the net charge of the protein is usually assumed to depend solely on pH, which may not be the case. Another widely used approach consists on the direct experimental measurement of $\Delta\varphi$ [9,11,19,20]. Some authors [9–11,20] used Ag/AgCl capillary-electrode apparatus to measure experimentally $\Delta\varphi$'s. But as some researchers noted [21] the obtention of unambiguous measurement of $\Delta\varphi$ according to this method is not simple. The similarity of the results is the strongest argument favoring their reliability.

In the last decade, Groβmann and Maurer [5] showed that $\Delta\varphi$ could be calculated exactly from the excess Gibbs energy of the solution, provided that there is no external electrical field and that the two-phase system is obtained by mixture of neutral components, and by introducing the condition of electroneutrality for each of the coexisting phases. They defined the electrical potential difference between both phases as the difference in the chemical potential of an arbitrarily chosen reference ionic species coexisting in those phases. According to these authors, the electric potential difference between phases is given by:

$$\Delta\varphi = \varphi^b - \varphi^t = \frac{RT\ln(a_k^t/a_k^b)}{Fz_k} \tag{2}$$

and the partition of any other ionic species present in the phases by

$$\ln K_i = \ln\left(\frac{m_i^t}{m_i^b}\right) = \ln\left(\frac{\gamma_i^b}{\gamma_i^t}\right) + \frac{Fz_i\,\Delta\varphi}{RT}$$
$$= \ln\left(\frac{\gamma_i^b}{\gamma_i^t}\right) + \frac{z_i}{z_k}\ln\left(\frac{a_k^t}{a_k^b}\right) \tag{3}$$

where a_k is the activity of the reference ion, z_k is the number of elementary charges on the reference species, and the superscripts t and b stand for top and bottom phases, respectively. The major drawback of this approach lies on the lack of experimental information, especially when applied to the partition of proteins. In fact, to use this theory we need separate experiments to assess the interaction parameters between proteins and the other components in the aqueous two-phase system. Unfortunately, in the literature these experimental data are scarce.

The application of the quasi-electrostatic-potential theory developed by Newman [22] is another widely used approach [8,9]. Here, an arbitrary reference ion k is selected, and all non-idealities in the electrochemical potential of k, μ_k, are assumed to be electrostatic in nature:

$$\mu_k = \mu_k^0 + RT\ln(m_k\gamma_k) = RT\ln(m_k) + z_kF\varphi \tag{4}$$

where $\mu_k^0$is the standard-state chemical potential of component k, m_k is the molality of ion k, γ_k is the activity coefficient of the same ion, and R, T, F, z_k and φ have the same meaning as above. From Eq. (4), the electrochemical potential of any other ionic specie i can be easily obtained [9]:

$$\mu_i = \mu_i^0 - \frac{z_i}{z_k}\mu_k^0 + RT\ln(m_i\gamma_i) - RT\frac{z_i}{z_k}\ln(\gamma_k) + z_iF\varphi \tag{5}$$

We can now apply the phase equilibrium condition for the ATPS, i.e., for bottom (b) and top (t) phases at constant temperature T and pressure P, for any ion present in both phases:

$$\mu_i^{\mathrm{t}} = \mu_i^{\mathrm{b}} \tag{6}$$

Substituting Eq. (4) into Eq. (6), and after some rearrangement, we can obtain, according to the quasi-electrostatic-potential theory [22], an expression for the electrical potential difference between phases:

$$\ln K_k = \ln\frac{m_k^{\mathrm{t}}}{m_k^{\mathrm{b}}} = \frac{z_kF}{RT}\Delta\varphi \tag{7}$$

where K_k is the partition coefficient of the reference ion and $\Delta\varphi = \varphi^{\mathrm{b}} - \varphi^{\mathrm{t}}$. The expression for the partition of other ionic species present in the systems is given by Eq. (3).

It is generally accepted to access the net charge of a protein based on acid/base titration coupled with isoelectric focusing experiments [23]. Thus, when we predict the partition coefficient of a specific protein, we are assuming that its net charge depends solely on pH.

In fact, the titration/electrophoresis experimental conditions (e.g., ionic strength) used to obtain the net charge are usually different from those in the ATPS. Since the net charge of a given protein seems to play an important role in its partition behavior, we believe that more experimental investigation should be directed to this specific area in order to assess the validity of the assumption.

Several models that attempt to calculate K_0 (see Eq. (1)) have been reported. The osmotic virial-expansion models and those based on the lattice theory are among the most widely used (for a discussion on the particularity of those models see, e.g., [21,24]).

In a previous paper, Xu et al. [25] presented a new modified Wilson equation to represent the vapor–liquid equilibrium (VLE) behavior of homologous aqueous polymer solutions, that incorporates some ideas from previous models, but in which the heat capacity is taken into account. Later, the model was extended to multicomponent systems to test its ability in correlating and predicting the LLE of polymer–polymer [26] and polymer–salt [27] aqueous two-phase systems. In this work, the modified Wilson model is tested as a tool to predict the protein partition (bovine serum albumin (BSA), lysozyme, glucosidase and catalase) in the Na_2SO_4/PEG6000 and K_2HPO_4/PEG6000 aqueous two-phase systems at 298.15 K. The data used to evaluate the capabilities of the model were collected from the literature.

2. Thermodynamic framework

The excess Gibbs energy is given as a sum of three contributions:

$$G^{\mathrm{E}} = G^{\mathrm{E}}_{\mathrm{LR}} + G^{\mathrm{E}}_{\mathrm{comb}} + G^{\mathrm{E}}_{\mathrm{SR}} \tag{8}$$

where the first term accounts for the contribution of long-range electrostatic interactions due to the presence of ionic species, the second term for the combinatorial contribution, that considers the size/shape of the molecules, and the last term for the short-range interactions, and reflects the interactions between segments of molecules.

According to Eq. (8), the activity coefficient may be written as:

$$\ln\gamma_i = \ln\gamma_{i,\mathrm{LR}} + \ln\gamma_{i,\mathrm{comb}} + \ln\gamma_{i,\mathrm{SR}} \tag{9}$$

The activity coefficients of all the solutes in ATPS are normalized to the infinite dilution reference state:

$$\ln\gamma_i^* = \ln\gamma_i - \ln\gamma_i^{\mathrm{ref}} (j \neq 1) \tag{10}$$

where γ_i^{ref} is the activity coefficient at the infinite dilution reference state.

2.1. Electrostatic interactions

The mean ionic activity coefficient of electrolyte i can be written as [28]:

$$(\ln\gamma_i)_{\mathrm{LR}} = \frac{A|Z_{\mathrm{a}}Z_{\mathrm{c}}|I^{1/2}}{1 + BI^{1/2}} \tag{11}$$

where Z_{a} and Z_{c} are the absolute charge number of the anion and cation respectively, and A is the usual Debye–Hückel parameter. B is, in this study, set equal to 1.2 [29] for all electrolyte solutions considered. I is the ionic strength of the

mixture in the molality scale. The contribution of the long-range forces to the activity coefficient of water is calculated according to the following equation [29]:

$$(\ln\gamma_{\mathrm{w}})_{\mathrm{LR}} = \frac{2AM_{\mathrm{w}}}{(10B)^3}\left((1+BI^{1/2}) - \frac{1}{1+BI^{1/2}} - 2\ln(1+BI^{1/2})\right) \tag{12}$$

The activity coefficient of ion j is calculated according to the following equation [29]:

$$(\ln\gamma_j)_{\mathrm{LR}} = -\frac{AZ_j^2 I^{1/2}}{(1+BI^{1/2})} \tag{13}$$

2.2. Combinatorial contribution

The expression of $G^{\mathrm{E}}_{\mathrm{comb}}$ for multicomponent ATPS is obtained by directly extending the equation for binary polymer aqueous solutions [25]:

$$\frac{G^{\mathrm{E}}_{\mathrm{comb}}}{RT} = \sum_i n_i \ln\frac{X_i}{x_i} + \frac{1}{\alpha}\sum_i n_i q_i \ln\frac{X_i}{\Phi_i} \tag{14}$$

where n_i is the mole numbers of species, Φ_i and x_i are the volume fraction and mole fraction, respectively. X_i is the hypothetical effective fraction of segment of polymer, and is given by:

$$X_i = \theta_i C_i, \quad (\text{if} \quad i = \text{ion},\ C_i = Z_i, \text{otherwise } C_i = 1) \tag{15}$$

$$\Phi_i = \frac{n_i r_i}{n_r}, \quad n_r = \sum n_{\mathrm{k}} r_{\mathrm{k}} \tag{16}$$

$$\theta_i = \frac{n_i q_i}{n_q}, \quad n_q = \sum n_{\mathrm{k}} q_{\mathrm{k}}, \quad q_i = r_i\left[1-\alpha\left(1-\frac{1}{r_i}\right)\right] \tag{17}$$

where r_i is the number of segments, θ_i is the effective segment fraction and q_i means the effective number of segment.

The expression of the activity coefficient for the combinatorial contribution is obtained from Eq. (14):

$$\ln\gamma_i = \ln\frac{X_i}{x_i} + \sum_j X_j\left(1-\frac{q_i}{q_j}\right) + \frac{q_i}{\alpha}\left[\ln\frac{X_i}{\Phi_i} + \sum_j\left(\frac{q_j r_i}{q_i r_j}-1\right)\Phi_j\right] \tag{18}$$

2.3. Short-range interactions

Based on the assumptions of local electroneutrality, like-ion repulsion [30] for the existing ions, and the hypothetical segment aggregate state for polymers, and following the same derivation procedure of Wu et al. [31] and Chen and Evan [32], the expression for the short-range term can be obtained by extending the short-range interaction for binary polymer aqueous solutions [25]:

$$\frac{G^{\mathrm{E}}_{\mathrm{SR}}}{n_q RT} = -\frac{1}{\alpha}\left(\sum_m X_m \ln\left(\sum_j X_j G_{jm}\right) + \sum_{\mathrm{c}} X_{\mathrm{c}} \sum_{\mathrm{a}'} \frac{X_{\mathrm{a}'}}{\sum_{\mathrm{a}''} X_{\mathrm{a}''}} \ln\left(\sum_j X_j G_{j,\mathrm{c},\mathrm{a}'\mathrm{c}}\right) + \sum_{\mathrm{a}} X_{\mathrm{a}} \sum_{\mathrm{c}'} \frac{X_{\mathrm{c}'}}{\sum_{\mathrm{c}''} X_{\mathrm{c}''}} \ln\left(\sum_j X_j G_{j,\mathrm{a},\mathrm{c}'\mathrm{a}}\right)\right) \tag{19}$$

where $G_{ij} = \exp(-\alpha_{ij}\tau_{ij})$, $G_{ji,ki} = \exp(-\alpha_{ji,ki}\tau_{ji,ki})$. After appropriate differentiation, the activity coefficient may be obtained:

$$\frac{1}{q_m}\ln\gamma_{m,\mathrm{SR}} = -\frac{1}{\alpha}\left(\ln\left(\sum_j X_j G_{jm}\right) + \sum_{m'} X_{m'}\frac{G_{mm'}}{\sum_j X_j G_{jm}} + \sum_{\mathrm{c}}\sum_{\mathrm{a}'}\frac{X_{\mathrm{a}'}}{\sum_{\mathrm{a}''} X_{\mathrm{a}''}}\frac{X_{\mathrm{c}} G_{m,\mathrm{c},\mathrm{a}'\mathrm{c}}}{\sum_j X_j G_{j,\mathrm{c},\mathrm{a}'\mathrm{c}}} + \sum_{\mathrm{a}}\sum_{\mathrm{c}'}\frac{X_{\mathrm{c}'}}{\sum_{\mathrm{c}''} X_{\mathrm{c}''}}\frac{X_{\mathrm{a}} G_{m,\mathrm{a},\mathrm{c}'\mathrm{a}}}{\sum_j X_j G_{j,\mathrm{a},\mathrm{c}'\mathrm{a}}} - 1\right) \tag{20a}$$

$$\frac{1}{Z_{\mathrm{c}}}\ln\gamma_{c,\mathrm{SR}} = -\frac{1}{\alpha}\left(\sum_{\mathrm{a}'}\frac{X_{\mathrm{a}'}}{\sum_{\mathrm{a}''} X_{\mathrm{a}''}}\ln\left(\sum_j X_j G_{j,\mathrm{c},\mathrm{a}'\mathrm{c}}\right) + \sum_m \frac{X_m G_{\mathrm{c}m}}{\sum_j X_j G_{jm}} + \sum_{\mathrm{a}}\sum_{\mathrm{c}'}\frac{X_{\mathrm{c}'}}{\sum_{\mathrm{a}''} X_{\mathrm{c}''}}\frac{X_{\mathrm{a}} G_{\mathrm{c},\mathrm{a},\mathrm{c}'\mathrm{a}}}{\sum_j X_j G_{j,\mathrm{a},\mathrm{c}'\mathrm{a}}} - \frac{1}{Z_{\mathrm{c}}}\right) \tag{20b}$$

$$\frac{1}{Z_{\mathrm{a}}}\ln\gamma_{a,\mathrm{SR}} = -\frac{1}{\alpha}\left(\sum_{\mathrm{c}'}\frac{X_{\mathrm{c}'}}{\sum_{\mathrm{c}''} X_{\mathrm{c}''}}\ln\left(\sum_j X_j G_{j,\mathrm{a},\mathrm{c}'\mathrm{a}}\right) + \sum_m \frac{X_m G_{\mathrm{a}m}}{\sum_j X_j G_{jm}} + \sum_{\mathrm{c}}\sum_{\mathrm{a}'}\frac{X_{\mathrm{a}'}}{\sum_{\mathrm{c}''} X_{\mathrm{a}''}}\frac{X_{\mathrm{c}} G_{\mathrm{a},\mathrm{c},\mathrm{a}'\mathrm{c}}}{\sum_j X_j G_{j,\mathrm{c},\mathrm{a}'\mathrm{c}}} - \frac{1}{Z_{\mathrm{a}}}\right) \tag{20c}$$

The following expressions are used here to describe the influence of temperature on τ [33]:

$$\tau_{ji} = a_{ji}^{(1)}\frac{T_0}{T} + a_{ji}^{(2)}\left(\frac{T_0}{T}\right)^2 \tag{21a}$$

$$\tau_{ij} = a_{ji}^{(1)}\frac{T_0}{T} + a_{ij}^{(2)}\left(\frac{T_0}{T}\right)^2 \tag{21b}$$

where $a_{ij}^{(1)}$, $a_{ji}^{(1)}$, $a_{ij}^{(2)}$, and $a_{ji}^{(2)}$, are adjustable model parameters (temperature and composition independent). $a_{ij}^{(2)}$ and $a_{ji}^{(2)}$ are set equal to zero, because it was concluded in a previous work [25] that $a_{ij}^{(1)}$ and $a_{ji}^{(1)}$ are enough to accurately describe the thermodynamic properties for binary polymer solutions.

3. Protein partition

Applying the quasi-electrostatic-potential theory (Eqs. (3)–(7)), the partition coefficient of a protein, K_p, is given by:

$$\ln K_p = \ln\left(\frac{\gamma_p^b}{\gamma_p^t}\right) + \frac{z_p}{z_k}\left[\ln\left(\frac{m_k^t}{m_k^b}\right) + \ln\left(\frac{\gamma_k^t}{k_k^b}\right)\right] \tag{22}$$

where the subscripts p and k stand for protein and reference ion, respectively, and the remaining symbols have the meaning already presented. Eq. (22) is the working equation for this essay.

4. Model interaction parameters

The several interaction parameters were obtained by fitting the modified Wilson model [25] to experimental data published in the literature:

(i) The interaction parameters between polymer and water were estimated from water-activity data [25] by minimizing the sum of squares:

$$\mathrm{SSQ} = \sum_{j=1}^{N}(a_w^{\mathrm{exp.}} - a_w^{\mathrm{calc.}})_j^2 \tag{23a}$$

(ii) Ion-water specific-interaction parameters were obtained from mean ionic activity coefficients available in the literature [28], using the following objective function:

$$\mathrm{OBJ} = \sum_{i}^{N}(\gamma_i^{*\mathrm{calc.}} - \gamma_i^{*\mathrm{exp.}}) \tag{23b}$$

(iii) Polymer-salt interaction parameters were estimated using LLE data with the isoactivity criterion between the two-liquid phases in ATPS [26,27]. The objective function used to correlate the LLE data in ATPS was the following;

$$\mathrm{OBJ} = \sum_{i}^{N_1}\sum_{j}^{N_2}\sum_{k}^{N_3}\left[1 - \frac{Q_{ijk}(\mathrm{calc.})}{Q_{ijk}(\mathrm{exp.})}\right]^2 \tag{23c}$$

where Q stands for any thermodynamic property.

For high dilute partitioning species, such as the case of the proteins studied here, their activity coefficients were assumed to be equal to the infinite dilution activity, i.e., it was assumed that the "charge effects" dominate the partition of a particular protein in an ATPS. Hence, its partition coefficient can be approximated to the second term on the right hand side of Eq. (22):

$$\ln K_p \cong \frac{z_p}{z_k}\left[\ln\left(\frac{m_k^t}{m_k^b}\right) + \ln\left(\frac{\gamma_k^t}{k_k^b}\right)\right] \tag{22a}$$

Given the very low protein concentration range studied here, we also assumed that the protein net charge effect on its partition is predominant. Thus, all the interaction parameters involving the proteins were set equal to zero. Besides, to our knowledge, there is no experimental information available in the literature that allows the calculation of these interaction parameters between protein and the phase-forming components.

5. Results and discussion

We tested the ability of the modified Wilson model in predicting the partition of bovine serum albumin (BSA), lysozyme, glucosidase and catalase in the Na_2SO_4/PEG6000 and K_2HPO_4/PEG6000 aqueous two-phase systems. The experimental data used were published by Brenneisen [23] and Groβmann et al. [7].

The interaction parameters between polymer and water, ion and water, and polymer and salt are given in Tables A.1–A.3, respectively (Appendix A).

The interaction parameters between PEG6000 and K_2HPO_4 were obtained by correlating the LLE data from Groβmann et al. [7], and the methodology used was identical to the one previously reported [27]. The interaction parameters between PEG6000 and Na_2SO_4 were assumed to be the same for the system PEG1000-Na_2SO_4 [27]. Fig. 1 compares the calculated phase diagrams with the experimental data for the system PEG6000-K_2HPO_4.

As can be seen from Fig. 1, the model predicts accurately the LLE formed by mixture of polymer and salt. The results are identical to those obtained for the other system studied (data not shown) confirming the results previously obtained [27].

Fig. 2 presents experimental and calculated partition coefficients for bovine serum albumin in the PEG6000-Na_2SO_4 ATPS at 298.15K at pH around 6.5. The arbitrarily reference ionic specie used in the calculation was, for this system, the anion (SO_4^{2-}). As can be seen from the figure, the model

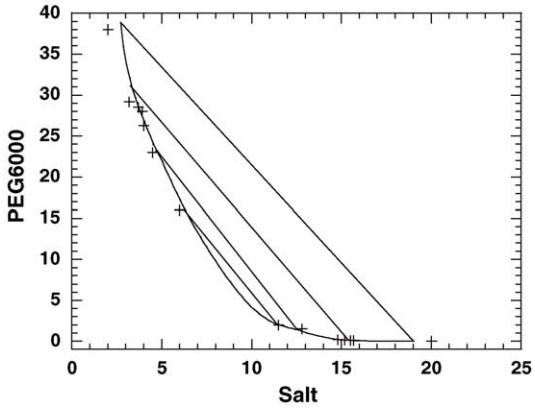

Fig. 1. Calculated and experimental (+) phase diagram for the PEG6000-K_2HPO_4 aqueous two-phase system at 298.15 K.

and methodology adopted are adequate to predict the partition coefficient of BSA in this system under these conditions.

For the same ATPS, at the same pH and temperature conditions, a similar agreement between experimental and calculated protein partition coefficient for glucosidase and catalase was found. However, the best correlation was obtained using different protein net charges from those measured. For instance, for pH around 6.5 the experimental charge obtained by Brenneisen [23] for glucosidase and catalase was −10 and −3, respectively, while the best model correlations were achieved with net charges of −4 and −7, respectively. Figs. 3 and 4 show experimental and calculated protein partition coefficients for these two proteins, in which the calculations were performed using the best fitting model protein net charges.

Table 1 resumes the several protein net charges that gave the best partition coefficients predictions for the proteins studied in the PEG6000-Na_2SO_4 aqueous two-phase system. Table 1 also presents the average relative deviation between experimental and calculated protein's partition coefficients using the best model fitting net charge.

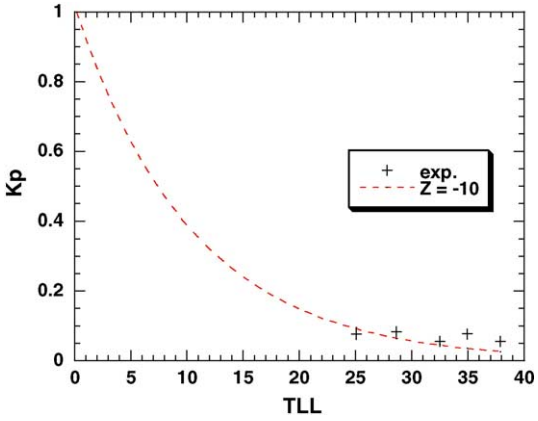

Fig. 2. Calculated and experimental (+) partition coefficient of BSA in the PEG6000-Na2SO4 aqueous two-phase system at 298.15 K.

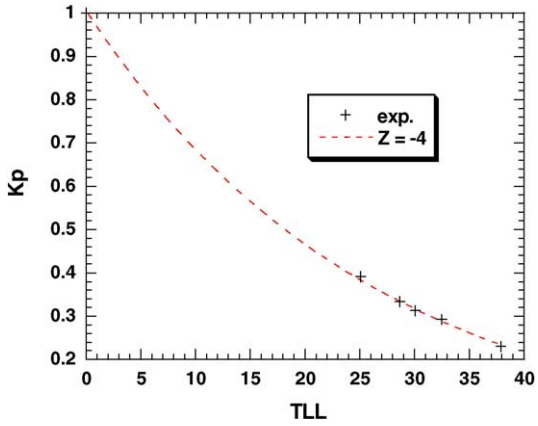

Fig. 3. Calculated and experimental (+) partition coefficient of glucosidase in the PEG6000-Na2SO4 aqueous two-phase system at 298.15 K.

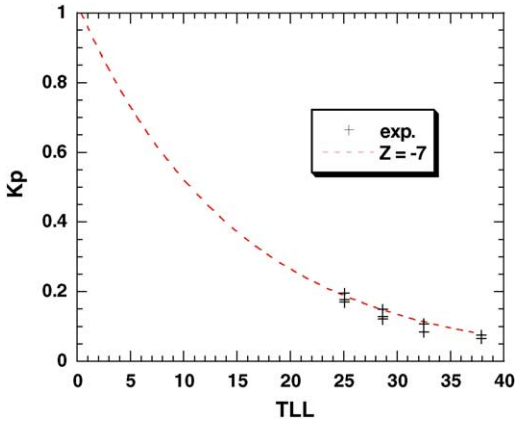

Fig. 4. Calculated and experimental (+) partition coefficient of catalase in the PEG6000-Na2SO4 aqueous two-phase system at 298.15 K.

As can be seen from Table 1 and Figs. 3 and 4, while the model predicts accurately, within the experimental uncertainty, the partition of BSA, glucosidase and catalase in the PEG6000-Na_2SO_4 aqueous two-phase system, there are large quantitative differences between prediction and experiment for the partition of lysozyme in the same system. Several reasons for that poor agreement may be found. For instance, the type and concentration of salt present in the system may

Table 1
Average relative deviations (ARD) between experimental and calculated partition coefficients of BSA, glucosidase, catalase and lysozyme in the PEG6000-Na_2SO_4 ATPS using the best model fitting net charges

Protein	PEG6000-Na_2SO_4		
	Experimental protein net charge[b]	Best model fitting net charge	ARD[a]
BSA	≈−10	−9	0.28
Glucosidase	≈−12	−4	0.13
Catalase	≈−3	−7	0.12
Lysozyme	≈+7	−6	0.23

[a] $\text{ARD} = \sum_{i=1}^{N} (|(k_i^{\text{exp.}} - k_i^{\text{calc.}})/k_i^{\text{exp.}}|)/N$.

[b] Data from Brenneisen [23].

Table 2
Average relative deviations (ARD) between experimental and calculated partition coefficients of BSA, glucosidase, catalase and lysozyme in the PEG6000-K_2HPO_4 ATPS using the best model fitting net charges

Protein	PEG6000-K_2HPO_4		
	Experimental protein net charge[b]	Best model fitting net charge	ARD[a]
BSA	≈−30	12	0.69
Glucosidase	≈−22	1	0.27
Catalase	≈−4	−2	0.54
Lysozyme	≈+2	+4	0.45

[a] ARD $\sum_{i=1}^{N}(|(k_i^{\text{exp.}} - k_i^{\text{calc.}})/k_i^{\text{exp.}}|)/N$.

[b] Data from Brenneisen [23].

originate attractive interactions between proteins (caused by electrostatic interactions with the salt), or can lead to conformational changes in the structure of the protein. It is worth to point out that the aqueous conditions during the titration and electrophoresis experiments were different from those in the partition experiments. Thus the right influence of the pH fluctuations in the ATPS on the charge number might not be obtained during the titration/electrophoresis experiments. Also, the assumption that the partition can be explained only in terms of electrical effects, i.e., that the first term on the right hand side of Eq. (22) can be neglected, might lead to larger deviations between predictions and experiments.

Table 2 resumes the several protein net charges that gave the best partition coefficients predictions for the proteins studied in the PEG6000-K_2HPO_4 aqueous two-phase system. Table 2 also presents the average relative deviation between experimental and calculated protein's partition coefficients using the best model fitting net charge.

Fig. 5 shows a comparison between experimental and calculated partition coefficients for lysozyme in the PEG6000-K_2HPO_4 ATPS at 298.15 K at pH around 9.5. The arbitrarily reference ionic species used in the calculation was, for this system, the cation (K^+). At this pH values, the net charge number of lysozyme is around +2. For this charge number

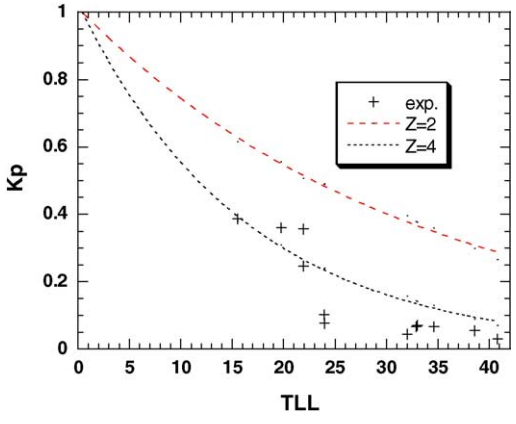

Fig. 5. Calculated and experimental (+) partition coefficient of lysozyme in the PEG6000- K_2HPO_4 aqueous two-phase system at 298.15 K.

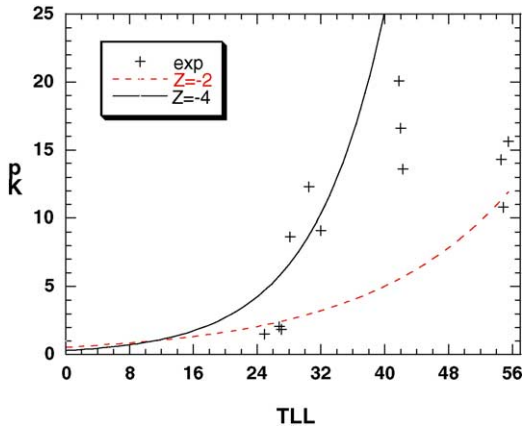

Fig. 6. Calculated and experimental (+) partition coefficient of catalase in the PEG6000-K_2HPO_4 aqueous two-phase system at 298.15 K.

predictions agree with the experimental results, within the experimental uncertainty, although the best predictions were obtained with lysozyme net charge number equal to +4 (Fig. 5). In the same system the model predicted the preference of catalase for the top phase (see Fig. 6), even though the net charge number that originates the best prediction results ($Z = -2$) was slightly different from the net charge obtained with the titration/electrophoresis experiments ($Z = -4$). For the other two proteins, i.e., BSA and glucosidase, the predicted results were considerably different from the experimental ones (see Table 2). Besides the reasons aforementioned for the discrepancies between predicted and experimental partition coefficient for lysozyme in the PEG6000-Na_2SO_4 aqueous two-phase system, the fact that we did not take into account the dissociation of the phosphate ion, might also have had influence on the deviations between experimental and predicted results observed for this system.

The experimental data available on partition coefficients of proteins in ATPS to date, does not allow us to accurately estimate interaction parameters between them and the other components present in the system (polymer, water and ions). Thus, although the model and methodology adopted to predict protein partition in ATPS are, in some cases, very satisfactory, the major discrepancies between prediction and experiment may be partially due to the lack of experimental data. Therefore additional experimental work should be carried out in order to test models and methodologies to predict protein partition in these systems. These experimental efforts should focus on the behaviour of proteins in aqueous solutions, and on how the presence of the phase forming components (polymers and salts) as well as buffers, will affect this behaviour, and ultimately its influence in the protein partition.

6. Conclusions

A modified Wilson model proposed previously in the literature [25] has been applied to the prediction of protein partition of BSA, lysozyme, glucosidase and catalase in the aque-

ous systems of Na_2SO_4/PEG6000 and K_2HPO_4/PEG6000, at 298.15 K. The electrostatic interactions were taken into account using the Debye–Hückel equation. Due to the lack of experimental information some parameters were assumed equal to zero. The results are, in most cases, satisfactory. Mainly, the observed deviations can be attributed to the assumptions made.

Acknowledgements

Pedro P. Madeira acknowledges the financial support from Laboratory of Separation and Reaction Engineering (LSRE) and Xin Xu acknowledges the postdoctoral fellowship provided by Fundação para a Ciência e a Tecnologia (FCT), Ministério da Ciência e Tecnologia, Portugal. The authors are very grateful to professor G. Maurer for all the material kindly provided and helpful discussions.

Appendix A

Table A.1
Interaction parameters between polymer (2) and water (1) [25]

Polymer	PEG6000
$a_{21}^{(1)}$	4.6259
$a_{12}^{(1)}$	−2.2849

Table A.2
Interaction parameters between salt (3) and water (1) [28]

Salt	$a_{31}^{(1)}$	$a_{13}^{(1)}$
Na_2SO_4	−10.5745	21.8676
KH_2PO_4	−6.5426	13.5299

Table A.3
Interaction parameters between polymer (2) and salt (3) [27]

System	$a_{32}^{(1)}$	$a_{23}^{(1)}$
PEG-Na_2SO_4	0.5296	65.7169
PEG-K_2HPO_4	0.9510	91.5033

References

[1] P-Å. Albertsson, Partition of cell particles and macromolecules, third ed., Wiley-Interscience, New York, 1986.
[2] J.N. Baskir, T.A. Hatton, U.W. Suter, Thermodynamics of the separation of biomaterials in two-phase aqueous polymer systems: effect of he phase-forming polymers, Macromolecules 20 (1987) 1300–1311.
[3] D. Forciniti, C.K. Hall, M-R. Kula, Influence of polymer molecular weight and temperature on phase composition in aqueous two-phase systems, Fluid Phase Equilib. 61 (1991) 243–252.
[4] D. Forciniti, C.K. Hall, M-R. Kula, Electrostatic effects on protein partitioning: Simultaneous effect of pH and polymer molecular weight, Chem. Eng. Sci. 47 (1992) 165–175.
[5] C. Groβmann, G. Maurer, On the calculation of phase equilibria in aqueous two-phase systems containing ionic solutes, Fluid Phase Equilib. 106 (1995) 17–25.
[6] C. Groβmann, R. Tintinger, J. Zhu, G. Maurer, Aqueous two-phase systems of poly(ethylene glycol) and dextran—experimental results and modeling of thermodynamic properties, Fluid Phase Equilib. 106 (1995) 111–138.
[7] C. Groβmann, R. Tintinger, J. Zhu, G. Maurer, Aqueous two-phase systems of poly(ethylene glycol) and di-potassium hydrogen phosphate with and without partitioning biomolecules—experimental results and modeling of thermodynamic properties, Ber. Bunsen. Phys. Chem. 99 (1995) 700–712.
[8] C.A. Haynes, H.W. Blanch, J.M. Prausnitz, Separation of protein mixtures by extraction: thermodynamic properties of aqueous two-phase polymer systems containing salts and proteins, Fluid Phase Equilib. 53 (1989) 463–474.
[9] C.A. Haynes, J. Carson, H.W. Blanch, J.M. Prausnitz, Electrostatic potentials and protein partitioning in aqueous two-phase systems, AIChE J. 37 (1991) 1401–1409.
[10] C.A. Haynes, F.J. Benitez, H.W. Blanch, J.M. Prausnitz, Application of integral-equation theory to aqueous two-phase partitioning systems, AIChE J. 39 (1993) 1539–1557.
[11] R.S. King, H.W. Blanch, J.M. Prausnitz, Molecular thermodynamics of aqueous two-phase systems for bioseparation, AIChE J. 34 (1988) 1585–1594.
[12] H. Walter, D.E. Brooks, D. Fisher, Partitioning in aqueous two-phase systems: theory, methods, uses, and applications to biotechnology, Academic Press, Orlando, FL, 1985.
[13] B.Y. Zaslavsky, Aqueous two-phase partitioning, physical chemistry and bioanalytical applications, Marcel Dekker, New York, 1995.
[14] P-Å. Albertsson, Chromatography and partition of cells and cell fragments, Nature 177 (1956) 771–774.
[15] W. Fan, U. Bakir, C.E. Glatz, Contribution of protein charge to partitioning in aqueous two-phase systems, Biotechnol. Bioeng. 59 (1998) 461–470.
[16] G. Johansson, Partition of proteins and micro-organisms in aqueous biphasic systems, Mol. Cell Biochem. 4 (1974) 169–180.
[17] G. Johansson, Comparison of two aqueous biphasic systems used for the partition of biological material, J. Chromatogr. 150 (1978) 63–71.
[18] G. Johansson, Determination of ionic charge by liquid-liquid partition, J. Chromatogr. 322 (1985) 425–432.
[19] S. Bamberger, G.V.F. Seaman, K.A. Sharp, D.E. Brooks, The effects of salts on the interfacial tension of aqueous dextran poly(ethylene glycol) phase systems, J. Colloid Interf. Sci. 99 (1984) 194–200.
[20] D.E. Brooks, K.A. Sharp, S. Bamberger, C.H. Tamblyn, G.V.F. Seaman, H. Walter, Electrostatic and electrokinetic potentials in two polymer aqueous phase systems, J. Colloid Interf. Sci. 102 (1984) 1–13.
[21] J. Jiang, J.M. Prausnitz, Molecular thermodynamics for partitioning of native and denatured proteins in aqueous two-phase systems, J. Phys. Chem. B. 104 (2000) 7197–7205.
[22] J.S. Newman, Electrochemical Systems, Prentice-Hall, Englewood Cliffs, NJ, 1973.
[23] Brenneisen J. Zur verteilung von proteinen auf wässrige zweiphasen-systeme. Phd thesis, Universität Kaiserslautern (2001).
[24] J.N. Baskir, T.A. Hatton, Suter, Protein partitioning in two-phase aqueous polymer systems, Biotechnol. Bioeng. 34 (1989) 541–558.
[25] X. Xu, P.P. Madeira, J.A. Teixeira, E.A. Macedo, A new modified Wilson equation for the calculation of vapor–liquid equilibrium of aqueous polymer solutions, Fluid Phase Equilib. 213 (2003) 53–63.
[26] Madeira PP, Xu X, Teixeira, JA, Macedo EA. Liquid-liquid equilibrium of aqueous polymer two-phase systems using the modified Wilson equation. Ind. Eng. Chem. Res. (2005), in press.
[27] X. Xu, P.P. Madeira, E.A. Macedo, Representation of liquid-liquid equilibria for polymer-salt aqueous two-phase systems, Chem. Eng. Sci. 59 (2004) 1153–1159.

[28] X. Xu, E.A. Macedo, A new modified Wilson model for electrolyte solutions, Ind. Eng. Chem. Res. 42 (2003) 5702–5707.
[29] A. Haghtalab, B. Mokhtarani, On extension of UNIQUAC-NRF model to study the phase behavior of aqueous two-phase polymer-salt systems, Fluid Phase Equilib. 180 (2001) 139–149.
[30] C.C. Chen, H.I. Britt, J.F. Boston, L.B. Evans, Local composition model for excess Gibbs energy of electrolyte systems. Part 1: single solvent, single completely dissociated electrolyte systems, AIChE J. 28 (4) (1982) 588–596.
[31] Y-T. Wu, D-Q. Lin, Z-Q. Zhu, Thermodynamics of aqueous two-phase systems-the effect of polymer molecular weight on liquid-liquid equilibrium phase diagrams by the modified NRTL model, Fluid Phase Equilib. 147 (1998) 25–43.
[32] C.C. Chen, L.B. Evan, A local composition model for the excess Gibbs energy of aqueous electrolyte systems, AIChE J 32 (3) (1986) 444–454.
[33] Y-T. Wu, Z-Q. Zhu, D-Q. Lin, L.H. Mei, A modified NRTL equation for the calculation of phase equilibrium of polymer solutions, Fluid Phase Equilib. 121 (1996) 125–139.