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Interaction probabilities in a four components aqueous two-phase system: polymer + salt + water + protein

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1. Introduction

The extraction of biomolecules and cell particles using two-phase systems is of interest as it allows the separation and purification of these substances in biocompatible surroundings under nondenaturation conditions (Mattiason & Ling, 1987, Chap. 21). Thus, the differentiation of compounds and their concentration is possible according to the affinity of each one to the phases. Most of the smaller soluble substances will partition to the more polar phase (bottom phase), while the proteins will go to the less polar phase (top phase). This is not always the case, though, because various conditions may influence the partition process and the degree of separation obtained depends on the surface properties of the solute (size, electric charge, hydrophobicity, etc.) and on the characteristics of the two-phase system formed (density, viscosity, pH, etc.) (Albertsson, Johansson, & Tjerneld, 1990, Chap. 10). The separation of proteins can be enhanced by altering some characteristics of the system, such as the average molecular mass of polymers, ionic species, ionic strength and hydrophobic groups. The partition coefficient (K) is used to quantify the degree of separation reached in an extraction process and is defined by

$$K = \frac{C_T}{C_B}, \tag{1}$$

where C_T and C_B are the substance concentrations in the top and bottom phases, respectively.

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1.1. Thermodynamic framework

The thermodynamic properties of aqueous two-phase systems are of interest in certain industrial applications (i.e. downstream processing biotechnology). In recent years aqueous two-phase polymer system investigations have focused on phase equilibrium thermodynamics (Kula, Kroner, & Hustedt, 1982; Kang & Sandler, 1988; Baskir, Hatton, & Suter, 1989; Diamond & Hsu, 1989; Haynes, Blanch, & Prausnitz, 1989; Hartounian, Floeter, Kaler, & Sandler, 1993; Li, Zhu, Wu, & Lin, 1998; Wu, Lin, & Zhu, 1998) but there is no universally applicable method available to describe this.

There exists several interesting prospects for the application of electrolyte/polymer aqueous two-phase systems to the separation and purification of proteins. Some authors have proposed models using the UNIFAC–Fowler–Guggenheim equation (Gao, Peng, Li, & Li, 1991; Peng, Li, & Li, 1994) and the Edmond and Ogston theory (King, Blanch, & Prausnitz, 1988) to the prediction of the phase diagram and protein partition coefficients giving complicated expressions with a high number of variables that are difficult to determine.

A protein interacts with the surrounding molecules within a phase via various bonds, such as hydrogen, ionic and hydrophobic interactions, together with other weak forces. The net effect of these interactions is likely to be different in the two phases and therefore the protein will be partitioned into the phase where the energy is more favourable. If the energy needed to move a protein from one phase to the other is ΔE, at equilibrium the relationship between the partition coefficient (K) and ΔE can be

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expressed as

$$K = \exp\left(\frac{\Delta E}{kT}\right), \tag{2}$$

where k is the Boltzman constant and T the absolute temperature. ΔE depends on the size of the partitioned molecule, since the larger it is, the greater will be the number of exposed atoms which can interact with the surrounding phase. Such energy is composed of subenergetic parts which result from independent interaction processes between the protein and the other components. In this case, the interaction energy will be for the following pairs: α-amylase/PEG, α-amylase/salt and α-amylase/water.

Each one of the interaction processes is expressed at equilibrium by a constant:

$$K_i = \exp\left(\frac{-\Delta G_i}{RT}\right) \tag{3}$$

where ΔG_i represent the free Gibbs energy of each interaction.

Thus the partition coefficient can be termed as a combination of such constants within an overall equilibrium constant:

$$K = \prod_{i=l}^{n} K_i. \tag{4}$$

A relationship between the equilibrium compositions of the quaternary system PEG 8000 + $MgSO_4 \cdot 7H_2O$ + H_2O + α-amylase at 298 K was developed and the thermodynamics that govern the process can be obtained. For this purpose, we used the equilibrium data of the PEG 8000 + $MgSO_4 \cdot 7H_2O$ + H_2O system (González-Tello, Camacho, Blázquez, & Alarcón, 1996), together with the rheological characteristics and densities of the phases (González-Tello, Camacho, & Blázquez, 1994) .

2. Results and discussion

The physical properties of the tie line extremes were known and new mixtures were prepared with precise quantities of α-amylase (1.25, 2.50 and 5.00 g l^{-1}). The points were chosen maintaining the initial salt concentration in the experiments at a constant value.

After separation, α-amylase concentration was determined in both phases using the Bradford method (Bradford, 1976) and the partition coefficients were calculated (Eq. (1)).

Initial and final values of PEG (w_1) and $MgSO_4$(w_2) concentrations are shown in Table 1, together with the final values of enzyme concentration (w_e). The partition coefficient values are shown in Table 2 for each initial enzyme concentration, together with the values of C_T and C_B (top and bottom phase enzyme concentration, respectively, in g l^{-1}).

2.1. Interaction probabilities

The partition coefficient, K, of the enzyme takes values depending on the tie line used in the extraction process. Thus, the enzyme concentration at the extremes also depends on the phase compositions and is related to its overall interaction with the other components. First, the study was made using the top phase compositions and then it was extended to the bottom ones.

2.1.1. Top phases

If we consider the quantity of salt to be negligible, C_T will only be related to such α-amylase interactions with water and PEG. The molecular binding with the more polar points of the enzyme explains its solubility in water. However, the enzyme portion transported to the top phase increases at the same rate as the increase of PEG concentration.

If α_e is the number of the total interacting points of the enzyme per area unit, the following relation can be assumed:

$$\alpha_e = \alpha_w + \alpha_{\mathrm{PEG}}, \tag{5}$$

where α_w and α_{PEG} are the number of binding points to water and PEG, respectively. A new configuration of Eq. (5) gives

$$1 = \frac{\alpha_w + \alpha_{\mathrm{PEG}}}{\alpha_e} = \frac{\alpha_w}{\alpha_e} + \frac{\alpha_{\mathrm{PEG}}}{\alpha_e}, \tag{6}$$

where α_w/α_e is the probability of occupation by water molecules, and $\alpha_{\mathrm{PEG}}/\alpha_e$ is that of PEG. Both probabilities must be related to the respective concentrations of water and PEG if the enzyme concentration is previously fixed (α_e).

A relationship between the number of points and concentrations can be expressed as follows (the superscripts are not indicated for simplicity, because only magnitudes of top phase are used):

$$\alpha_e = AC_T, \quad \alpha_w = Bw_3^a, \quad \alpha_{\mathrm{PEG}} = Cw_1^b,$$

where w_3 is the water mass fraction and w_1 is that of PEG. The powers (a, b) are related with the stoichometry of the individual interactions: a molecules of water and b molecules of PEG per enzyme unit. Parameters A, B and C should give the proportion of these molecules that are effectively linked. The expression α_e is the only one that depends on the concentration, in terms of g l^{-1}. The transformation of concentrations to mass fraction is related by the density of the phase, and its effects can be included into the A parameter. Thus, Eq. (6) takes the following form:

$$1 = A^* \frac{w_3^a}{C_T} + B^* \frac{w_1^b}{C_T}. \tag{7}$$

Table 1
Partition of α-amylase (w_e) in aqueous two-phase systems PEG 8000 (w_1) + $MgSO_4$ (w_2) + H_2O, at 298 K. Initial compositions (w_1^0, w_2^0: initial mass fractions; C_0: initial enzyme concentration, g l^{-1}) and tie line compositions (w_1'', w_2'', w_e'': top phase mass fractions; w_1', w_2', w_e': bottom phase mass fractions)

w_1^0	w_2^0	w_1''	w_2''	w_1'	w_2'	$C_0 = 1.25$ g l^{-1}		$C_0 = 2.50$ g l^{-1}		$C_0 = 5.00$ g l^{-1}	
						w_e''	w_e'	w_e''	w_e'	w_e''	w_e'
0.1266	0.1200	0.1954	0.0699	0.0047	0.2124	0.0004	0.0022	0.0004	0.0048	0.0004	0.0073
0.1700	0.1200	0.2781	0.0418	0.0000	0.2450	0.0006	0.0023	0.0004	0.0043	0.0005	0.0099
0.2312	0.1200	0.378	0.0239	0.0000	0.2951	0.0007	0.0026	0.0007	0.0041	0.0006	0.0115
0.2750	0.1200	0.4094	0.0140	0.0000	0.3275	0.0007	0.0029	0.0009	0.0046	0.0009	0.0120
0.3190	0.1200	0.4563	0.0108	0.0000	0.3788	0.0011	0.0017	0.0015	0.0035	0.0006	0.0105

Table 2
Partition coefficients of α-amylase in aqueous two-phase systems PEG 8000 + $MgSO_4$ + H_2O, at 298 K, corresponding to previous tie lines (C_T, C_B, in g l^{-1})

$C_0 = 1.25$ g l^{-1}			$C_0 = 2.50$ g l^{-1}			$C_0 = 5.00$ g l^{-1}		
C_T	C_B	K	C_T	C_B	K	C_T	C_B	K
0.44	2.40	0.183	0.45	5.35	0.084	0.41	8.53	0.048
0.63	2.60	0.242	0.48	4.78	0.100	0.56	11.20	0.050
0.70	3.50	0.200	0.72	4.74	0.152	0.67	13.51	0.050
0.80	3.20	0.250	0.97	5.41	0.179	0.93	14.30	0.065
1.20	1.85	0.649	1.57	4.21	0.373	0.61	12.85	0.048

As shown in Table 2, the enzyme concentration in the top phase reaches a mean value at each tie line. These values are employed in the following operations.

The enzyme–water interaction process is thermodynamically favourable with its own free Gibbs energy term

$$\Delta G_w = -RT \ln K_w, \tag{8}$$

where K_w is the equilibrium constant enzyme–H_2O that is generalized to all the possible unions.

$$\text{enzyme} + a\ H_2O \rightleftharpoons \text{enzyme} - H_2O$$

$$K_w = \frac{[\text{enzyme} - H_2O]}{C_T w_3^a}, \tag{9}$$

from which

$$[\text{enzyme} - H_2O] = \exp\left(\frac{-\Delta G_w}{RT}\right) C_T w_3^a. \tag{10}$$

The A^* parameter is related to the left hand term:

$$A^* = X[\text{enzyme} - H_2O] = X\exp\left(\frac{-\Delta G_w}{RT}\right) C_T w_3^a. \tag{11}$$

If a similar operation is performed with B^* by using the equilibrium between enzyme and PEG (with ΔG_{PEG} as the free energy for enzyme–PEG interaction):

$$B^* = Y[\text{enzyme} - \text{PEG}] = Y\exp\left(\frac{-\Delta G_{PEG}}{RT}\right) C_T w_1^b. \tag{12}$$

X and Y would represent the extension of each intermediate formation, because it is probably that not all the interactions between species could be effective.

The total equilibrium constant of the concurrent processes in the top phase is K_T and is given by its own free Gibbs energy, ΔG_T:

$$K_T = \exp\left(\frac{-\Delta G_T}{RT}\right) = \exp\left(\frac{-\Delta G_w}{RT}\right) \exp\left(\frac{-\Delta G_{PEG}}{RT}\right). \tag{13}$$

By combining Eqs. (11) and (12):

$$A^* = \frac{ZC_T^2 w_3^a w_l^b}{B^*}, \tag{14}$$

where $Z = XYK_T$.

By substituting in Eq. (7) and taking into account that w_3 is high, a new constant can be included to obtain:

$$w_1 = \left(\frac{C_T}{\beta C_T^2 + B^*}\right)^{1/b} \tag{15}$$

which can be treated with a nonlinear regression to give the following values:

$$\beta = 0.7020, \quad B^* = 0.5104, \quad (1/b) = 4.5059.$$

The dependence between experimental (PEG exp) and calculated (PEG cal) data using Eq. (15) is shown in Fig. 1.

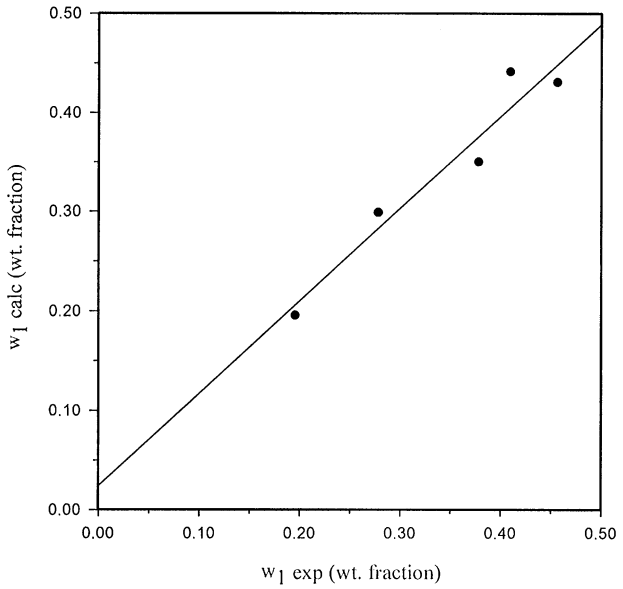

Fig. 1. PEG calculated vs experimental concentrations, Eq. (15).

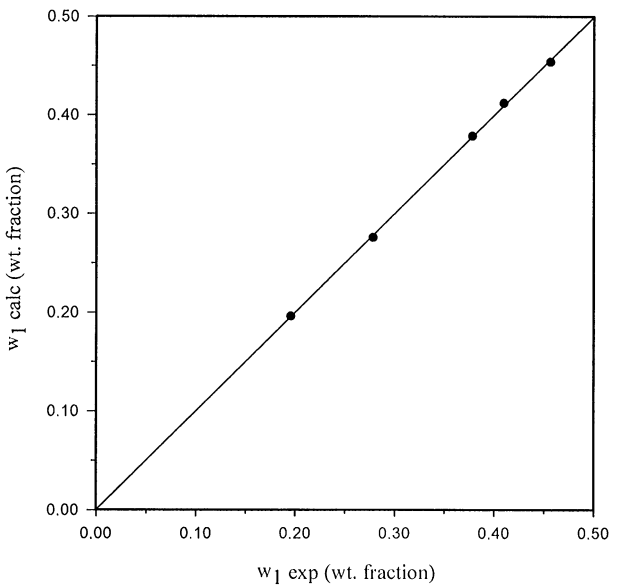

Fig. 2. PEG calculated vs experimental concentrtations, Eq. (18).

There exists a concentration of salt in the top phase that might be appreciable given the small quantities of enzyme employed, perhaps producing alterations. Thus, a similar operation could be performed, but taking the salt presence into consideration.

$$1 = \frac{\alpha_w}{\alpha_e} + \frac{\alpha_{\mathrm{PEG}}}{\alpha_e} + \frac{\alpha_s}{\alpha_e}, \tag{16}$$

where α_s/α_e is the probability of salt occupation. We consider

$$\alpha_e = AC_T, \quad \alpha_w = Bw_3^a, \quad \alpha_{\mathrm{PEG}} = Cw_1^b, \quad \alpha_s = Dw_2^c,$$

then

$$1 = A^* \frac{w_3^a}{C_T} + B^* \frac{w_1^b}{C_T} + C^* \frac{w_2^c}{C_T}. \tag{17}$$

Here C^* has a similar expression to those of A^* and B^*, as a function of w_2; by isolating A^* and operating, w_1 can be related to the enzyme and salt concentrations in the same phase:

$$w_1 = \left(\frac{C_T - C^* w_2^c}{\beta' C_T^3 w_2^c + B^*} \right)^{1/b}. \tag{18}$$

Using nonlinear regression:

$$C^* = 3.8223, \quad c = 0.9935, \quad \beta' = 275.9115,$$

$$B^* = 0.7311, \quad (1/b) = 1.$$

Eq. (18) offers greater precision and more exactitude in the correlation of the experimental data, as shown in Fig. 2. A more exact expression was obtained, relating the experimental data from a quaternary system, where water composition is considered to be constant. The enzyme concentration appears in Eq. (18) at power 3, which indicates the number of compounds taken into account.

Table 3
Mean values of C_T (g l^{-1}) and Gibbs free energy (J mol^{-1}) of top phases

C_T	$-\Delta G_T$	K_T
0.46	−17761.63	1298.68
0.56	−18226.66	1566.66
0.61	−18915.44	2068.75
0.90	−19136.09	2261.31
0.96	−19538.27	2660.05

If we include the calculated parameters in the expressions of $B^*, C^* y\beta'$, the values of free energies can be evaluated, as shown in Table 3. R, T, w_3, X, YyZ must be positive numbers and ΔG_T presents negative values (isobaric and isothermic spontaneous processes).

We made some simplifications to facilitate estimations:

(1) X, Y and Z are parameters introduced to link A^*, B^* and C^* with the intermediate forms enzyme–H_2O, enzyme–PEG and enzyme–salt, respectively. If the latter interactions of the enzyme are assumed to be complete, the three parameters present a value of one.

(2) Although w_3 is assumed to be constant, these values lie within an interval (from $\sim$ 50% to $\sim$ 75% in mass). Thus, unitary value is not assumed, but a approaches unity, as it occurs with PEG and salt. The data in Table 3 are generated under this condition.

2.1.2. Bottom phases

Table 1 shows that bottom phases are only formed by H_2O, $MgSO_4$ and the corresponding enzyme concentration, C_B(PEG quantity is negligible). Due to the constant value of C_B for any C_0 the latter treatment cannot be applied.

In the overall process, the partition coefficient can be expressed as:

$$K = \exp\left(\frac{\Delta E}{kT}\right) = \left[\exp\left(\frac{-\Delta G_{\mathrm{PEG}}}{RT}\right)\exp\left(\frac{-\Delta G_w}{RT}\right)\exp\left(\frac{-\Delta G_s}{RT}\right)\right]_T \times \left[\exp\left(\frac{-\Delta G_w}{RT}\right)\exp\left(\frac{-\Delta G_s}{RT}\right)\right]_B = K_T K_B, \quad (19)$$

where T and B represent the top and bottom phases, respectively. ΔG_{PEG}, ΔG_w and ΔG_s represent the free energy of the respective interaction processes of enzyme and PEG, water and salt.

K_B has to be constant for each initial enzyme concentration. Then, K_B can be determined using the experimental data of K and the calculated values of K_T giving a way to estimate the free energy of the bottom phase (ΔG_B). The free energy takes a constant value for each initial enzyme concentration employed, probably due to the absence of PEG in the phase that disturbs the compensation relationship between salt and water in the top phases.

$$\Delta G_B(1.25\ \mathrm{g\ l^{-1}}) = -22008.70\ \mathrm{J\ mol^{-1}},$$

$$\Delta G_B(2.50\ \mathrm{g\ l^{-1}}) = -23325.64\ \mathrm{J\ mol^{-1}},$$

$$\Delta G_B(5.00\ \mathrm{g\ l^{-1}}) = -25612.24\ \mathrm{J\ mol^{-1}}.$$

The free energies show that bottom phases are more favourable at first, under these conditions, but as the quantity of PEG increases the tendency is for the top phases to become more favourable.

3. Conclusion

A model was developed to justify the behaviour of proteins in extraction phenomena based on interaction probabilities with the other compounds. This gave rise to an expression giving the energy parameters of the total interaction processes, offering the possibility of estimating the same in bottom phase.

Notation

A^*, B^*, C^*, D^*	parameters
a, b, c	powers of water, polymer and salt mass fractions, respectively
C_0	initial enzyme concentration, g l^{-1}
C_B, C_T	bottom phase and top phase enzyme concentrations, g l^{-1}
E	total partition energy, J
G_i	Gibbs free energy of each interaction, J mol^{-1}
G_B, G_T	Gibss free energy of bottom and top phase, J mol^{-1}
K	partition coefficient
K_B	global constant of bottom phase equilibria
K_i	individual equilibrium constant
K_T	global constant of top phase equilibria
k	Boltzman's constant, J K^{-1}
T	temperature, K
w_1, w_2, w_3, w_e	mass fraction of polymer, salt, water and enzyme, respectively
X, Y, Z	parameters

Greek letters

α_i	number of interacting points
β, β'	energy-related parameters of Eqs. (15) and (18), respectively
Δ	variation of magnitude

Subscripts

i	PEG, s, w, e (polymer, salt, water and enzyme, respectively)

Superscripts

0	initial values
$'$, $''$	magnitude from bottom and top phase, respectively

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