Journal of Chromatography B, 877 (2009) 2579–2584

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Thermodynamic studies of partitioning behavior of lysozyme and conalbumin in aqueous two-phase systems

Rita de Cássia Superbi de Sousa[a], Jane Sélia dos Reis Coimbra[a,*], Luis Henrique Mendes da Silva[b], Maria do Carmo Hespanhol da Silva[b], Edwin Elard Garcia Rojas[c], Antonio António Augusto Vicente[d]

[a] Separation Process Laboratory (LPS), Department of Food Technology, Federal University of Viçosa (UFV), P.H. Rolfs Av., s/n, 36570-000 Viçosa, MG, Brazil
[b] Department of Chemistry, Federal University of Viçosa (UFV), P.H. Rolfs Av., s/n, 36570-000 Viçosa, MG, Brazil
[c] Department of Agribusiness and Administration Engineering, Federal University Fluminense (UFF), Trabalhadores Av. 420, 27225-250 Volta Redonda, RJ, Brazil
[d] IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

ARTICLE INFO

Article history:
Received 8 January 2009
Accepted 1 July 2009
Available online 7 July 2009

Keywords:
Aqueous two-phase systems
Egg white protein
Conalbumin
Lysozyme
Thermodynamic parameters

ABSTRACT

The objective of this study was to determine the thermodynamic parameters ($\Delta_{tr}G$, $\Delta_{tr}H$ and $\Delta_{tr}S$) associated with lysozyme and conalbumin partitioning in aqueous two-phases systems (ATPS). Influence of salt type and polyethylene glycol (PEG) concentrations on the partition coefficient of lysozyme and conalbumin from egg white was studied. The evaluated ATPS were composed of PEG 1500 and inorganic salts (sodium citrate and sodium sulfate) at a temperature of 25 °C and pH 7.0, with PEG 1500 g mol^{-1} concentrations of 14%, 16% and 18% (mass basis). Partitioning of lysozyme in PEG–citrate ATPS was enthalpically driven, however the PEG–sulfate ATPS was entropically driven. The tested systems can be employed for the separation of these two proteins in egg white, due to the fact that lysozyme migrates toward the polymeric phase and conalbumin to the saline phase in both ATPS. A high recovery of conalbumin in the saline phase of the PEG–sulfate ATPS was determined to be enthalpically driven.

1. Introduction

Egg white is a natural protein source with high nutritional and biological value. Among these proteins, lysozyme and conalbumin are of particular interest due to their high nutritional and functional value, highlighting the interest in the development of new techniques for their separation and purification while maintaining their functional characteristics unchanged [1–3].

Aqueous two-phase systems have achieved continued applications in recent decades for concentration, isolation and separation of proteins. These systems are composed of two immiscible phases which provoke biomolecule separation under gentle conditions and an suitable environment, allowing biomolecules to maintain their functionality. The high concentration of water (between 65% and 90% in mass) in these systems favors protein stability during separation when compared with traditional liquid extraction systems composed of organic solvents. ATPS can be a good alternative for the first stage of protein purification since these systems permit the removal of diverse contaminants using a simple and low cost system [4–7].

Difficulty in prediction protein partitioning in ATPS is due to the lack of thermodynamic understanding which controls this process. Theoretical thermodynamic comprehension of aqueous phase systems is valuable for comparison with actual protein partitioning from experimental results. Tubío et al. [8] and Picó et al. [9] have published data regarding the understanding of thermodynamic partitioning properties.

Therefore, the objectives of this work were: (i) to evaluate the use of ATPS composed of polyethylene glycol (PEG), salt and water as a separation technique for lysozyme and conalbumin; (ii) to study the influence of salt type (sodium citrate and sodium sulfate) and the concentration of PEG (14%, 16% and 18% (w/w)), on protein partitioning and (iii) to obtain the thermodynamic parameters associated with protein transfer to each of the phases.

2. Materials and methods

2.1. Materials

Lysozyme and conalbumin were purchased from Sigma–Aldrich (USA). Polyethylene glycol (PEG) 1500 g mol^{-1} (SYNTH, Brazil), sodium sulfate and sodium citrate (CROMOLINE, Brazil) were used in the preparation of the ATPS. All reagents used were of analytical grade requiring no greater purification. In the experiments, ultrapure water (Milli-Q system, Millipore Inc., USA) was used.

* Corresponding author. Tel.: +55 31 38992229; fax: +55 31 38992208.
E-mail address: jcoimbra@ufv.br (J.S. dos Reis Coimbra).

1570-0232/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2009.07.002

2.2. Preparation of the aqueous two-phase systems (ATPS)

Biphasic systems were prepared by mixing PEG 1500 and an inorganic salt (sodium citrate or sodium sulfate) according to liquid–liquid equilibrium data cited by Carvalho et al. [10] and Martins et al. [11].

A stock solution of PEG 1500 (50% (w/w)) was first prepared. Sodium sulfate and sodium citrate were subsequently added by weighting their solid masses. The pH values of the saline solutions used were close to 7.0, requiring no adjustments. Proteins (lysozyme and conalbumin) were the last components to be added to the system (5.0 mg—analytical balance, M-310, DENVER INSTRUMENTS, USA). All systems were prepared in graduated centrifuge tubes with a total system mass of 12 g for each tube. Tubes were manually agitated for approximately 3 min and centrifuged (EPPENDORF 5702, Germany) at 400 rpm for 10 min to accelerate phase formation. The tubes were placed in a thermostatic bath (TE-184, TECNAL, Brazil) at the working temperature (25 °C) and left to rest for 20 h. After this treatment, protein concentration in each of the phases was determined by taking samples the phases using a pipette for the upper phase and a syringe with a long, thin needle (in order to minimize disturbances to the upper phase) for the lower phase.

2.3. Quantification of proteins present in the phases

The concentration of proteins (lysozyme and conalbumin) present in both the saline and polymeric phases was determined by high performance liquid chromatography (HPLC) in a ÄKTA Purifier® 10/100 system (Amersham Pharmacia Biotech, Sweden), using a Mono S® HR 5/5 cation exchange column (Pharmacia Biotech, Sweden). The eluent was monitored by UV absorption (UV-900) at 215 nm and its conductivity was measured on-line by a pH/C-900 flow cell. Samples were passed through 0.22 μm Millipore filters and injected through a 50 μl loop. For elution of the proteins, a mobile phase gradient composed of phosphate pH 7.0 buffer (*A*) and phosphate pH 7.0 buffer +0.5 mol L^{-1} NaCl (*B*), at a flow rate of 1.0 mL min^{-1}, was used (Table 1). Maximum pressure on the column bed was 2.0 MPa and due to its high viscosity, the polymeric phase was diluted 1:2 (sample:water) before injection.

2.4. Calculation of the partition coefficient

After chromatographic analysis, the protein concentration in each phase was found by relating the area of the protein peak with the calibration curve (area versus concentration), obtained using pure proteins. After determining the concentration of each protein in each phase, the partition coefficient for the individual proteins was determined. In the case of lysozyme, the partition coefficient (K_L) was calculated as the ratio between $[P]_{top}$ and $[P]_{bottom}$, where $[P]_{top}$ and $[P]_{bottom}$ are the equilibrium protein concentrations in the PEG (upper) and salt (lower) rich phases, respectively. In the case of conalbumin, the partition coefficient (K_C) was calculated inversely as the ratio between $[P]_{bottom}$ and $[P]_{top}$. All experiments were performed in duplicate and the partition coefficients were used to quantify the degree of separation achieved in the extraction process. To select the ATPS with the best protein purification capacity, a theoretical recovery (y, %) in the upper and lower phases was calculated using the following equations:

$$y_{top}(\%) = \frac{100}{1 + (1/R_L K_L)} \quad (1)$$

$$y_{bottom}(\%) = \frac{100}{1 + (1/R_C K_C)} \quad (2)$$

in which $R_L = V_{top}/V_{bottom}$ and $R_C = V_{bottom}/V_{top}$, where V_{top} and V_{bottom} are the volumes of the upper and lower phases, respectively.

Table 1
Mobile phase gradient for the elution of the proteins.

Time (min)	% Buffer	
	A	*B*
0	100	0
1	100	0
3	65	35
7	65	35
9	25	75
14	25	75
15	0	100
18	0	100

2.5. Determination of the enthalpy of transfer ($\Delta_{tr}H$)

In this study, $\Delta_{tr}H$ was determined directly by isothermal titration microcalorimetry. This technique permitted the evaluation of the energy of transfer by the addition of an extractor phase (polymeric in the case of lysozyme and saline in the case of conalbumin) directly on the phase where the dissolved protein was found (lysozyme in the salt phase and conalbumin in the polymeric phase). When compared with solution microcalorimetry, this technique shows advantages including the smaller amount of reagent necessary for determination and the possibility of performing the transfer experiment with the two phases present, therefore allowing simultaneous the transfer of other ATPS components together with the proteins.

Energy transfer measurements were performed using an isothermal titration microcalorimeter CRC (USA, model ITC 4200). The microcalorimeter was composed of a pair of cells with a capacity of 1.8 mL of solution, in which one was for the sample and the other for the reference material. A syringe added specified volumes of the titration solution (extractor phase) at the same time that the solution was agitated at a given temperature. The total heat of transfer (Q_T) was obtained by experiments which consisted of consecutive injection of 10 μL samples of the upper phase (at a pre-established PEG concentration) to the calorimeter containing 1.8 mL of the lower phase with 3 mg mL^{-1} of the protein (lysozyme). To obtain the net heat of transfer (Q_{net}), energy associated with the formation of microphases from the upper phase dispersed in the lower phase (Q_{ph}) was discounted from the total heat of transfer (Eq. (3)). For this calculation, samples of the upper phase were also injected in the pure lower phase (absence of protein).

$$Q_{net} = Q_T - Q_{ph} \quad (3)$$

The amount of protein present in the saline phase to be transferred to a drop of the added polymeric solution registered peaks and their areas were proportional to the relative energy transferred from the protein phase to the upper phase present in the syringe. Integration of the heating power versus time curves provides the resulting enthalpy of the transfer process ($\Delta_{tr}H$).

The enthalpy of transfer was calculated considering the number of moles of protein transferred (n_p) in each injection (Eq. (4)) using the partition coefficients obtained experimentally. In each experiment, 20 injections were executed.

$$\Delta_{tr}H = \frac{Q_{net}}{n_p} \quad (4)$$

In the case of conalbumin, the procedure was the inverse of that of lysozyme; in this case, successive injections of the saline phase in the upper phase containing the dissolved protein were performed.

Table 2
Partition coefficients and theoretical recovery of lysozyme (polymeric phase) and conalbumin (saline phase) for the ATPS PEG–salt systems at 25 °C, pH 7.0.

ATPS	PEG (% m/m)	Lysozyme		Conalbumin	
		K_L	y_{top} (%)	K_C	y_{bottom} (%)
PEG–sodium citrate	14.00	1.72 ± 0.04	60.63 ± 0.50	0.31 ± 0.02	78.33 ± 0.96
	16.00	2.30 ± 0.01	70.44 ± 0.06	024 ± 0.03	82.63 ± 2.04
	18.00	3.85 ± 0.17	77.39 ± 0.79	0.21 ± 0.01	84.28 ± 0.82
PEG–sodium sulfate	14.00	1.01 ± 0.03	63.97 ± 4.46	0.20 ± 0.03	80.94 ± 0.29
	16.00	1.18 ± 0.01	66.90 ± 1.63	0.18 ± 0.04	83.33 ± 0.05
	18.00	1.46 ± 0.21	72.44 ± 0.28	0.18 ± 0.01	82.61 ± 0.05

3. Results and discussion

Aqueous two-phase systems (ATPS) composed of PEG 1500 and salt were applied to investigate the partitioning behavior of egg white proteins (lysozyme and conalbumin). Changes in the type and concentration of the salt can alter the partitioning behavior of biological materials [7]. The PEG–citrate and PEG–sulfate ATPS are adequate for large-scale purification of biological material and permit the use of traditional liquid–liquid extraction equipment [12–13].

3.1. Influence of the tie line length (TLL) on protein partitioning

In Table 2, the partition coefficients (K_L and K_C) and the theoretical recovery (y, %) for lysozyme and conalbumin are shown. It was observed that lysozyme concentrated itself in the upper phase while conalbumin preferentially transferred to the lower phase. The greatest recovery percentages of lysozyme and conalbumin were found when using the PEG 1500–sodium citrate systems.

Tie line length (TLL) is proportional to the difference between intensive thermodynamic properties for the upper and lower phases. The partitioning behavior of proteins should preferentially be expressed as a function of TLL and not relative to only salt or polymer concentration [14]. The TLL was calculated according to Eq. (5):

$$TLL = \sqrt{[\Delta PEG]^2 + [\Delta Sal]^2} \quad (5)$$

where [ΔPEG] and [ΔSal] correspond to difference in the PEG and salt concentrations (% mass), respectively, between upper and lower phases.

Tables 3 and 4 show the behavior of $\ln K_L$ and $\ln K_C$ as functions of the TLL for lysozyme and conalbumin, respectively. The results show that partitioning of lysozyme to the upper phase increased with the augment of the TLL, while the contrary was observed for conalbumin.

For lysozyme, it was verified that the increase in the partition coefficient was due to the greater PEG concentration in the upper phase and the higher salt concentration in the lower phase. In the case of conalbumin, it can be observed from Table 2 that increases in PEG concentrations resulted in a decrease of protein partitioning to the upper phase.

Table 3
Influence of the TLL on $\ln K_L$ for lysozyme.

ATPS	TLL	$\ln K_L$
PEG–sodium citrate	28.91	0.556
	39.22	0.956
	42.15	1.348
PEG–sodium sulfate	26.79	0.013
	29.74	0.162
	35.12	0.379

Table 4
Influence of the TLL on $\ln K_C$ for conalbumin.

ATPS	TLL	$\ln K_L$
PEG–sodium citrate	28.91	−1.179
	39.22	−1.401
	42.15	−1.561
PEG–sodium sulfate	26.79	−1.595
	29.74	−1.689
	35.12	−1.698

3.2. Thermodynamic analysis of the partition

The free energy of transfer ($\Delta_{tr}G$) is defined as the free molar energy associated with the protein transfer process from the saline phase to the polymeric phase and is calculated with the classic thermodynamic equation (Eq. (6)), using the partition coefficient (K) for each of the proteins:

$$\Delta_{tr}G = -RT \ \ln K \quad (6)$$

The relationships between variables of the two studied ATPS at the temperature of 25 °C are shown in Figs. 1 and 2. For lysozyme, a linear reduction in the free energy of the system was observed with the increase of the TLL. However, the opposite was verified for conalbumin, showing that the transfer of this protein from the lower phase to the upper phase is an unfavorable thermodynamic process.

Two components make up the $\Delta_{tr}G$, one enthalpic ($\Delta_{tr}H$) and the other entropic ($\Delta_{tr}S$). The theoretical model developed by Johansson et al. [15], based on the Flory and Huggins theory, describes the driving force which rules the partition of a solute in an ATPS using two analytical equations, one related to the enthalpy contribution and the other to the entropy contribution.

The enthalpy of transfer ($\Delta_{tr}H$) was obtained experimentally by isothermal titration microcalorimetry and used to investigate the

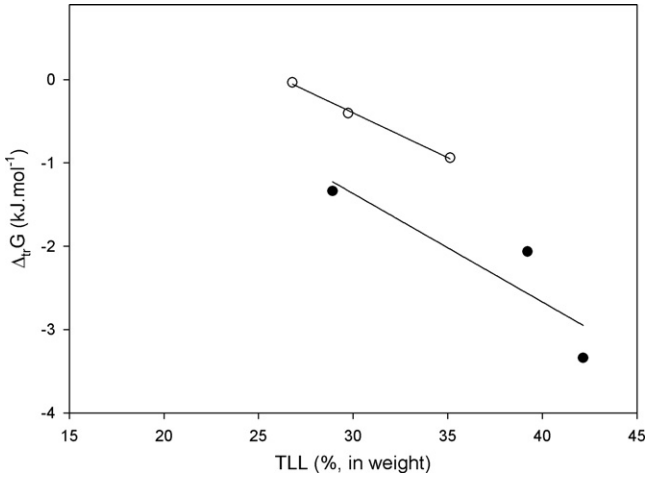

Fig. 1. Influence of the TLL on $\Delta_{tr}G$ for lysozyme (●: sodium citrate; ○: sodium sulfate).

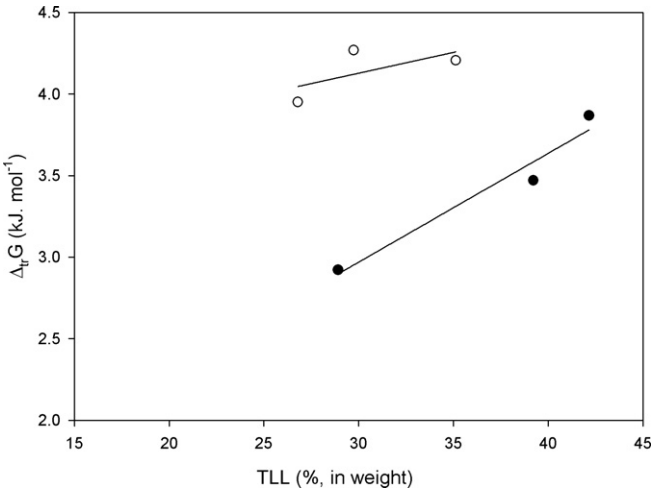

Fig. 2. Influence of the TLL on $\Delta_{tr}G$ for conalbumin (●: sodium citrate; ○: sodium sulfate).

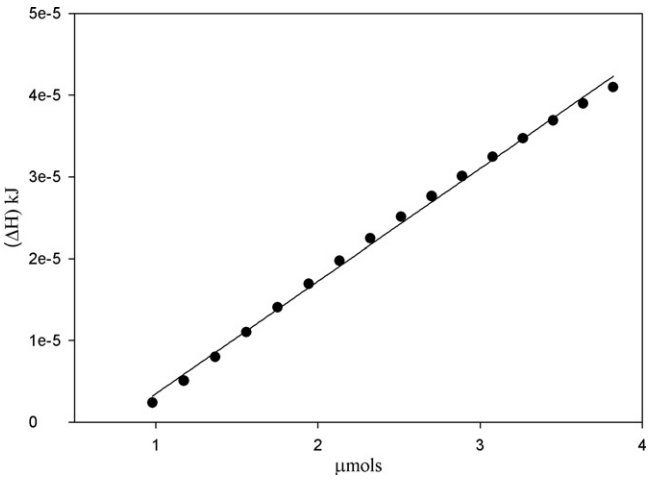

Fig. 4. The integral enthalpy of transfer as a function of the number of conalbumin moles transferred to the extract phase in ATPS PEG 1500–sodium citrate.

contribution of the enthalpic molecular interactions between the different ATPS components and the protein. To obtain $\Delta_{tr}H$, the number of protein moles transferred to the extractor phase in each injection was calculated. The integral enthalpy of transfer was then plotted as a function of the amount of protein transferred and two typical results are shown in Figs. 3 and 4. After a linear regression, the value of $\Delta_{tr}H$ was obtained from the angular coefficient of the line.

From the values of $\Delta_{tr}G$ and $\Delta_{tr}H$, it was possible to calculate the entropy of transfer using the classic thermodynamic equation (Eq. (7)):

$$\Delta_{tr}S = \frac{\Delta_{tr}H - \Delta_{tr}G}{T} \tag{7}$$

In Johansson's model [13], the entropy contribution in the transfer process of a solute to one of the phases is described by Eq. (8):

$$\ln K = \frac{MM_S}{\rho}\left(\frac{n^S}{V^S} - \frac{n^i}{V^i}\right) \tag{8}$$

where K is the partition coefficient of the protein, MM is the molecular mass, ρ is the numeric global molecular density of the ATPS, n^S and V^S are the number of molecules present in the upper phase and its volume, respectively and n^i and V^i correspond to the number of molecules in the lower phase and its corresponding volume.

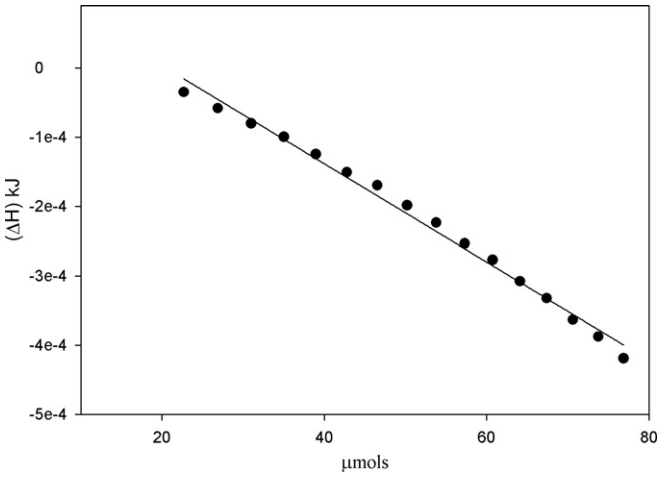

Fig. 3. The integral enthalpy of transfer as a function of the number of lysozyme moles transferred to the extract phase in ATPS PEG 1500–sodium citrate.

Eq. (8) is applied when the absence of enthalpy contributions for solute partitioning is considered. This way, the solute will preferentially transfer to one phase only if the number of molecules per unit volume of the phases is different. According to these conditions, partitioning will occur to a phase which possesses the greater numeric density of molecules (n/V) which is principally determined by the number of water molecules in each phase. The phase with the greater numeric density is capable of accommodating a solute of various different forms (different configurations), increasing the mixture entropy for that phase.

Considering that entropy contributions are negligible, or that the numeric densities of the phases of an ATPS are identical, the enthalpy contribution for solute partitioning is given by Eq. (9):

$$\ln K = -\frac{MM_S}{RT}\left[\sum_{i=1}^{3}(\phi_i^S - \phi_i^I)w_{iS} - \sum_{i=1}^{2}\sum_{j=2}^{3}(\phi_i^S\phi_j^S - \phi_i^I\phi_j^I)w_{ij}\right] \tag{9}$$

where $\Phi_i{}^S$ and $\Phi_i{}^I$ are the volumetric fractions of the component "i" in the upper and lower phase, respectively, and w_{ij} or w_{iS} are the potential effective pairs, given by Eq. (10):

$$w_{ij} = z\left[\varepsilon_{ij} - \frac{1}{2}(\varepsilon_{ii} - \varepsilon_{jj})\right] \tag{10}$$

where w_{ij} is the potential effective pair between the ATPS components formed by "i" and "j"; w_{iS} is the potential effective pair between the component "i" in the upper or lower phase with the solute "s"; z is the total number of potential pairs which occur between the components of the ATPS "i" and "j" or with the solute "S" and; ε_{ij}, ε_{ii} and ε_{jj} are the potential pairs. A positive value of w_{ij} indicates an endothermic interaction (enthalpically repulsive) between the components i and j.

Eq. (9) shows two enthalpy terms which reflect the different causes which provoke solute partitioning. The first term $\left[\sum_{i=1}^{3}(\phi_i^S - \phi_i^I)w_{iS}\right]$, refers to the difference in energy due to all types of interactions which occur between the protein and the components present in both the upper and lower phases. This term reflects the tendency of the protein to transfer to a phase with a greater concentration of the component with which it has a greater interaction, signified by a lesser value of w_{iS}. The second term of the equation, $\sum_{i=1}^{2}\sum_{j=2}^{3}(\phi_i^S\phi_j^S - \phi_i^I\phi_j^I)w_{ij}$, represents the difference of the enthalpy content between the phases, being that this energy only comes from the different interactions between components of

the ATPS. This term does not express the interactions caused by the proteins.

Therefore, $\sum_{i=1}^{2}\sum_{j=2}^{3}(\phi_i^S\phi_j^S)w_{ij}$ defines the self-energy of each phase. The dependence of K on the self-energies of the phases is related to the fact that the insertion of proteins in one phase requires the breach of interactions between its components to create a cavity where the proteins collect themselves. If these interactions are attractive, their disruption will be energetically unfavorable. However, if the interactions are repulsive, the formation of a cavity is favorable. Consequently, the protein will be transferred to a phase with greater (more positive) self-energy, where less energy is required to break and form new interactions. From the experimental thermodynamic transfer variables, it could be understood why lysozyme concentrates in the upper phase and conalbumin in the lower phase.

Table 5 presents the thermodynamic parameters associated with each of the ATPS studied for lysozyme. It can be observed that the partition of lysozyme to the upper phase is a favorable process ($\Delta_{tr}G<0$). In the PEG–citrate system, the enthalpic contribution ($\Delta_{tr}H<0$) drives lysozyme to the upper phase. Therefore, it could be concluded that this partition is governed by molecular interactions. It can also be noted that when the PEG concentration in the upper phase is increased, and consequently the salt concentration in the lower phase is increased, the enthalpy value becomes more and more negative, thus rendering the transfer process more favorable. This favorable enthalpy, according to Eq. (9), has two contributions: one due to the molecular interaction between the protein and the components of each phase and the other due to the self-energy of the phases. Increasing the PEG concentration causes the rise in intermolecular interactions between the protein and the polymer and therefore more energy is released to the system. For example, in the PEG–citrate system, the PEG phase has greater self-energy (less energy spent to separate the PEG–water bond than the water–salt bond) because of the significant concentrations of PEG and salt which repel each other. Hence, with the increase of the PEG concentration, the self-energy of the upper phase also increases, favoring the partition of the protein to that phase. The greatest numeric density in the saline phase was found in the PEG–citrate system and for this reason the protein partition to the upper phase resulted in an entropy decrease. For lysozyme, the enthalpy effect compensated for the high numeric density of the saline phase, causing the protein to partition to the upper phase.

When analyzing the thermodynamic parameters for lysozyme in the ATPS containing sodium sulfate (Table 5), it was concluded that partitioning is governed by entropy; however, this entropy is not configurational entropy. This is due to the fact that lysozyme presumably migrates to the upper phase since water molecules which were once interacting with the PEG were liberated and the system entropy increased.

Table 6 presents the thermodynamic parameters related to the transfer of conalbumin from the lower phase to the upper phase of the PEG–citrate and PEG–sulfate systems. For the PEG–citrate systems, it was concluded that the transfer of conalbumin to the lower phase is spontaneous and the system absorbs energy, making the process endothermic. This signifies that the partition is not governed by intermolecular interactions but by the number of configurations that the protein can assume in the saline phase, due to the greater content of molecules present in this phase (higher configuration entropy). The transfer of conalbumin to the upper phase is enthalpically favorable since the entropic contribution to the transfer is overshadowed by that of enthalpy. According to Eq. (8), the protein will partition to the phase with the greatest numeric density (saline phase). This fact proves that entropy increases proportionally with the number of distinct configurations the protein is capable of assuming in that phase. This entropic force increases linearly with the size of the solute and, because conalbumin has a large molecular weight (77,700 g mol^{-1}), its concentration in the saline phase is entropically favorable [15]. It was also noticed that as the TLL increases, the difference in the water concentration between the two phases increases, making the partition of conalbumin to the lower phase more entropically favorable.

Regarding to sodium sulfate, Table 6 indicates that the partitioning of conalbumin to the lower phase is not governed by enthalpy. This absence of an enthalpy contribution may be due to the unfavorable molecular interactions between conalbumin and sodium sulfate in the lower phase.

Table 5
Thermodynamic parameters for lysozyme in ATPS composed of PEG 1500 and different salts at 25 °C.

ATPS	TLL	$\Delta_{tr}G$ (kJ mol^{-1})	$\Delta_{tr}H$ (kJ mol^{-1})	$T\Delta_{tr}S$ (kJ mol^{-1})
PEG–sodium citrate	28.91	−1.34	−1.31	−0.03
	39.22	−2.06	−6.90	−4.84
	42.15	−3.34	−9.16	−5.82
PEG–sodium sulfate	26.79	−0.03	2.32	2.35
	29.74	−0.40	–	–
	35.12	−0.94	6.44	7.37

Table 6
Thermodynamic parameters for conalbumin in ATPS composed of PEG 1500 and different salts at 25 °C.

ATPS	TLL	$\Delta_{tr}G$ (kJ mol^{-1})	$\Delta_{tr}H$ (kJ mol^{-1})	$T\Delta_{tr}S$ (kJ mol^{-1})
PEG–sodium citrate	28.91	2.92	−13.80	−16.72
	39.22	3.47	−46.64	−50.11
	42.15	3.87	−48.90	−52.77
PEG–sodium sulfate	26.79	3.95	42.47	46.42
	29.74	4.27	80.03	75.76
	35.12	4.21	23.57	19.37

4. Conclusion

The aqueous two-phase systems (ATPS) can be utilized for the separation of lysozyme and conalbumin since the results obtained in this study showed that lysozyme tends to concentrate itself in the upper phase and conalbumin in the lower phase in all ATPS evaluated. The higher indices of lysozyme recovery in the upper phase and of conalbumin in the lower phase were encountered in the PEG–citrate systems. The driving force (enthalpy or entropy) governing the partitioning behavior of these proteins depends on the protein structure and on the nature of the salt.

Acknowledgments

The authors wish to acknowledge the National Council of Technological and Scientific Development (CNPq) and the Foundation to Research Support of the Minas Gerais State (FAPEMIG) for their financial support.

References

[1] E.E.G. Rojas, J.S.R. Coimbra, L.A. Minim, S.H. Saraiva, C.A.S. Silva, J. Chromatogr. B 840 (2006) 85.
[2] C. Guérin-Dubiard, M. Pasco, A. Hietanen, A. Quiros del Bosque, F. Nau, T. Croguennec, J. Chromatogr. A 1090 (2005) 58.
[3] W.J. Stadelman, O.J. Cotterill, Egg Science and Technology, AVI Publisher Company, Wesport, 1995.
[4] C.S. Da Silva, J.S.R. Coimbra, E.E.G. Rojas, L.A. Minim, L.H.M. Da Silva, J. Chromatogr. B 858 (2007) 205.
[5] C.K. Su, B.H. Chiang, Process Biochem. 41 (2006) 257.
[6] B.Y. Zaslavsky, Aqueous Two-Phase Partitioning: Physical Chemistry and Bioanalytical Applications, Marcel Dekker Inc., New York, 1994.

[7] P.A. Albertsson, Partition of Cell and Macromolecules, John Wiley and Sons, New York, 1986.
[8] G. Tubío, B. Nerli, G. Picó, J. Chromatogr. 207 (2008) 244.
[9] G. Picó, G. Bassani, B. Farruggia, B. Nerli, Int. J. Biol. Macromol. 40 (2007) 268.
[10] C.P. Carvalho, J.S.R. Coimbra, I.A.F. Costa, L.A. Minim, M.C. Maffia, Quim. Nova 31 (2008) 209.
[11] J.P. Martins, C.P. Carvalho, L.H.M. da Silva, J.S.R. Coimbra, M.C.H. da Silva, G.D. Rodrigues, L.A. Minim, J. Chem. Eng. Data 53 (2008) 238.
[12] A.D. Giraldo-Zuniga, J.S.R. Coimbra, L.A. Minim, E.E.G. Rojas, J. Food Eng. 72 (2006) 302.
[13] J.D. Coimbra, J. Thommes, A.J. Meirelles, M.R. Kula, Bioseparation 5 (1995) 259.
[14] L. Capezio, D. Romanini, G.A. Pico, B. Nerli, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 819 (2005) 25.
[15] H.O. Johansson, G. Karlström, F. Tjerneld, C.A. Haynes, J. Chromatogr. B 711 (1998) 3.