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Application of the aqueous two-phase systems of ethylene and propylene oxide copolymer-maltodextrin for protein purification

Belén Bolognese, Bibiana Nerli, Guillermo Picó*

Physical Chemistry Department and CONICET, Faculty of Biochemistry and Pharmaceutical Sciences, National University of Rosario, Suipacha 570 (S2002RLK) Rosario, Argentina

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Abstract

In this study, the effect of several factors that govern the partitioning behaviour of three model proteins, such as bovine serum albumin, lysozyme and trypsin was analysed in a two-phase system formed by maltodextrin and a copolymer of ethylene and propylene oxides. The protein partition coefficient (K_r) showed to be very sensitive to temperature changes, protein molecular weight, pH medium and the lyotropic ion presence. The phase diagram obtained for these novel polymer–polymer two-phase systems shows two phases with high polymer concentrations. The maltodextrin is enriched in the bottom phase while the copolymer of ethylene and propylene oxides is found in the upper phase. Since this copolymer is thermoreactive, the upper phase can be removed and heated above the copolymer's cloud point resulting in the formation of a new two-phase system with a lower water phase, containing the target protein and an upper copolymer-rich phase. Our results show that systems formed by maltodextrin and a copolymer of ethylene and propylene oxides may be considered as an interesting alternative to be used in protein purification due to their low cost, and also because they offer a viable solution to problems of polymer removal and recycling.

Keywords: Ethylene oxide propylene oxide random copolymer; Maltodextrin; Temperature-induced two-phase system; Partitioning

1. Introduction

Aqueous two-phase systems (ATPSs) are widely used for the separation and purification of biological macromolecules.

In most instances, these systems can be formed by combining either two water-soluble polymers differing in their chemical structure, or a polymer and a salt in water above a certain critical concentration [1,2]. For the large-scale isolation of enzymes, the inexpensive polyethyleneglycol (PEG)/salt systems are being used, but they are not very selective and can damage fragile proteins. In some cases, when high salt concentration is used, they become a waste disposal problem. In contrast, polymer–polymer systems can be more selective by incorporating appropiate ions or ligands in the system. The most common polymer–polymer system is composed by dextran and PEG, but this system is very expensive (due to the high cost of dextran) for scaling up. This problem may be solved by the use of alternative economical polymers that substitute for dextran with equivalent partition properties. Moreover, polymer–polymer systems show difficulty in separating target molecules from polymer solution. These systems would also be more cost efficient if the polymer could be readily recycled without costly ultrafiltration or chromatography steps. Temperature-induced phase partitioning avoids these problems [3]. A group of non-ionic, linear polymers composed by ethylene and propylene oxides, which are normally soluble in water, separates from solution when sufficiently heated. The temperature at which this

Abbreviations: EO-PO, ethylene oxide-propylene oxide random copolymer; Mdx, maltodextrin; BSA, bovine serum albumin; TRY, trypsin; LYS, lysozyme; M_v, viscosity-average molecular weight; M_n, number-average molecular weight; K_r, partition coefficient; R, protein recovery; ANS, 1-anilino-8-naphthalene sulfonate; S_o, protein surface hydrophobicity; ATPS, aqueous two-phase system; pI, isoelectrical point

* Corresponding author. Fax: +54 341 480 4598.
E-mail address: gpico@fbioyf.unr.edu.ar (G. Picó).

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phenomenon occurs is known as the cloud point (CP) of the polymer [4]. This phase separation results in a water-rich phase and a copolymer-rich phase. An adequate combination of temperature-induced separation with two-phase partitioning offers a viable solution to both the polymer removal and the recycling problem. A simple purification scheme includes a first step where the target molecules are partitioned at room temperature in a system composed by two polymers, one of them being thermoreactive. Medium conditions are manipulated in order to displace the partition equilibrium of the target protein to the concentrated thermosensible polymer phase. After separation, the thermosensible polymer-rich phase is removed and its temperature increased above the CP. This results in the formation of a new two-phase system with a water-buffer phase, containing the biomolecule and a polymer-rich-phase from which the copolymer can easily be recovered and recycled [5].

In order to develop new biphasic systems suitable for large-scale processes, the use of both a low-cost starch derivative (maltodextrin) as replacement for dextran and a copolymer of ethylene and propylene oxides (thermoreactive) were investigated. The partitioning behaviour of three model proteins: bovine serum albumin, lysozyme and trypsin was analysed in order to evaluate the capability of this novel aqueous two-phase system for protein separation.

2. Experimental

2.1. Chemicals

Bovine serum albumin (BSA), lysozyme (LYS), trypsin (TRY) and 1 anilino-8- naphthalene sulfonate (ANS) were purchased from Sigma Chemical Co. and used without further purification. The random copolymer of ethylene and propylene oxides was a gift from Dow Chem Co (San Lorenzo, Argentina). Maltodextrin was commercially available as “Polimerosa”, an inexpensive dietary supply made by Kasdorf SA (Buenos Aires, Argentina). All the other reagents were of analytical grade.

2.2. Molecular weight determination

The viscosity-average molecular weight (M_v) and the polydispersity index (M_v/M_n) of the employed polymers were determined by viscosimetry and osmotic pressure measurements (M_n). The viscosity experiments were carried out in a Brookfield DV-II viscosimeter, the temperature was maintained constant at 24 °C and measured with an accuracy of 0.1 °C.

2.3. Phase diagrams

The phase diagram for EO–PO/Mdx system was determined at room temperature (24 °C). Mixtures consisting of known weights of polymer stock solutions (EO–PO, 36% w/w and Mdx, 30% w/w) were prepared on an analytic balance. The prepared two-phase systems were allowed to equilibrate for approximately 1 h, in a Haake water bath. After this treatment, the two phases became clear and transparent, and the interface was well defined. The concentrations of the polymers in the phases were determined with two independent methods. First, the Mdx concentrations in both phases were determined by polarimetry [6]. The EO–PO copolymer showed no optical activity. Second, the concentration of EO–PO copolymer was determined according to a colorimetric assay based on the partitioning of a chromophore present in ammonium ferrothiocyanate reagent from an aqueous to an organic (chloroform) phase in presence of EO–PO [7].

2.4. Preparation of the aqueous two-phase systems

To prepare the ATPSs, stock solutions of the phase components EO–PO, 36% w/w and Mdx, 30% w/w, and different buffer solutions (Table 1) were mixed in order to obtain a total system composition of approximately EO–PO, 8.9% w/w and Mdx, 22.5% w/w. Low-speed centrifugation (at 2000 × g for 3 min) to speed up phase separation was used after thorough gentle mixing of the system components, then 2 g of each phase were mixed to reconstitute several two-phase systems in which the protein partitioning was assayed.

2.5. Determination of the protein partition coefficient (K_r)

Protein partitioning in both phases was analysed by dissolving increasing amounts of protein (15–45 uM of total system concentration) in the two-phase pre-formed systems. Aliquots of the protein stock solution (1500 μM) added to the systems varied from 40 to 120 μL, the change of the total volume of each phase being negligible. After mixing by inversion for 1 min and leaving it to settle for at least 30 min, the system was centrifuged at low speed (at 2000 × g for 3 min) for the two-phase separation. Samples were withdrawn from the separated phases, and after dilution the protein content in each phase was determined by measurement of light absorption at 280 nm. Equally diluted samples from identical phase systems without protein were used as blanks, which had been prepared in parallel. The partition coefficient (K_r) is defined as:

$$K_r = \frac{[P]_{top}}{[P]_{bottom}} \quad (1)$$

Table 1
Medium composition of different ATPSs employed

System	Composition
A	Na Pi, 50 mM; pH 7.4
B	Na Pi, 50 mM, pH 7.4 + $NaClO_4$, 100 mM
C	NaAc, 100 mM; pH 5.0
D	Glutamic acid, 10 mM; pH 3.1
E	Glutamic acid, 10 mM; pH 3.1 + $NaClO_4$, 100 mM

where $[P]_{top}$ and $[P]_{bottom}$ are the equilibrium concentrations of partitioned protein in EO–PO (top) and Mdx (bottom) enriched phases, respectively. In the assayed range of protein concentrations, a plot of $[P]_{top}$ versus $[P]_{botton}$ showed a linear behaviour with a slope equal to K_r. Absorbance measurements were carried out in a Spekol 2000 spectrophotometer.

2.6. Determination of thermodynamic parameters associated to the protein partitioning

The partition coefficient (K_{r_1}, K_{r_2}) was determined at two different temperatures ($T_1 = 8\,^{\circ}C$ and $T_2 = 24\,^{\circ}C$) and by applying the van't Hoff equation:

$$\ln\frac{K_{r_2}}{K_{r_1}} = \frac{\Delta H^{\circ}}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \quad (2)$$

the enthalpic change (ΔH°) associated to the protein partitioning was calculated. The free energy change (ΔG°) was determined from the $\Delta G^{\circ} = -RT\ln K_r$ and the entropic change (ΔS°) from:

$$\Delta S^{\circ} = \frac{(\Delta H^{\circ} - \Delta G^{\circ})}{T} \quad (3)$$

2.7. Measurements of the protein relative surface hydrophobicity (S_o)

The relative surface hydrophobicity of the protein was determined by applying the optical method previously reported [8]. Stock solutions of 8 mM ANS and 1.5 mM of assayed proteins were prepared. Aliquots of the protein (from 4 to 40 μL) were added to a sample, containing ANS (final concentration 20 μM) in different media according to Table 1, in absence and presence of either EO–PO, 5% w/w or Mdx, 5% w/w. The polymer presence did not affect the fluorescence signal (data not shown). The protein concentration varied from 0 to 20 uM. The fluorescence emission intensity at 484 nm (while exciting at 365 nm) for each protein concentration was measured at a Jasco FP 770 spectrofluorometer at 24 °C. Under the above-mentioned experimental conditions (with ANS excess), the initial slope (S_o) of the fluorescence intensity versus the protein concentration plot has been shown to be correlated to the relative surface hydrophobicity of the protein (S_o).

2.8. Cloud point determination

The cloud point temperature (CP) was determined by placing solutions of EO–PO (from 1 to 5% w/w) in different buffer media, according to Table 1, kept in a cuvette with a screw cap, in an Aminco-Bowman Series 2 spectrophotometer. The heating rate was 1 °C/min. The CP was graphically determined from the recorded absorbance at 600 nm versus the time chart [9]. The effect of sodium sulphate presence (0.2 and 0.3 M) on the polymer CP was evaluated.

2.9. Temperature-induced two-phase systems

Two-phase preformed systems of EO–PO/Mdx (final mass system 10 g) were prepared according to compositions of item 2.4. Aliquots of sodium sulphate (2 M) and protein (1.5 mM) stock solutions were added to 4 mL of the removed top-phase sample (EO–PO enriched). The thermoseparation was performed by placing the systems in graduated capped glass tubes in a bath at 5 °C above the polymer previously determined CP, for 3 h. The water-rich phase volume (V_{water}) was measured and aliquots of this phase were removed at 1, 2 and 3 h after thermoseparation began. The protein concentration ($[P]_{water}$) was determined spectrophotometrically at 280 nm and the percent protein recovery (R) in the water phase was calculated according to:

$$R(\%) = \frac{[P]_{water}V_{water}}{[P]^{\circ}V^{\circ}} \quad (4)$$

where $[P]^{\circ}$ and V° are the initial protein concentration and the system volume, respectively.

3. Results and discussion

3.1. Characterization of ATPSs of EO–PO/Mdx

The molecular mass and the sample polydispersity of phase-forming polymers are important factors in the equilibrium distribution [10]. The viscosity-average molecular weight (M_v) by viscosimetry and the number-average molecular weight (M_n) by osmotic pressure measurements were determined, and the polydispersity index (M_v/M_n) was calculated for EO–PO and Mdx. The results are summarized in Table 2. As can be seen the molecular weights of both polymer fractions are narrowly distributed (polydispersity index of about one), thus indicating that nearly all the polymer chains can be assumed to be of a single molecular weight. The binodal curve for EO–PO/Mdx system at 24 °C is shown in Fig. 1. This curve was determined by fitting a sigmoidal equation to the experimental data. The tie lines were determined by linear regression of each corresponding set of the total, bottom and top phase concentrations. The tie lines were confirmed by performing mass balances on the top and bottom equilibrium compositions to determine the amounts of EO–PO and Mdx used to generate the total mixture. The experimental compositions for all the systems are given in Table 3. The concentration of Mdx required to

Table 2
Molecular weights and polydispersity parameters of the phase-forming polymers

Polymer	M_v[a]	Polydispersity index (M_v/M_n[b])
EO–PO	1228	1.16
Mdx	922	1.10

[a] Determined by viscosimetry.
[b] Determined by osmotic pressure measurements.

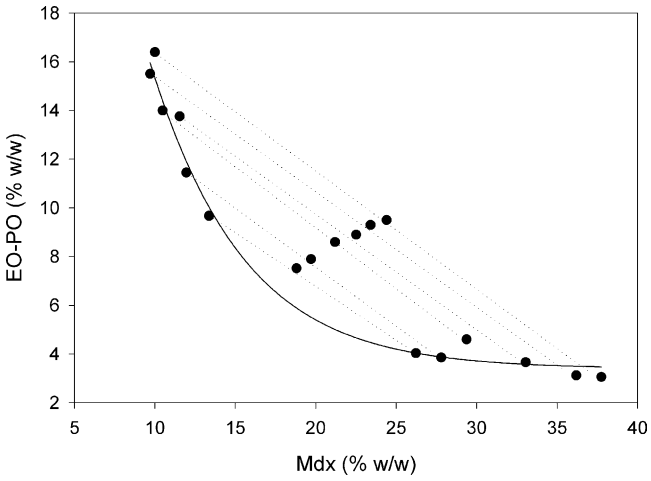

Fig. 1. Binodal diagram corresponding to the aqueous two-phase system EO–PO/Mdx in buffer NaPi 50 mM, pH 7.4 at 24 °C.

form two phases with EO–PO higher than that of dextran in a PEG/Dx systems can be correlated to its lower molecular weight (M = 922) compared with Dx (M = 500,000). EO–PO is enriched at the top phase, while Mdx is enriched at the bottom phase. All EO–PO/Mdx systems are characterised by the presence of considerable quantities of Mdx in the top phase when compared to systems formed with PEG/Dx. In contrast, the EO–PO at the bottom phase is very small, the EO–PO being excluded from this phase.

On the other hand, the slope of the tie lines is practically constant, which implies that they are parallel to each other, thus allowing to know the coexisting phase compositions for any given total polymer phase-forming composition.

3.2. The effects of salts and pH on protein partitioning

When the anion and cation of a salt have different relative affinities for different phase-forming polymers, and consequently for each phase, the requirement of electroneutrality in each phase results in a Donnan-type electrostatic potential difference between the phases. This potential difference appears to have large effects on partitioning of charged solutes (such as proteins), therefore the choice of salt is an easy way to influence the target biomolecule partitioning. The most hydrophobic anions or cations will drive the partitioning of their counterions to the most hydrophobic phase. Co-ions, which are less hydrophobic, will partition to the hydrophilic phase. The affinity of ions for different phases agrees with the Hofmeister or lyotropic series. The anions used in this work can be arranged in the following order, with regards to hydrophobicity [11,12]:

$$ClO_4^- > CH_3COO^- > PO_4^{3-}/HPO_4^{2-}/H_2PO_4^-$$

For example, anion ClO_4^- has a tendency to partition to the more hydrophobic phase (in our case the EO–PO-enriched one), while PO_4^{3-} has an affinity for the more hydrophilic phase (Mdx). The sodium ion was used in all the cases. On the other hand, when zwitterionic amino acids, such as glutamic acid at its pI (isoelectric pH) are used as buffers, the electrostatic interfacial potential is reduced, becomes negligible and the partitioning behaviour depends more on other parameters, such as the molecular weight and the protein hydrophobicity.

3.2.1. BSA

Fig. 2 shows the BSA partitioning behaviour in the different buffer media, according to Table 1. K_r values lower

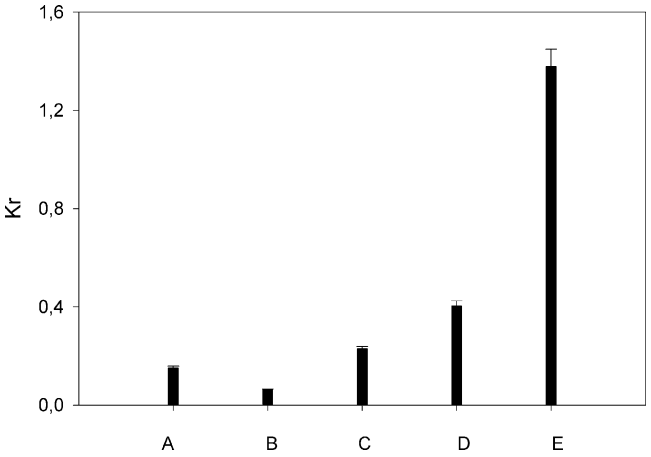

Fig. 2. Effect of pH changes and lyotropic ion presence on the BSA partitioning behaviour in aqueous two-phase systems of EO–PO/Mdx at 24 °C. Total polymer concentrations: [EO–PO] = 8.9% w/w and [Mdx] = 22.5% w/w. Letters A, B, C, D and E on x-axis represent the different buffer media according to Table 1.

Table 3
Phase compositions for EO–PO/Mdx systems at 24 °C

Total system composition (% w/w)		Top phase composition (% w/w)		Bottom phase composition (% w/w)		Tie line slope
EO–PO	Mdx	EO–PO	Mdx	EO–PO	Mdx	
9.50	24.40	16.40	10.00	3.06	37.75	−0.480
9.30	23.40	15.50	9.70	3.12	36.18	−0.467
8.90	22.50	13.76	11.54	3.66	33.04	−0.469
8.60	21.20	14.00	10.48	4.60	29.36	−0.498
7.90	19.70	11.45	11.95	3.86	27.79	−0.479
7.50	18.80	9.67	13.37	4.04	26.22	−0.430
av						−0.471 ± 0.022

av, slope tie line average.

than 1 were observed for nearly all the ATPSs assayed, thus indicating that the partition equilibrium is displaced to the Mdx-enriched phase. This protein exhibits a p*I* equal to 5.0 [13], therefore it will be positively charged at a pH lower than p*I* and negatively charged at a pH higher than p*I*.

A (NaPi) and B (NaPi + $NaClO_4$) media: BSA has a negative charge of approximately −18 [13] in both media since pH is 7.40. As can be seen the ClO_4^- presence (B medium) induces a decrease of K_r with respect to the value in solutions with only NaPi (A medium). This fact may be explained assuming that ClO_4^- has more affinity for the EO–PO phase, thereby inducing a negative potential on this polymer side of the phase and then displacing the protein to the opposite phase (Mdx-enriched).

C (NaAc, pH 5.0) and D (glutamic acid, pH 3.1) media: In both cases, the electrostatic component of K_r is negligible because in C medium, BSA is not charged since pH is equal to its p*I* and in D medium, due to the presence of a zwitterionic species as buffers. At pHs lower than 4.0, the higher protein charge density, and therefore the increase in the repulsive forces between charged residues induce a conformational change from the BSA normal form (*N*) to the fast migrating form (*F*) [14]. This transition involve an abrupt opening of the molecule and an increase in the exposed number of certain hydrophobic amino acids. Moreover, the *F* form is characterized by a dramatic increase in viscosity, much lower solubility, and a significant loss in helical content. In this way, it is reasonable to think that the K_r values in D medium were higher (the protein further displaced to the more hydrophobic EO–PO phase) than in C due to an increase of the protein hydrophobic surface.

E (glutamic acid, pH 3.1 + $NaClO_4$) medium: The higher K_r values observed for this system may be explained because the BSA is positively charged at pH 3.1 and then the negative potential on the EO–PO side of the phase due to the ClO_4^- presence induces a displacement of the BSA partition equilibrium to the top phase.

3.2.2. TRY and LYS

Since p*I*s (isoelectric points) for TRY and LYS are 10.4 and 11.0, respectively [13], both proteins are positively charged in all the pHs assayed. Fig. 3 shows the partitioning behaviour of TRY and LYS for the five medium systems.

3.2.2.1. A and B media. In the A medium, a negative potential on the Mdx side of the phase due to the high affinity of phosphate anion for this polymer induces a protein partitioning equilibrium displacement to the bottom phase, reaching K_r values lower than one. However, in the B medium, the ClO_4^- presence may cause a sign inversion of the electrostatic difference between the phases, and therefore an increase in the K_r [11,12].

3.2.2.2. C medium. In this case, the K_r values for TRY adopt intermediate values between the corresponding to medium A and B in correspondence with the intermediate position of acetate ion, between ClO_4^- and phosphate, in the lyotropic series.

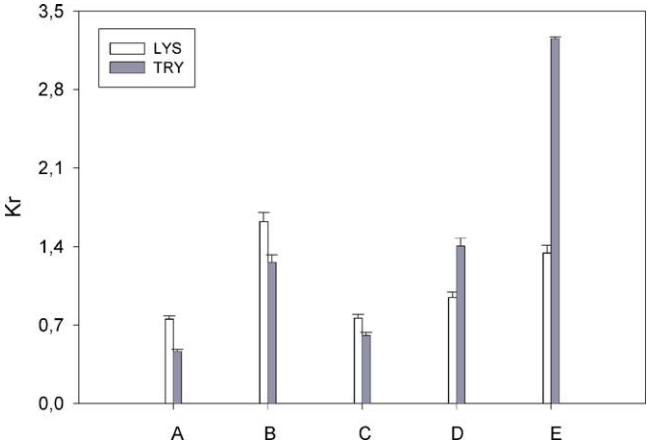

Fig. 3. Effect of pH changes and lyotropic ion presence on the TRY and LYS partitioning behaviour in aqueous two-phase systems of EO–PO/Mdx at 24 °C. Total polymer concentrations: [EO–PO] = 8.9% w/w and [Mdx] = 22.5% w/w. Letters A, B, C, D and E on *x*-axis represent the different buffer media according to Table 1.

3.2.2.3. D and E media. In the E medium, a significant increase in the K_r values with respect to D medium is observed. In E medium the net positive charge of both proteins is responsible of their preferential partitioning to the top phase. Moreover, both the acidic medium conditions and the lyotropic ion (ClO_4^-) presence would induce an exposition of hydrophobic amino acid residues in the molecule, and thus a higher displacement of the two proteins to the EO–PO-enriched phase.

3.3. Thermodynamic parameters associated to protein partitioning

Figs. 4 and 5 show the thermodynamic parameters, ΔH° and ΔS°, associated to protein partitioning in the five systems. A differential behaviour for BSA and TRY with respect to LYS is observed, thus suggesting different molecular mechanisms for protein partitioning. The transfer of BSA and TRY from Mdx to the EO–PO phase is an endothermic process, entropically favoured for almost all the assayed media. This behaviour is similar to that observed for BSA in PEG/Dx systems [15] and suggests the interaction between the hydrophobic surface area of EO–PO molecule and hydrophobic residues in the protein. On the other hand, the transfer of LYS to the top phase is enthalpically driven, indicating that the forces involved in the EO–PO–LYS interaction would be of electrostatic nature, probably between the EO–PO polymer and exposed tryptophans in the protein [16] or between the positively-charged amino acid residues of the protein and the polymer.

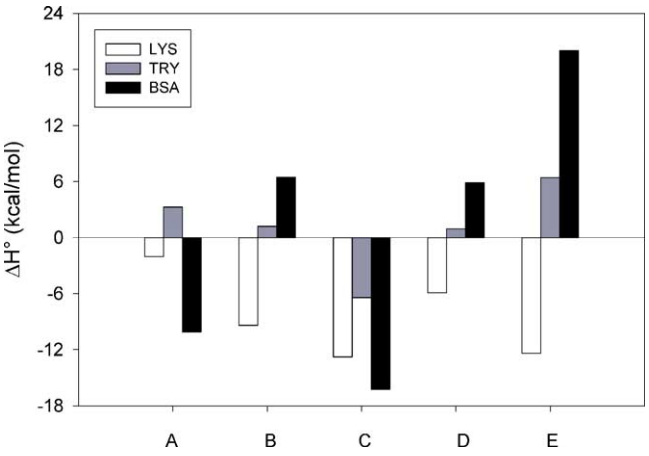

Fig. 4. Enthalpic changes (ΔH°) associated to the transfer of BSA, TRY and LYS from the Mdx-enriched phase to the EO–PO-enriched phase in the five assayed buffer media (A, B, C, D and E) according to Table 1.

3.4. Protein relative surface hydrophobicity (S_o)

Fig. 6 summarizes the measured S_o values for the three proteins. For nearly all the assayed buffer media (Table 1), the following relative surface hydrophobicity order was observed:

$$S_{o_{LYS}} < S_{o_{TRY}} \lll S_{o_{BSA}}$$

This sequence is in agreement with the thermodynamics associated to the partitioning [17], since those proteins whose transfer to EO–PO phase are entropically driven, show the higher S_o values (TRY and BSA), while LYS, whose transfer is enthalpically driven, shows the S_o lowest value. The ClO_4^- presence (B and E media) induced a significant increase of S_o with respect to the values in A and D media for TRY and LYS. This chaotropic anion might cause a disorder increase of the water molecules at the vicinity of protein, thus conducing to an increase of the protein surface accessible to the fluorescent probe.

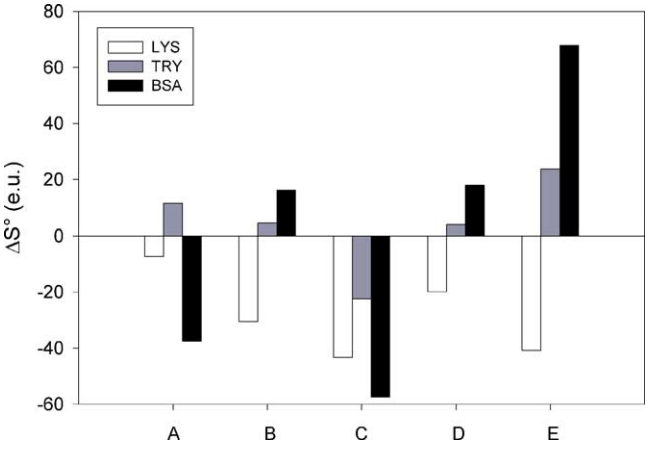

Fig. 5. Entropic changes (ΔS°) associated to the transfer of BSA, TRY and LYS from the Mdx-enriched phase to the EO–PO-enriched phase in the five assayed buffer media (A, B, C, D and E) according to Table 1.

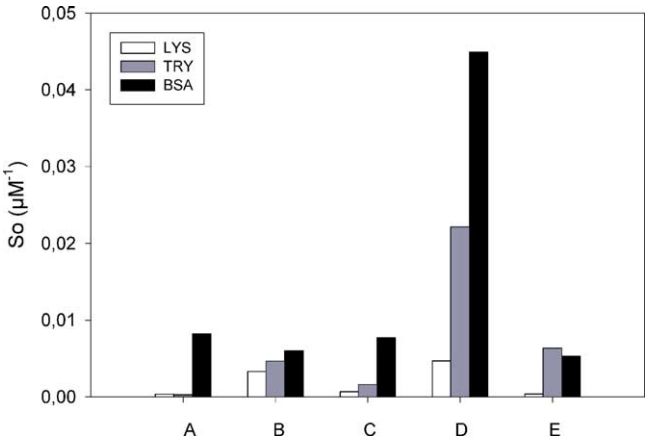

Fig. 6. Relative surface hydrophobicities (S_o) of BSA, TRY and LYS in the five assayed media. For BSA, S_o values must be multiplied by a factor of 50.

3.5. Effect of inorganic salts on EO–PO cloud point

Thermoseparation results from the increase in the net repulsive interaction between solvent and polymer segments (different polarity) at high temperature [18]. The addition of some water-soluble compounds, such as inorganic salts, to nonionic polymer solutions induces a decrease in the polymer cloud point. This effect is the consequence of a preferential interaction of such compounds with water, which makes the solvent more polar and increases the difference in polarity between solvent and polymer [19]. The presence of Na_2SO_4 at different concentrations (0.2 and 0.3 M) on EO–PO cloud point was evaluated (Fig. 7). As can be seen, the thermoreactive polymer CP, close to 57 °C, decreases to values between 37 and 41 °C for the five assayed systems. In this way, the temperature-induced systems formed by heating EO–PO-enriched phases can be employed in protein purification, since the temperatures at which the thermoseparation occurs are far away from the unfolding temperatures of the three assayed proteins.

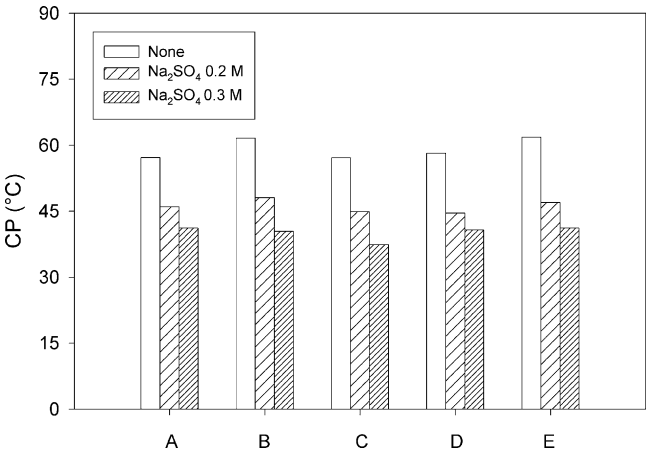

Fig. 7. Effect of the sodium sulphate presence on the cloud point of EO–PO in the five assayed media. EO–PO, 5% w/w.

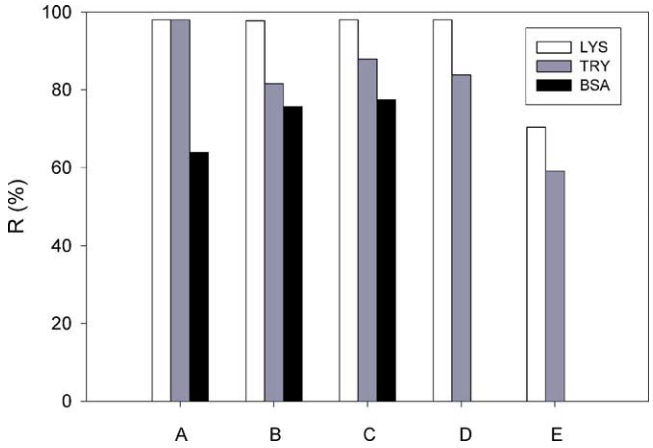

Fig. 8. Percent protein recoveries (*R*) for BSA, TRY and LYS in the water-enriched phase of a temperature-induced two-phase system obtained after heating the top phase of a EO–PO/Mdx system in different media.

3.6. Protein partitioning in temperature-induced two-phase systems

After the Na_2SO_4 addition, the increase in temperature of the EO–PO enriched phase of EO–PO/Mdx systems 5 °C above the polymer CP results in the formation of a new two-phase system. When separated, the bottom phase is composed of approximately 95% of water and the upper phase is EO–PO-enriched. Fig. 8 shows the percent protein recovery (*R*) in the water phase obtained 3 h after the thermoseparation begins. A significant *R* value, between 60 and 98% is observed in all the media. *R* values around 80% were obtained for all the assayed proteins in B and C media. The high affinity of the three proteins for the lower water phase is due to both the strong interaction between charged groups in the protein and the polar water molecule (protein hydrophilic character) and the excluded volume effect in the polymer-enriched phase. Moreover, the following sequence was observed:

$$R_{BSA} < R_{TRY} < R_{LYS}$$

which corresponds to the inverse hydrophobicity order.

Recoveries for BSA in D and E media could not be calculated probably due to a protein precipitation in the interface.

4. Conclusions

In this work, the separation properties of a novel two-phase system formed by maltodextrin and a copolymer of ethylene and propylene oxides were evaluated.

The EO–PO/Mdx system was found to have similar characteristics to the well known PEG/Dx system. Top phase has a high EO–PO concentration, while bottom is Mdx-enriched. The tie lines of its binodal diagram are practically parallel, thus allowing us to determine the top and bottom compositions for any given total polymer composition. The protein partitioning in these systems showed to be very sensitive to pH changes and lyotropic ion presence. In this way, an adequate choice of medium conditions can direct the target protein to the more hydrophobic EO–PO phase. A temperature increase of this phase, above the polymer cloud point (lowered by sodium sulphate addition), results in a second temperature-induced two-phase system with an EO–PO-enriched upper phase and a water-enriched lower phase, containing most of initial protein.

Finally, we conclude that EO–PO/Mdx systems may be considered as an interesting alternative for protein purification due to the low cost of the phase-forming polymers (suitable for large-scaling), the maltodextrin biodegradability and their possibility of forming temperature-induced systems which will let both the protein in a water-buffer medium be recovered and the copolymer recycled.

Acknowledgments

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References

[1] B.Y. Zaslavsky, in: B.Y. Zaslavsky (Ed.), Physical Chemistry and Bioanalytical Applications, Marcel-Dekker Inc., New York, 1994.
[2] P.Å. Albertsson, Partition of Cell Particles and Macromolecules, second ed., John Wiley and Sons, New York, 1971.
[3] P. Alred, A. Kozlowski, M. Harris, F. Tjerneld, J. Chromatogr. A 659 (1994) 289.
[4] I. Galaev, B. Mattiason, Enzyme Microb. Technol. 15 (1993) 354.
[5] P.A. Harris, G. Karlstrom, F. Tjerneld, Bioseparation 2 (1991) 237.
[6] H. Walter, D. Brooks, D. Fisher (Eds.), Partitioning in aqueous two-phase systems. Theory, methods, uses, and applications to biotechnology, vol. 1, Academic Press Inc., Albertsson, Orlando, 1985.
[7] A. Nag, G. Mitra, P.C. Ghosh, Anal. Biochem. 237 (1996) 224.
[8] C.A. Haskard, E.C. Li-Chan, J. Agric. Food Chem. 46 (1998) 2671.
[9] P. Alexandridis, J. Holzwarth, A. Hatton, Macromolecules 27 (1994) 2414.
[10] C. Kang, S. Sandler, Macromolecules 21 (1988) 3088.
[11] H. Walter, G. Johansson, Anal. Biochem. 155 (1986) 215.
[12] H. Johansson, G. Lundh, G. Karlstrom, F. Tjerneld, Biochim. Biophys. Acta 1290 (1996) 289.
[13] M. Svensson, K. Berggren, A. Veide, F. Tjerneld, J. Chromatogr. A 839 (1999) 71.
[14] D. Carter, J. Ho, Adv. Protein Chem. 45 (1994) 153.
[15] B. Nerli, M. Espariz, G. Picó, Biotechnol. Bioeng 72 (2001) 468.
[16] A. Nilsson, H. Johansson, M. Mannesse, F. Tjerneld, Biochim. Biophys. Acta 1601 (2002) 138.
[17] G. Tubio, B. Nerli, G. Picó, J. Chromatogr. B 799 (2004) 293.
[18] J. Persson, H. Johansson, I. Galaev, B. Mattiasson, F. Tjerneld, Bioseparation 9 (2000) 105.
[19] G. Karlstrom, A. Carlsson, B. Lindman, J. Phys. Chem. 94 (1990) 5005.