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Polyethyleneglycol molecular mass and polydispersivity effect on protein partitioning in aqueous two-phase systems

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Abstract

The partitioning of model proteins (bovine serum albumin, ovalbumin, trypsin and lysozyme) was assayed in aqueous two-phase systems formed by a salt (potassium phosphate, sodium sulfate and ammonium sulfate) and a mixture of two polyethyleneglycols of different molecular mass. The ratio between the PEG masses in the mixtures was changed in order to obtain different polymer average molecular mass. The effect of polymer molecular mass and polydispersivity on the protein partition coefficient was studied. The relationship between the logarithm of the protein partition coefficient and the average molecular mass of the phase-forming polymer was found to depend on the polyethyleneglycol molecular mass, the salt type in the bottom phase and the molecular weight of the partitioned protein. The polymer polydispersivity proved to be a very useful tool to increase the separation between two proteins having similar isoelectrical point.
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1. Introduction

Aqueous two-phase systems have been successfully used for the separation and purification of proteins because of their advantages as regards the traditional methods. These systems have good resolution, a high yield and are easy to scale up [1]. Due to the high water content of the two phases (80–90%, w/w) and the low interfacial tension between them, the two phases are mild and they are not deleterious to labile structures, thus increasing the protein thermodynamic stability.

The macromolecule partition coefficient depends on many variables such as: hydrophobicity, molecular mass and concentration of the macromolecule and the flexible chain polymers, macromolecule net electrical charge, temperature, etc. [2]. The addition of salts and the change of pH or ionic strength have often been used to alter the partitioning of a biomolecule and

improve the selectivity of the two-phase extraction. However, when the proteins to be isolated have similar properties such as isoelectric point value, molecular mass or hydrophobicity, a significant difference in their partition coefficients is not easily available.

The influence of the molecular mass of the flexible polymer on the protein partitioning has been well reported [3]. In general, the polymer molecular mass increase induces the protein transfer to the phase where the polymer is present at the lower concentration. This partitioning behaviour is governed by the combination of different factors such as an exclusion effect of the polymer from the protein domain and the forces involved in the polymer–protein interaction.

The polymer solutions are not of a single molecular weight (monodispersed) since they content molecules of a range of molecular weights (polydispersed). The polydispersivity parameter of a polymer sample is given by the ratio between the weight-average molecular weight and the number-average molecular weight. When molecular weights of a polymer are narrowly distributed the polydispersivity parameter adopts values near the unity and the polymer can be considered monodispersed. In the study of the phase equilibrium of polymer solutions, polydisperse systems are usually treated as monodisperse systems with

averaged distributed intensive properties such as the average molecular mass. However, there are important differences; for example, the sharp phase boundaries found in monodisperse mixtures are not found in disperse systems. Kang and Sandler [4] have developed a thermodynamical treatment to predict the effect of polydispersivity on binodal diagram compositions for a polyethyleneglycol/dextran system. They found that polymer polydispersivity increased the liquid–liquid inmiscible region and induced a polymer fractionation with larger PEG molecules tending to remain in the top phase while the smallest ones tending to remain in the bottom phase. However, at present, the molecular mass polydispersivity is a variable whose effect on the protein partition coefficient has not yet been explored.

In a previous work [5], we have isolated alpha-1-antitrypsin from human serum plasma and separated it from albumin, the most abundant plasma protein (albumin/antitrypsin concentration ratio 20:1) by using partitioning in aqueous two-phase system of polyethyleneglycol-phosphate. In a system of polyethyleneglycol 600-phosphate both proteins have a tendency to be partitioned to the polymer-rich phase, while in a system formed by polyethyleneglycol 1000-phosphate, both proteins tend to go to the phosphate-rich phase. However, a selective separation between the partitioning coefficient values of both proteins was observed for a system formed by phosphate and a mixture of PEG600 and PEG1000.

In this study, the effect of the polyethyleneglycol molecular mass and polydispersivity on the partition coefficient of model proteins has been examined so as to apply this variable to improve the selectivity of the aqueous two-phase systems.

2. Materials and methods

2.1. Chemicals

Polyethyleneglycols of the following average molecular masses: 600 (PEG600), 1500 (PEG1500), 4000 (PEG4000) and 8000 (PEG8000), bovine serum albumin (BSA), trypsin (TRY), ovalbumin (OVA) and lysozyme (LYS) were purchased from Sigma Chem. Co. and used without further purification.

2.2. Preparation of the aqueous two-phase systems

Biphasic systems were prepared by a mixture of two PEGs of different molecular mass and an inorganic salt (potassium phosphate, ammonium sulfate or sodium sulfate). To prepare aqueous two-phase systems, stock solutions of the phase components: polymers 40% (w/w), potassium phosphate, pH 7.0, 25% (w/w) were mixed in order to obtain a total system composition of PEG 13.6% (w/w) and salt 13.2% (w/w) (see Section 2.4). Sodium sulfate or ammonium sulfate was employed by weighing the solid form. The medium pH of PEG-ammonium sulfate ATPSs was 5.60 (given by the ammonium proteolysis) while for the sodium sulfate ATPSs, it was adjusted to 7.00 by the addition of a 100 mM buffer solution of sodium phosphate. Systems of 40 g each (PEG-potassium phosphate, PEG sodium sulfate and PEG-ammonium sulfate systems) were prepared, stirred for 2 h for equilibration and the phases were then allowed to settle

overnight at 8 °C. After phase separation, 1 g of each phase was mixed to reconstitute several two-phase systems in which the protein partition was assayed. In order to speed up phase separation, low-speed centrifugation was used after a gentle mixing of the system components [1,2].

2.3. Determination of the protein partition coefficient (K_p)

Protein partitioning was analysed by dissolving increasing amounts of proteins (2–10 μM of total concentration) in the two-phase preformed systems containing 1 ml of each equilibrated phase. The aliquots of protein stock solution (1000 μM) added to the system varied from 5 to 20 μL, the total volume change of each phase being negligible. After mixing by inversion for 30 min and leaving it to settle for at least 1 h, the system was centrifuged at low speed for the two-phase separation. Samples were withdrawn from the separated phases, and after dilution, the protein content in each phase was determined by measuring the absorbance 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_p) was defined as:

$$K_p = \frac{[P]_{top}}{[P]_{bottom}} \tag{1}$$

where $[P]_{top}$ and $[P]_{bottom}$ are the equilibrium concentrations of the partitioned protein in the PEG (top) and salt (bottom) enriched phases, respectively. In the assayed protein concentration range, a plot of $[P]_{top}$ versus $[P]_{bottom}$ showed linear behaviour, the K_p value being its slope. All the operations were carried out at 8 °C.

2.4. Composition of assayed aqueous two-phase systems with different PEG average molecular mass

To evaluate the effect of polymer polydispersivity on protein partitioning behaviour, different groups of two-phase systems were used (A–D). Each group was prepared with two PEGs of different molecular mass (PEG$_{(A)}$ and PEG$_{(B)}$) and an inorganic salt. Within the group, different PEG compositions were obtained by increasing the amount of one of the PEGs and decreasing the amount of the other polymer in parallel, the total mass of PEG and salt remaining constant. $x_{PEG(A)}$ and $x_{PEG(B)}$ stand for the feed compositions in mass fraction of each PEG in the mixture:

$$x_{PEG(A)} = \frac{m_{PEG(A)}}{m_{PEG(A)} + m_{PEG(B)}},$$

$$x_{PEG(B)} = \frac{m_{PEG(B)}}{m_{PEG(A)} + m_{PEG(B)}} \tag{2}$$

Therefore, the weight-average molecular mass M_w can be calculated according to:

$$M_w = M_{PEG(A)}x_{PEG(A)} + M_{PEG(B)}x_{PEG(B)},$$

$$M_w = (M_{PEG(A)} - M_{PEG(B)})x_{PEG(A)} + M_{PEG(B)} \tag{3}$$

Table 1
Composition of different SBAs

System A: Mixtures of PEG600 and PEG1500								
PEG600 (%, w/w)	13.47	12.12	9.43	8.08	6.73	5.12	2.69	1.89	0
PEG1500 (%, w/w)	0	1.35	4.04	5.39	6.73	8.35	10.78	11.58	13.47
M_w	600	690	870	960	1050	1158	1320	1374	1500
System B: Mixtures of PEG1500 and PEG4000								
PEG1500 (%, w/w)	13.47	12.12	9.43	8.08	6.73	5.12	2.69	1.89	0
PEG4000 (%, w/w)	0	1.35	4.04	5.39	6.73	8.35	10.78	11.58	13.47
M_w	1500	1750	2250	2500	2750	3050	3500	3650	4000
System C: Mixtures of PEG4000 and PEG8000								
PEG4000 (%, w/w)	13.60	11.69	10.88	8.16	6.80	5.44	4.08	1.36	0
PEG8000 (%, w/w)	0	1.90	2.72	5.44	6.80	8.16	9.52	12.24	13.60
M_w	4000	4560	4800	5600	6000	6400	6800	7600	8000
System D: Mixtures of PEG600 and PEG4000								
PEG600 (%, w/w)	15.00	14.44	14.21	13.41	13.01	12.62	12.22	11.42	11.03
PEG4000 (%, w/w)	0	0.56	0.76	1.59	1.99	2.38	2.78	3.58	3.94
M_w	600	727	772	960	1050	1140	1230	1410	1495

Potassium phosphate total concentration is 13.2% (w/w) in all the assayed systems.

where $M_{PEG(A)}$ and $M_{PEG(B)}$ are the average molecular masses of narrow standard polyethyleneglycol fractions, thus considered monodisperse systems. It should be noted that the feeds of all the assayed systems have the same total PEG concentration but a different average molecular mass. Table 1 shows the employed range of PEGs compositions and the corresponding M_w.

3. Results and discussion

3.1. Protein partitioning in PEG-potassium phosphate system

Fig. 1 shows the influence of the relative composition of PEG mixture of average molecular mass between 600 and 1500 on the partition coefficient of four model proteins. A decrease of K_p can be seen when the PEG molecular mass increases for all the assayed proteins in agreement with the predicted effect of the polymer molecular mass on the protein partitioning. The ln K_p change with M_w followed a linear behaviour for the smallest pro-

teins (LYS, TRY and OVA), while a non linear function showed to be the best fitting for BSA partitioning patterns.

Fig. 2 shows the dependence of ln K_p on the M_w for PEG mixtures when the molecular mass varies from 1500 to 4000. The data fitting showed that there is a non-linear relationship for BSA and TRY, while OVA and LYS showed a linear behavior when changing PEG molecular mass. The linear dependence of ln K_p on the PEG average molecular mass suggests that the free energy change (ΔG°) for the protein transfer between the phases is a direct function of the PEG average molecular mass in the mixture (see that $\Delta G^\circ = -RT \ln K_p$). As a result of this behaviour, the two PEGs interact with OVA and LYS in a different way and the proportion of each polymer in the mixture will influence the protein K_p. OVA showed an anomalous behaviour because its K_p increased with the increase of the average PEG molecular mass.

Fig. 3 shows a significant decrease of BSA K_p at increasing PEG average molecular mass in systems formed by mixtures of PEG4000 and PEG8000, K_p of OVA and TRY decreased
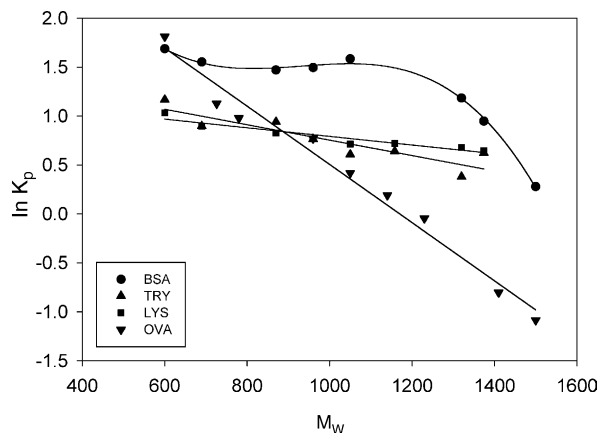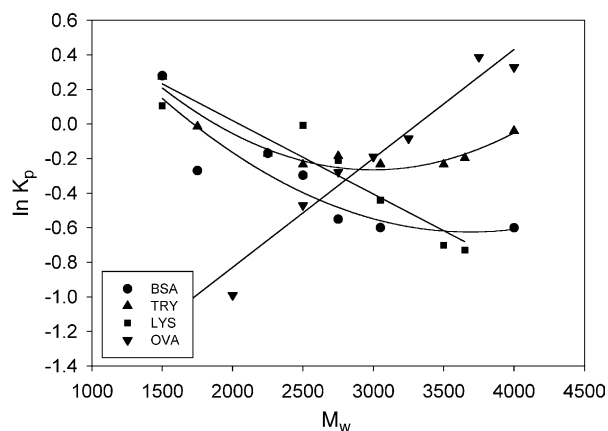

Fig. 1. Dependence of the partitioning behaviour of several proteins on the PEG average molecular mass. ATPSs formed by a mixture of PEG600 and PEG1500–phosphate, pH 7.0 (see composition in Table 1). Temperature 8 °C.

Fig. 2. Dependence of the partitioning behaviour of several proteins on the PEG average molecular mass. ATPSs formed by a mixture of PEG1500 and PEG4000–phosphate, pH 7.0 (see composition in Table 1). Temperature 8 °C.
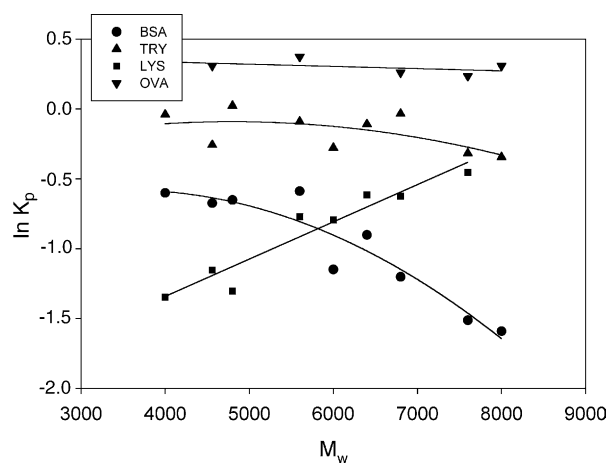
Fig. 3. Dependence of the partitioning behaviour of several proteins on the PEG average molecular mass. ATPSs formed by a mixture of PEG4000 and PEG8000–phosphate, pH 7.0 (see composition in Table 1). Temperature 8 °C.

slightly, while LYS showed the opposite effect. This last behavior, observed for the partitioning of other proteins in aqueous biphasic systems, may be due to the low molecular mass and hydrophobicity of LYS [6].

The former results demonstrate that there is not a general rule for the behaviour of the four assayed proteins in the three PEG mixtures (A–C). One of the reasons could be the different spacial conformation that PEGs of different molecular mass adopt in solution [7], which leads to a differential polymer-protein interaction according to the relative composition of the PEG mixture. To support this possibility, systems with the same average molecular mass as system A but formed by mixtures of PEG600 and PEG4000 were assayed (see system D in Table 1).

Fig. 4 shows the partition coefficient of LYS and TRY in ATPSs of similar PEG average molecular mass but different PEG composition. For both LYS and TRY, the K_p values observed in system D are lower than those corresponding to system A. This finding is a proof that the effect of PEG molecular mass on the
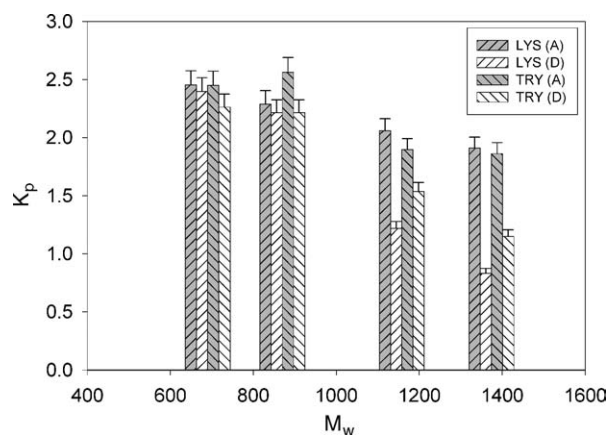

Fig. 4. Effect of polydispersivity on the partition coefficient of TRY and LYS. (A) ATPSs formed by phosphate and a mixture of PEG600 and PEG1500 (see compositions in Table 1). (D) ATPSs formed by phosphate and a mixture of PEG600 and PEG4000 (see compositions in Table 1). Temperature 8 °C, pH 7.0.
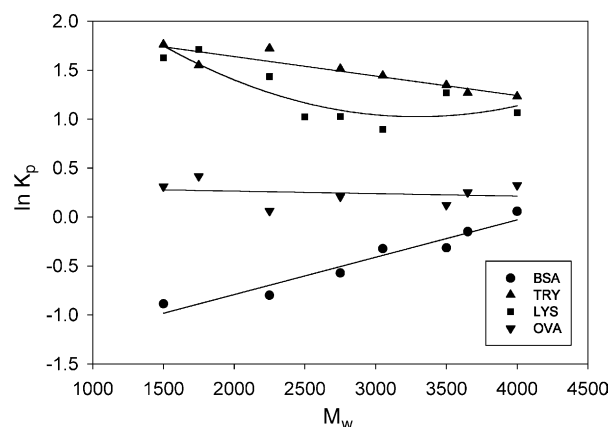

Fig. 5. Dependence of the partitioning behaviour of several proteins on the PEG average molecular mass. ATPSs formed by a mixture of PEG1500 and PEG4000–ammonium sulfate, pH 5.6. Temperature 8 °C.

protein K_p is not exclusively the result of the average M_w value according to the excluded volume theory [8]. Hartounian and Sandler [9] have shown that the average molecular mass of a polymer in the two phases are quite different, the polydispersity parameter of a polymer being lesser than in the feed. Therefore, the largest PEG molecules (molecular mass 4000) concentrate in the top phase while the smallest PEG molecules concentrate in the bottom phase. As a consequence, the average molecular mass in the top phase of system D will be larger than the polymer average molecular mass in the A systems, thus inducing a displacement of the protein partitioning equilibria to the bottom phase due to an exclusion effect. This behaviour was found to depend on the PEG average molecular mass, the largest displacement being observed for systems of higher PEG molecular mass.

3.2. Protein partitioning in other PEG-salt systems

PEG sodium sulfate and PEG-ammonium sulfate are aqueous two-phase systems widely used in the isolation and purification of proteins. Since most used ATPSs are formed by the former salts and PEGs of molecular mass 1500 and 4000, we selected this molecular mass range (1500–4000) to compare their results with those obtained from the PEG-phosphate system.

3.2.1. PEG1500–4000/ammonium sulfate system

This system is frequently used in the isolation of several proteins by the partitioning method [10]. The system pH value 5.6 is given by the concentrated ammonium sulfate solution. Fig. 5 shows a non-linear relationship between the ln K_p and M_w for LYS, while a linear relationship is observed for the other three proteins. For BSA, an increase in the K_p values is observed at increasing PEG average molecular mass while a slight decrease is observed for the other proteins. In PEG/potassium phosphate ATPSs (system A) higher slope values ($\partial \ln K_p / \partial M_w$) were obtained with respect to those observed for PEG/ammonium sulfate ATPSs (see Tables 2 and 3).

Table 2
Values of the $\partial \ln K_p / \partial M_w$ for ATPSs PEG/potassium phosphate, calculated from Figs. 1–3

Protein	ATPS		
	System A (PEG600/PEG1500)	System B (PEG1500/PEG4000)	System C (PEG4000/PEG8000)
$\partial \ln K_p / \partial M_w$			
BSA	Non linear	Non linear	Non linear
OVA	$(-2.9 \pm 0.2) \times 10^{-3}$	$(0.63 \pm 0.05) \times 10^{-3}$	$(-0.16 \pm 0.01) \times 10^{-3}$
TRY	$(-0.78 \pm 0.02) \times 10^{-3}$	Non linear	Non linear
LYS	$(-0.44 \pm 0.06) \times 10^{-3}$	$(-0.42 \pm 0.06) \times 10^{-3}$	$(0.26 \pm 0.03) \times 10^{-3}$

Table 3
Values of the $\partial \ln K_p / \partial M_w$ for ATPSs PEG/ammonium sulfate and PEG/sodium sulfate, calculated from Figs 5 and 6

Protein	ATPS	
	PEG/ammonium sulfate	PEG/sodium sulfate
$\partial \ln K_p / \partial M_w$		
BSA	$(0.38 \pm 0.04) \times 10^{-3}$	$(0.21 \pm 0.05) \times 10^{-3}$
OVA	$(-0.26 \pm 0.05) \times 10^{-3}$	Non linear
TRY	$(-0.19 \pm 0.03) \times 10^{-3}$	$(0.18 \pm 0.02) \times 10^{-3}$
LYS	Non linear	$(-0.22 \pm 0.04) \times 10^{-3}$

3.2.2. PEG1500–4000/sodium sulfate system

Fig. 6 summarizes the effect of PEG molecular mass on the protein partitioning in a PEG/sodium sulfate system at pH 7.0. The $\ln K_p$ versus M_w relationship was non linear for OVA, while the other protein yielded a linear relationship. K_p for TRY and BSA showed an increase at increasing average molecular mass, while LYS showed the opposite effect. For all the proteins, the slope values were lower than those corresponding to PEG/potassium phosphate ATPSs (see Tables 2 and 3).

3.3. How the PEG molecular mass affects the recovery of a protein in one of the phases

The aim of a successful protein extraction by ATPSs is to find a suitable phase system where the selective separation is achieved with the best yield. In this way, the molecular mass
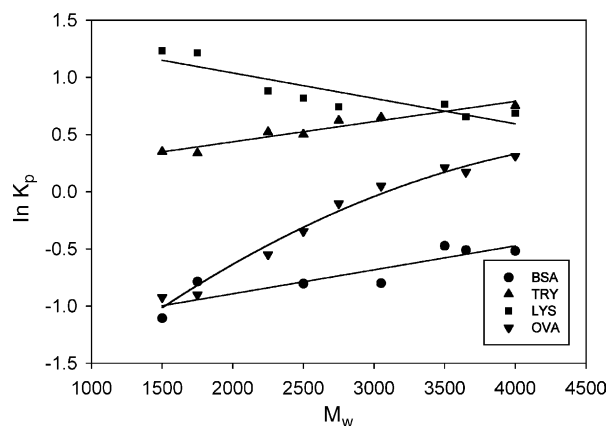

Fig. 6. Dependence of the partitioning behaviour of several proteins on the PEG average molecular mass. ATPSs formed by a mixture of PEG1500 and PEG4000–sodium sulfate, pH 7.0. Temperature 8 °C.

effect of PEG on K_p must be studied for a target protein and its impurities. Sometimes the best theoretical yield is reached for an average PEG molecular mass which is not commercially available. For example, Mayerhoff et al. [11] studied the extraction of a reductase by using a yield function which depended on the PEG molecular mass and the tie line values. These authors found that the best yield was obtained for PEG of molecular mass 744. Obviously, this PEG is not commercially available, however, this problem can be solved using a mixture of two PEGs of different M_w with the appropriate mass composition in order to obtain the M_w value.

From a visual inspection of Fig. 1, the largest K_p difference between OVA and LYS, OVA and TRY or TRY and LYS in PEG600–PEG1500 ATPSs (system A) can be seen for systems prepared with a single PEG (extreme systems). On the other hand, the maximal difference between the K_p values for TRY, LYS and OVA with respect to BSA is reached for systems formed by an appropriate mixture of PEGs, which shows that these systems have the highest separation capability. The theoretical protein yield of a given protein (i.e. protein A) in the top phase after only one partition step from a mixture of identical quantities of two proteins (A and B) can be calculated according to [12]:

$$y_{A(top)} \, (\%) = \frac{100 R K_{p,A}}{1 + R K_{p,A}} \tag{4}$$

where $R = V_T / V_B$, V_B and V_T are the bottom and top phase volumes, respectively (see that $y_{A(bottom)}$ is $100 - y_{A(top)}$). The protein A purity, thus the ratio between the content of protein A and the total protein in a given phase, can be calculated as:

$$P_A \, (\%) = \frac{100 y_A}{y_A + y_B} \tag{5}$$

It must be taken into account that within a phase P_A plus P_B is 100%.

Fig. 7 shows the dependence of the theoretical BSA purity in the top phase on M_w for three mixtures: BSA/OVA, BSA/TRY and BSA/LYS. The V_T / V_B was assumed to be one. Systems of approximately 1200 average molecular mass can be seen to have the best separating capability for mixtures of BSA with LYS and TRY and systems with PEG average molecular mass of 1400 separate mixtures OVA/BSA. Those systems that allow the maximal purity value for a given protein in the top phase will allow us to obtain the maximal purification factor of its impurity in the bottom phase. ATPSs provide maximal purity
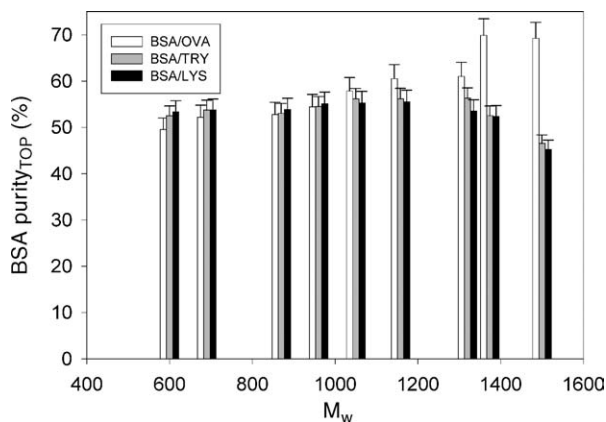
Fig. 7. Dependence of the calculated BSA purity in the top phase on the PEG average molecular mass for three binary mixtures: BSA/OVA, BSA/TRY and BSA/LYS. Assumptions: V_T/V_B equal to one, only one partition step and the mixtures formed by identical quantities of the two proteins. ATPSs formed by phosphate and a mixture of PEG600 and PEG1500, pH 7.0. Temperature 8 °C.

for intermediate PEG molecular mass as consequence of the non-linear behaviour of ln K_p versus M_w, i.e. the dependence of the $\partial \ln K_p / \partial M_w$ value with M_w.

The effect of PEG molecular mass on the partitioning of proteins have been studied by several authors [13]. Forciniti and coworkers [3,6] studied the partitioning of lysine, chymotrypsin and bovine albumin in a system of PEG (M_w 4000–20,000)-dextran (500,000) and found that the increase of the PEG molecular mass induces a protein transfer to the PEG rich phase. The K_p for the smallest protein such as LYS proved to be poorly affected by the M_w change, while the BSA partitioning was four fold decreased when the M_w varied from 4000 to 20,000. Similar behaviours were observed for most of the assayed proteins in this work with the exception of OVA and LYS in systems B and C.

Albertsson [14] applied the Flory Huggins theory to predict the change of ln K_p with polymer molecular mass (holding phase composition constant) yielding the following equation:

$$\ln K_{p,PEG_{Mw1}} - \ln K_{p,PEG_{Mw2}} = M_{w,prot} C \left(\frac{1}{M_{w1}} - \frac{1}{M_{w2}} \right) \tag{6}$$

where $K_{p,PEG_{Mw1}}$ and $K_{p,PEG_{Mw2}}$ are the partition coefficients of the protein in systems formed by PEG of M_{w1} and M_{w2} molecular mass, respectively and C is a constant given by the concentration of PEG in the top phase minus that in the bottom. Eq. (6) predicts that increasing PEG M_w will lead to a decrease in the protein K_p. Eq. (6) also predicts that the effect will increase linearly with the protein molecular mass. Thus, the partitioning of large proteins is more sensitive to changes in polymer molecular mass. However, Eq. (6) cannot be applied to all biphasic systems since it can not explain either the poor effect of the protein molecular mass on the partition for ATPSs of PEG/sodium sulfate and PEG/ammonium sulfate or the TRY and OVA behaviours, whose K_p values slighly increased when the PEG molecular mass increased.

One of the reasons could be that Eq. (6) was derived considering only the effect of the molecular mass change of the polymer in the density number of one of the phases and assuming that the enthalpic contributions are independent of PEG molecular mass. Baskir et al. [15] stated that the protein partition is driven by not only an entropic mechanim but by enthalpic effects as well. According to Picó and coworkers [16], from a molecular point of view, PEG of low molecular mass may interacts strongly with proteins while PEGs of higher molecular mass have ability to form intra molecular bonds. The protein transfer to one of the phases requires the breaking of the interaction between the phase components to create a cavity where the protein will be included. Therefore, the energy balance can be negative or positive according to whether protein/polymer interactions are attractive or repulsive, thus it depends on the PEG molecular mass [14]. The PEG-salt interaction must also be considered, for example, in the PEG/phosphate system, due to the repulsion between the PEG and phosphate molecules, the PEG phase has higher self energy than the phosphate phase. The interaction PEG-sulphate medium is more stronger than the PEG-phosphate interaction, hence, it is easier for a protein to get into the PEG-sulfate phase than to a PEG-phosphate phase.

Another reason for anomalous behaviour of some of assayed proteins may be the polydispersivity of the systems. In practise, flexible polymer chains are more or less polydisperse in molecular mass, depending on their origin. The commercial dextrans are reactives whose polydispersivity increases with the increase in their molecular mass, because they are products from the metabolism of a microorganism. On the other hand, PEG, which is obtained from industrial synthesis, can be considered to be monodisperse since its narrow molecular mass distribution according to the manufacturer information. Average distributed properties, such as an average molecular mass in polymer systems, have been used to treat polydisperse systems as monodisperse [17]. However, there are important differences between monodisperse and polydisperse systems. For example, the sharp phase transition boundaries that exist in monodisperse mixtures are not found in systems containing polydispersed species. Moreover, a PEG fractionation occurs with the larger PEG molecules tending to remain in the top phase. In this way, when PEG average molecular mass changes, phase composition also changes, thus affecting the protein partitioning behaviour. From inspection of Fig. 4, small differences between the K_p values of TRY and LYS for ATPSs (system A) of 1158 and 1374 average molecular mass are enhanced and inverted in systems of similar M_w but higher polidispersivity (system D).

4. Conclusion

In bioseparation processes, partitioning is used to separate the target species from unwanted impurities We demonstrated that polymer molecular mass and polydispersivity of phase-forming polymers are parameters of the system that can be manipulated to maximize the differences in the partition coefficient between target protein and impurities. Our results showed that the choice of the adequate system (in polydispersivity and molecular mass of the polymers) led to an efficient separation of mixtures of two

proteins with similar isoelectrical point such as OVA/BSA and TRY/LYS.

Acknowledgements

References

[1] P.Å. Albertsson, Partition of Cell Particles and Macromolecules, second ed., John Wiley and Sons, New York, 1971.

[2] B.Y. Zaslavsky, Aqueous Two-phase Partitioning. Physical Chemistry and Bioanalytical Applications, Marcel Dekker Inc., New York, 1994.

[3] P.A. Albertsson, A. Cajarville, D.E. Brooks, F. Tjerneld, Biochim. Biophys. Acta 926 (1987) 87.

[4] C.H. Kang, S.I. Sandler, Macromolecules 21 (1988) 3088.

[5] G. Reh, B. Nerli, G. Picó, J. Chromatogr. B 780 (2002) 389.

[6] D. Forciniti, C.K. Hall, M.R. Kula, Bioseparation 2 (1991) 115.

[7] L.-Y. Lee, J.C. Lee, Biochemistry 26 (1987) 7813.

[8] N.L. Abbot, D. Blankstein, T.A. Hatton, Macromolecules 26 (1996) 825.

[9] H. Hartounian, S.I. Sandler, Biotechnol. Prog. 7 (1991) 279.

[10] D.P. Harris, A.T. Andrews, G. Wright, D.L. Pyle, J.A. Asenjo, Bioseparation 7 (1997) 31.

[11] Z.D.V.L. Mayerhoff, I.C. Roberto, T. Franco, Biochem. Eng. J. 18 (2004) 217.

[12] H. Walter, D. Brooks, D. Fisher (Eds.), Partitioning in Aqueous Two-phase Systems. Theory, Methods, Uses, and Applications to Biotechnology, Academic Press Inc., Orlando, 1985.

[13] D.Q. Lin, Y.T. Wu, L.H. Mei, Z.Q. Zhu, S.J. Yao, Chem. Eng. Sci. 58 (2003) 2963.

[14] H.O. Johansson, G. Karlstrom, F. Tjerneld, C.A. Haynes, J. Chromatogr. B 711 (1998) 3.

[15] J.N. Baskir, T.A. Hatton, U.W. Suter, Macromolecules 20 (1987) 1300.

[16] B. Farruggia, B. Nerli, H. Di Nucci, R. Rigatuso, G. Picó, Int. J. Biol. Macromol. 26 (1999) 23.

[17] C.-H. Kang, C.-K. Lee, S.I. Sandler, Ind. Eng. Chem. Res. 28 (1989) 1537.