ELECTROPHORETIC AQUEOUS TWO-PHASE PARTITION OF PROTEINS

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ABSTRACT. An electrical field was applied to an aqueous two-phase system. The integration of these techniques in one step is used to improve the partition of biological molecules. Experiments were performed for an individual protein - BSA - and for a mixture of proteins - lysozyme and egg albumin - at different polarities and opposite charges of the biomolecules for the aqueous two-phase system Reppal PES - PEG8000. The separation between those two proteins improved more than two-fold.

KEYWORDS. Two-Phase Systems / Electrical partition

INTRODUCTION

Aqueous two-phase systems (ATPS) are obtained when two water soluble polymers or one polymer and a salt are added to water and, as a consequence, two immiscible aqueous phases are formed (Venâncio *et al.*, 1993). In recent years, ATPS have found applications in various areas of biotechnology (Walter and Johansson, 1994). The main advantage of this separation technique lies on the gentle environment that is provided for biomolecules separation due to the high water content of both phases.

Biomolecules can also be separated by applying an electrical field to an aqueous solution. This technique, exploited essencially at small scale in processes such as electrophoresis, isotachophoresis and isoelectric focusing (Westermeier, 1993), is quite effective in the obtention of pure fractions of the molecule to be isolated.

One way of increasing separation performance and yield in bioproduct recovery is to couple in one step two (or more) purification processes.

In this way, the simultaneous utilization of ATPS and electrophoretic purification - two techniques based on different principles - may become an important contribution for the improvement of biomolecules concentration and purification. This method (electrophoretic aqueous two-phase systems - EATPS) will also take advantage of the stable interface that is formed between the two aqueous phases, thereby reducing the convective flux caused by the application of an electrical field.

Results describing the application of an electrical field in the partition of a pure protein - BSA - and of a mixture of two proteins - lysozyme and egg albumin - in aqueous two-phase systems are presented in this work.

MATERIALS AND METHODS

Chemicals

Poly(ethylene glycol) (PEG8000), number average molecular mass of (7-9)x10^3 Da was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Reppal PES 100 (Rep) was obtained from Reppe AB (Vaxjo, Sweden). All other chemicals were analytical grade and water was distilled and passed through a mixed ion exchanger.

Proteins

Lysozyme from chicken egg white (pI = 10.5), Egg Albumin-grade III (pI = 4.63) and Bovine Serum Albumin (BSA) were obtained from Sigma Chemical Co (St. Louis, Mo, USA).

Aqueous two-phase systems

ATPS were prepared by weighting out appropriate amounts of all chemicals, polymers and buffering salts, and dissolving them in distilled water, as described elsewhere (Venâncio *et al.*, 1996). In all experiments phosphate buffer was employed, except for the partition of BSA at pH 3.6. In the later case, citric acid buffer was employed.

Protein partition coeficients were defined as the ratio between the protein concentration in the upper and bottom phases.

Electropartition device

A modification of the electropartition device described by Marando and Clark (1993) was constructed. This device is divided into three chambers (figure 1) in a vertical alignment. The top and bottom chambers contain the buffer solution (electrolyte) and the middle chamber contains the two-phase system. The buffer solution is separated from the ATPS by dialysis membranes These membranes retain all phase forming polymers and proteins and allow for the passage of electrical current. The buffer solution circulates between top and bottom chambers and is constantly cooled with ice cold water to prevent protein denaturation.

Protein analysis

BSA was assayed by measuring the optical density at 280 nm. Lysozyme and Egg Albumin were analysed by FPLC, using BIORAD Econo column with a Macro-Prep High Q anion exchange support.

RESULTS AND DISCUSSION

The partition behaviour of BSA in ATPS under different conditions was studied. Experiments were performed at different polarities and different charge of the BSA molecule (i.e., with different buffer solutions).

In the absence of electrical field, negatively charged BSA was directed towards the lower phase (Table 1). With the application of an electrical field, with the anode in the upper phase, BSA migrates towards the upper phase and a drastic increase in the partition coefficient of BSA was obtained.

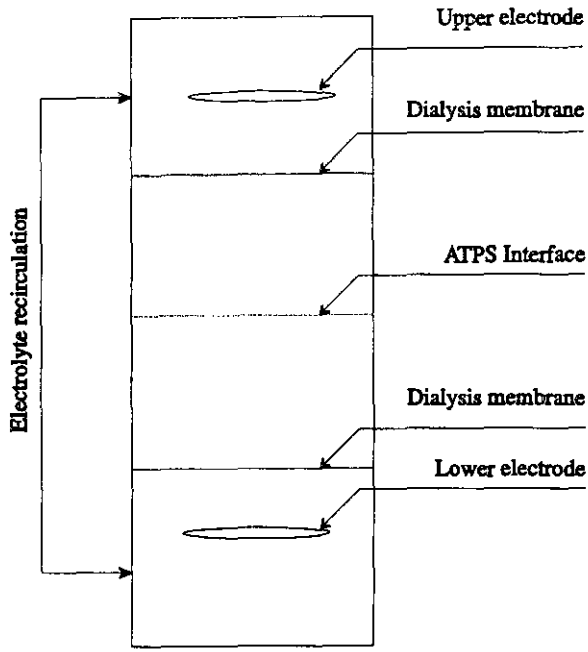

Figure 1 Electropartition device.

In another experiment, the anode was placed in the lower phase and BSA was made positive by a change in pH. In this case, an increase in partition coefficient was also observed. Although not being as drastic as in the previous experimental conditions, the obtained values confirm the principles underlying the methodology.

Table 1 Partition coefficient, K_{BSA}, of BSA before and after the application of the electrical field, in an aqueous two-phase system with composition: 15% (w/w) Reppal PES, 5% (w/w) PEG8000

electrical field (V)	pH	BSA charge	time (min)	anode	K_{BSA}	$\Delta\log K_{BSA}$*
0	6.8	-			0.472	
90	6.8	-	40	upper phase	2.131	0.65
0	6.8	-	remixing and phase separation		0.482	
0	3.6	+			0.231	
90	3.6	+	20	lower phase	0.385	0.42
0	3.6	+	remixing and phase separation		0.146	

* $\Delta\log K_{BSA} = \log K_{BSA/el} - \log K_{BSA}$

These results indicate that the application of an electrical field in ATPS may be an effective technique to separate proteins with different charges that present similar partition coefficients when no electrical field is applied.

The separation of two proteins with similar partition coefficients in the Reppal PES-PEG8000 system and with different pI's was assayed. Selected proteins were lysozyme (pI = 10.5) and egg albumin (pI = 4.6), both from egg white. Experiments were performed at a pH of 7.6 in order to have proteins with opposite charges.

As can be seen in table 2, with the application of EATPS and the anode in the upper phase, the separation of these two proteins can be improved by a factor of two. In agreement with results reported by other authors (Clark, 1992) and in this work for BSA, when considering individual proteins, in the presence of the electrical field, egg albumin moved towards the upper phase and lysozyme concentration increased in the bottom phase.

Table 2 Partition of two hen egg proteins, Lysozyme and Egg Albumin, in an aqueous two-phase system: 15% (w/w) Reppal PES, 5% (w/w) PEG8000, pH 7.6

electrical field*	time (min)	$K_{egg\ albumin}$	$K_{lysozyme}$	P**
0	-	0.77	0.44	1.75
90 V	60	0.82	0.23	3.57

* anode in the upper phase.

** defined as the ratio between egg albumin partition coefficient and lysozyme partition coefficient.

CONCLUSIONS

Tha experiments performed above and below the isoelectric point of BSA (Table 1) showed that the application of an electrical field, coupled with the manipulation of the charge of biological molecules, can be used to control and improve the partition of charged molecules in ATPS. The application of this technique - EAPTS - as demonstrated for the separation of a mixture of proteins, can also be used to enhance biomolecules purification.

This set of experiments proved that electro aqueous two-phase partition may be used as a powerful tool in the separation of biological molecules with different electrical mobilities. EATPS can and should be used as a final refinement step in a purification procedure.

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