PARTITION OF BIOMOLECULES USING AQUEOUS TWO-PHASE SYSTEMS: COMPARISON OF BATCH AND CONTINUOUS PROCESSES.

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Key words: Aqueous two-phase system / Biomolecule / Continuous

Introduction. Aqueous two-phase systems (ATPS) have proved to be a practical alternative to establish batch processes for the recovery and purification of a wide range of target products [1]. They have been previously proposed as a suitable step to overcome challenges in the large-scale purification of a desired protein from fermentation broths containing a wide range of contaminant biomolecules [2], however the lack of continuous devices to jump from lab to industrial scale limits its utilization.
The objective of the present work is to evaluate the use of a novel continuous device (**Fig.1**) to perform ATP partitioning of biomolecules obtaining similar or improved partition coefficients and recovery when compared to batch operation.

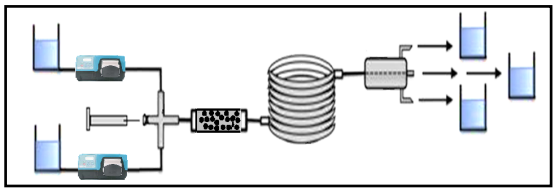

Fig. 1 Prototype scheme of the continuous device.

Methods. ATPS with different compositions of poly(ethylen-glycol) (M.W. 1000 kDa) and potassium phosphate at room temperature were employed in all experiments. Proofs of concept were performed using four different experimental models. Dye partitioning was monitored measuring phase absorbance at the corresponding λ_{max} (Bio-Tek Instruments, VT, U.S.A.). Total protein concentration from the phases was determined using Bradford's method [3]. Enzymatic activity of the α-amylase protein was measured using the micro-plate based assay reported by the Xiao *et al*. for enzyme quantification [4]. Some samples from top and bottom phases were taken and analyzed by SDS-PAGE electrophoresis according to the Laemmli protocol [5].

Results. The study of phase formation inside the device was performed using Gentian violet, ensuring dye transfer from bottom to top phase (**Fig. 2**). Next, bovine serum albumin (BSA) was used as a model protein and it was observed that the turbulent regime created at the entrance of the device by a static mixer, contributed to an efficient mass transfer to reach a similar partition coefficient (K_P) but in shorter equilibration times than batch system. After that, whey protein isolate (WPI), characterized by the presence of two main proteins (α-lactalbumin and β-lactoglobulin) with different phase affinities, was successfully processed resulting in differential protein partitioning with total recoveries up to 90% (top + bottom phase) and minimal protein precipitation at the interface. The prototype was also proved for the recovery of α-amylase from soybean extracts as another model system. This system was selected as an example of low-abundant protein present in complex mixtures. Similar Kp (>4) with a higher top phase enzyme recovery (81%) and a purification factor 40-fold higher than batch experiments was achieved.

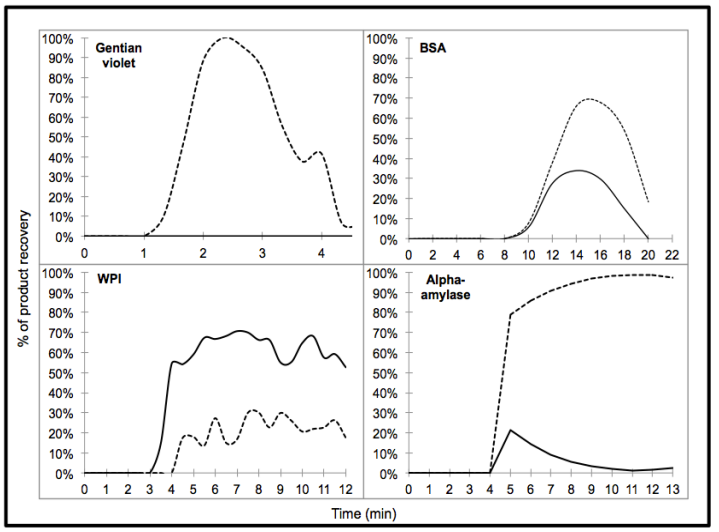

Fig. 2 Recovery percentages (w/w) of the different experimental models explained in the text for top (⋯) and bottom (—) phases.

Conclusions. Compared with batch systems, continuous operation in this prototype seems to increase partition coefficient with higher recovery efficiencies. The obtained results suggested that the continuous recovery and purification of protein products in a defined phase could be feasible, by controlling the flow rates of the ATPS components. These findings demonstrate the potential application of the proposed device for the continuous protein recovery from complex mixtures. The promising performance of this prototype can raise the attention of the industry for the adoption of aqueous two-phase system based strategies.

Acknowledgements. To the Bioprocesses research chair (CAT161), FEMSA Nutrigenomic Research Chair and the CONACyT for the scholarships of P. Vázquez-Villegas No. 228073 and E. Espitia-Saloma No. 246739.

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