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Towards continuous aqueous two-phase extraction (CATPE)

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[1] M.J. Abrahamson, E. Vazquez-Figueroa, N.B. Woodall, J.C. Moore, A.S. Bommarius, "Development of a Novel Amine Dehydrogenase for Synthesis of Chiral Amines", Angew. Chem. Intl. Ed. 2012, 51, 3969-3972 [2] B. Bommarius, H. Gieren, K.E. Jaeger, A.S. Bommarius, "Scale-up and large-scale fermentation of amine dehydrogenase and formate dehydrogenase", to be submitted [3] Schembecker, G., van Winssen, F.A., Burghoff (2012), B. Process for separation/purification of biomolecules, European Patent Office, Patent no. WO2012130855 A1 [4] Van Winssen, F.A., Merz, J., Czerwonka, L.-M., Schembecker, G., Separation and Purification Technology 2014, 136, 123-129. [5] Van Winssen, F.A., Merz, J., Schembecker, G., Journal of Chromatography A 2014, 1329, 38-44.

TOWARDS CONTINUOUS AQUEOUS TWO-PHASE EXTRACTION (CATPE)

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Aqueous Two-Phase Extraction (ATPE) in mixer-settlers offers a gentle and biocompatible environment to separate proteins from complex mixtures. We have developed an aqueous two-phase system with inexpensive and biocompatible PEG 1500 or 4000 and ammonium citrate. We have purified several dehydrogenases [1] to near homogeneity after forward extraction into a PEG-heavy top phase at pH > 9 and back extraction into a bottom phase at pH 4-6; in selected cases, we were able to obtain pure protein in the bottom phase without forward extraction into the top phase. We have scaled up the PEG 1500/4000-ammonium citrate to a 5-10 L scale, with phase separation times of less than five minutes.[2] We currently extend the system to the separation of Qα virus-like particles.

However, ATPE technology is characterized by complex phase separation and very limited number of separation stages not offering enough separation efficiency. These limitations can be overcome by the novel Tunable Aqueous Polymer Phase Impregnated Resins (TAPPIR) technology which immobilizes one phase out of a biphasic aqueous extraction system in porous material (Figure 1) [3]. By immobilizing these impregnated resins in columns continuous operation similar to Simulated Moving Bed systems become possible. TAPPIR provides high separation efficiency along with high capacity, avoids long phase separation times (especially for highly viscous polymer phases) and offers an answer to the non-ecological image of ATPE through immobilizing and re-using phase forming material.

The application of the TAPPIR technology has been shown for the separation of lysozyme and myoglobin using a polyethylene glycol 4000/citrate aqueous two-phase system in batch experiments [4]. In addition, the influence on protein partitioning of the porous solids' properties like solid material, particle and pore size has been investigated. It could be demonstrated that the same partitioning levels can be reached for the TAPPIR as for classical ATPE mixer/settler experiments and that the leaching of the immobilized phase is negligible [5].

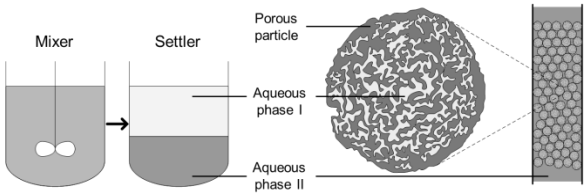

Figure 1 - Mixer-settler setup and TAPPIR

The presentation will introduce the TAPPIR technology, describe the advantages over chromatography and present a process concept for continuous operation with zero waste.

[1] M.J. Abrahamson, E. Vazquez-Figueroa, N.B. Woodall, J.C. Moore, A.S. Bommarius, "Development of a Novel Amine Dehydrogenase for Synthesis of Chiral Amines", *Angew. Chem. Intl. Ed.* **2012**, *51*, 3969-3972
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[5] Van Winssen, F.A., Merz, J., Schembecker, G., *Journal of Chromatography A* **2014**, *1329*, 38-44.