PROGRESS TOWARDS CONTINUOUS AQUEOUS TWO-PHASE EXTRACTION VIA TAPPIR

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At ICB II, we presented Aqueous Two-Phase Extraction (ATPE) as a non-chromatographic alternative for protein purification. We had developed an aqueous two-phase system with inexpensive and biocompatible PEG 1500 or 4000 and ammonium citrate. We purified several enzymes, more specifically a series of 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. Scale-up of the PEG 1500/4000-ammonium citrate to 5-10 L scale still often gave phase separation times of less than five minutes.[2] However, ATPE technology is characterized by complex phase separation and very limited number of separation stages not offering enough separation efficiency. Furthermore, conventional ATPE does not lend itself to continuous operation.

These limitations can be overcome by the novel Tunable Aqueous Polymer Phase Impregnated Resins (TAPPIR) technology which immobilizes one of the two phases of the aqueous extraction system in porous material (Figure 1) [3]. By immobilizing these impregnated resins in columns, continuous operation similar to Simulated Moving Bed systems becomes feasible. TAPPIR provides high separation efficiency along with high capacity, avoids long phase separation times (especially for highly viscous polymer phases) and improves ecological compatibility through immobilization and re-use of phase forming material. TAPPIR technology has been applied to the separation of lysozyme and myoglobin using a polyethylene glycol 4000/citrate aqueous two-phase system in batch experiments [4]. 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].

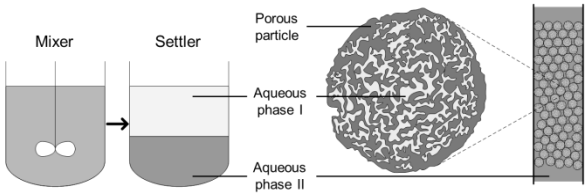

Figure 1 - Mixer-settler setup and TAPPIR

We report on our efforts to develop continuous purification of dehydrogenase enzymes via TAPPIR. We often observed precipitation of protein at the interface, which is incompatible with operation of TAPPIR. However, we did not necessarily lose significant levels of activity. Interfacial precipitation often did prevent partitioning, as the interface presented a physical barrier and target protein was immobilized in or near the interface. In such a case, neither the raffinate phase nor the extract phase showed much active protein. We will report how we still achieved partitioning in single-stage ATPE under interfacial precipitation conditions (though not in TAPPIR). Stirring of the ATPE phases continually renews the interface, which might allow overcoming blockage via interfacial precipitation. To succeed in running TAPPIR for separation, we screened for conditions of low or absent protein precipitation at the interface. We will conclude the presentation by describing methods for efficient search for such conditions.

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