TAILOR-MADE AQUEOUS TWO-PHASE SYSTEMS FOR APPLICATION IN CONTINUOUS SEPARATION OF POTENT BIOMOLECULES

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Aqueous Two-Phase Extraction (ATPE) using Aqueous Two-Phase Systems (ATPS) has long been shown to be a viable and promising alternative in the work-up of potent biomolecules (e.g. enzymes, proteins, therapeutics) from fermentation broth. Although ATPE has significant advantages over common separation strategies, such as a high biocompatibility, gentle separation profile due to low interfacial tension, good scalability and high efficiencies, industrial applications have not yet been realized.
Reasons typically given are based on the ATPS “physiochemical” properties such as viscosities and low density differences between the phases, which lead to long phase separation times. However, these challenges can be addressed using advanced technology such as the “Tunable Aqueous Polymer-Phase Impregnated Resins” (TAPPIR)-Technology immobilizing one phase of an ATPS inside porous solids, which are then transferred into a chromatography column. The second aqueous phase serves as mobile phase. The main advantage of this technique is the simple and efficient emulsification and liquid–liquid phase separation through the packed-bed column design. In addition, the extraction phases, i.e. both the back extraction phase and the immobilized phase, can be reused enabling a low-waste production process.

The remaining bottleneck for an industrial application is the identification of the “base” ATPS, which enables the desired extraction of the biomolecule with the required yield and purity to be competitive to existing processes. State-of-the-art ATPS design so far is based on a “trial-and-error” based approach identifying ATPS that work for a given task but often perform in suboptimal fashion.
In the present work, we will present a novel thermodynamics-based strategy for the identification and characterization of tailor-made ATPS for the continuous separation of highly potent industrial enzymes by ATPE. By consideration of the molecular interactions in solution, we are able to define potentially suitable ATPS based on a predictive modeling approach using *e*PC-SAFT, a state-of-the-art equation of state. The objective of this step is to supply a thermodynamically optimized combination of ATPS-phase formers that lead to optimal water condition (low concentration of phase formers, large process window), in principal enabling optimal separation. This initial selection is refined by taking into account molecular interactions of the biomolecule (enzyme), by measuring and modeling biomolecule-biomolecule and biomolecule-phase former interactions. These interactions are experimentally captured using advanced light scattering techniques that are both time and cost efficient. It will be shown that, based on the description of molecular interactions through osmotic virial coefficients (B_{22} and B_{23}) as well as the diffusion interaction parameter (k_D) between the molecules in solution, the phase behavior of the biomolecule in an ATPS can be made accessible, but was previously inaccessible with other phase diagram estimation strategies
One major advantage of our predictive modeling approach is the estimation of the partition coefficient of the biomolecule between the two aqueous phases based on a minimal set of experimental data, i.e. B_{22}, B_{23}, k_D, and phase composition data. Furthermore, the influence of the ATPS phase-formers on protein solubility and stability can be judged qualitatively, an ideal complement in the development of ATPS.
Lastly, we applied the thermodynamics-based strategy to the separation of an industrially relevant dehydrogenase from fermentation broth. The design-driven process development led to the identification of a tailor made ATPS that outperformed the reference ATPS from previous works in terms of solubility and stability of the biomolecule enabling a cost-efficient use of the TAPPIR technology.