**Transfer of the TAPPIR®-Technology to a Packed Bed for Separation of Biomolecules**

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**Highlights**

* Semi continuous aqueous two-phase extraction
* Packed bed application of the TAPPIR®-Technology in a column
* Impregnation stability of porous particles in incident flow

**1. Introduction**

Since gentle and cost efficient separation techniques for biochemical products are of major interest for the biotech-industry, Aqueous Two-Phase Extraction (ATPE) has been shown to be a promising approach in this regard [1]. This is mainly based on the high biocompatibility of ATPE resulting from the water content of up to 70-90 wt.-%. However, long phase separation times in “conventional” ATPE design (Mixer-Settler-Approach) are the major drawback of this technique, requiring additional equipment and energy to enable faster phase separation [2]. To circumvent this bottleneck, the Tunable Aqueous Polymer Phase Impregnated Resins (TAPPIR®)-Technology is applied. As one phase of the Aqueous Two-Phase System (ATPS) is immobilized in porous particles enabling extraction through dispersion of these particles in the other aqueous phase, a subsequent liquid-liquid separation is no longer necessary. The impregnation stability [3] and competitive mass transfer kinetics of biomolecules within the TAPPIR®-Technology compared to “conventional” ATPE were already proven in batch experiments. In order to implement this technique in an industrial process, in this work the TAPPIR®-Technology is transferred to a column enabling a semi continuous flow-through separation of biomolecules in a packed bed. Consequently, one aqueous phase is used as the mobile phase, whereas the other aqueous phase is stationary impregnated in the particles providing an easy and cost efficient separation process. Within this work, we focused on the impregnation stability of the stationary phase in porous particles in flow through operation as well as the extraction performance in the packed bed.

**2. Methods**

A polyethylene glycol - tri-sodium citrate (polymer-salt) ATPS was used in this work. If applicable, sodium chloride was utilized as displacement agent. After preparation, the ATPS is equilibrated and transferred to a dropping funnel for settling. Immunoglobulin G serves as a model biomolecule within this work and is introduced into the system using the salt-rich phase of the APTS. Polymer-rich phase impregnated micro porous ceramic particles are packed in a semi-preparative YMC ECOPlus glass column. An ÄKTApurifier from GE Healthcare is used as utility to ensure stable process conditions. The impregnation status can either be determined by adding a dye to the stationary phase or the retention time of Bovine serum albumin, introduced as a tracer that partitions selectively to the salt-rich phase. An UV detector is used for the analysis of the biomolecule concentrations.

**3. Results and discussion**

In order to transfer the TAPPIR®-Technology to a semi continuous operation mode in a packed bed column, several factors need to be evaluated: (1) impregnation stability of the stationary phase, (2) uniform flow within the packed bed, (3) extraction performance comprising the kinetics in continuous flow. The impregnation stability in batch experiments of the polymer-rich phase in porous ceramic particles showed negligible leaching as soon as the three-phase contact angle (measured from the polymer-rich phase on solid material in salt-rich phase) showed values < 90 °. However, upon transfer to a column, in which the impregnated particles have to withstand a steady incident flow, several factors influence stability not encountered in batch mode. It is shown that a uniform packing induces slower leaching rates. Also, packed beds formed by larger particles have a higher impregnation stability than those with smaller particles of the same material. Moderate flowrates (e.g. 1 ml/min in a column with a diameter of 1 cm) additionally lower the leaching of the impregnated phase out of the pores. Changing the ATPS composition itself (e.g. using polyethylene glycol with a higher molecular weight), a stable impregnation in steady flow could be achieved for more than 48 hours. This now allows for further characterization of the new setup towards process parameters that enable an optimal extraction efficiency.

**4. Conclusions**

Within this work, it was shown that the transfer of the TAPPIR®-Technology to a packed bed in a column was possible enabling a semi continuous two-phase extraction. Thereby, additional downstream treatment of the emulsion compared to classical ATPE was avoided. In the packed bed application, factors influencing the impregnation stability of the polymer-rich phase in the porous particles were identified allowing to apply optimal process conditions in semi continuous mode. The extraction performance was competitive to conventional ATPE and can be optimized further by adjusting design and process parameters. It was shown that the TAPPIR®-Technology in a column offers an improved approach for the implementation of ATPE as a separation technique for biomolecules.

**References**

1. J. A. Asenjo, B. A. Andrews, J. Chem. Technol. Biotechnol. 83 (2) (2008) 117-120.
2. U. Gündüz, A. Tolga, J. Chromatogr. B 807 (1) (2008) 13-16.
3. I. Kaplanow, M. Schmalenberg, I. Borgmann, G. Schembecker, J. Merz, Sep. Purif. Technol. 190 (2018) 1-8.