



Tunable aqueous polymer-phase impregnated resins-technology—A novel approach to aqueous two-phase extraction



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ABSTRACT

Aqueous Two-Phase Extraction (ATPE) represents a promising unit operation for downstream processing of biotechnological products. The technique provides several advantages such as a biocompatible environment for the extraction of sensitive and biologically active compounds. However, the tendency of some aqueous two-phase systems to form intensive and stable emulsions can lead to long phase separation times causing an increased footprint for the required mixer-settler devices or the need for additional equipment such as centrifuges. In this work, a novel approach to improve ATPE for downstream processing applications called 'Tunable Aqueous Polymer-Phase Impregnated Resins' (TAPPIR®)-Technology is presented. The technology is based on the immobilization of one aqueous phase inside the pores of a solid support. The second aqueous phase forms the bulk liquid around the impregnated solids. Due to the immobilization of one phase, phase emulsification and phase separation of ATPE are realized in a single step. In this study, a biodegradable and sustainable aqueous two-phase system consisting of aqueous polyethylene glycol/sodiumcitrate solutions was chosen. The impregnation of different macroporous glass and ceramic solids was investigated and could be proven to be stable. Additionally, the separation of the dye Patent blue V was successfully performed with the TAPPIR®-Technology. Thus, the "proof of principle" of this technology is presented.

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1. Introduction

Nowadays, a variety of specialized biotechnological products is needed in food, cosmetic and pharmaceutical industry and the demand for these products increases steadily [1]. Developments in the upstream section are rather fast, while Downstream Processing (DSP) often represents the bottleneck of the whole production process [2,3]. Product sensitivities towards high temperatures, pH values or organic solvents, comparatively low product concentrations, a high number of unspecified impurities and strict regulatory laws for final product quality complicate the DSP [4]. Current DSP strategies are often expensive and limited by product loss or irreversible product deactivation. Thus, the need for stable, selective and cost-effective DSP unit operations is of main importance. One of these unit operations is Aqueous Two-Phase Extraction (ATPE) [5], where two partially immiscible aqueous phases are used as extraction system. The main advantage of ATPE as a DSP unit operation for biotechnological molecules is the biocompatibility due to the high water content of 70–90 wt.% [6]. The low interfacial tension between the two aqueous phases allows fast mass

transfer rates [7] and emulsification is reached at lower agitation speed than in conventional solvent extraction systems. However, the low interfacial tension and low density differences between the two aqueous phases lead to intensive and stable emulsifications and therefore, to long phase separation times [8]. The phase separation time is a crucial parameter for ATPE equipment choice, design and performance as the time needed for complete phase separation determines the volume of the settler unit [9,10]. A newly developed approach to overcome the long settling times of classical ATPE is the so called 'Tunable Aqueous Polymer-Phase Impregnated Resins' (TAPPIR®)-Technology [11]. This approach eliminates the need of phase emulsification and separation by immobilizing one liquid phase inside porous solids prior to extraction. The phase contact is achieved by the suspension of the impregnated solids in the second liquid phase. The idea of the TAPPIR®-Technology is based on the solvent impregnated resins (SIR) invented by Warshawsky [12]. In contrast to SIR, which involves only organic/aqueous systems, the TAPPIR®-Technology is meant to be used with two aqueous phases to make the technique applicable to separate biotechnological products. Thus, organic solvents should be avoided because of possible denaturing effects. The used aqueous two-phase systems (ATPS) create a biocompatible environment for gentle extraction conditions. However, the physicochemical properties of ATPS pose additional challenges concerning the setup. While comparing

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the physicochemical properties of classical organic-aqueous phase systems used for SIR and ATPS used for TAPPIR[®], different emulsification and demixing behaviors are present. This might also result in different impregnation efficiencies and stabilities. A sufficient impregnation and the prevention of immobilized phase leaching during the process are essential to make the SIR- as well as the TAPPIR[®]-Technology competitive to other techniques and determine their applicability. Besides the impregnation, the selection of ATPS determines the partitioning of product and contaminants between the immobilized aqueous polymer phase and bulk aqueous salt phase. Additionally, the solids used to immobilize one of the aqueous phases can influence the impregnation stability, transport processes and separation efficiency in TAPPIR[®] due to their form, size and pore size distribution.

In this study, the application of the TAPPIR[®]-Technology as new extraction method is presented. A polyethylene glycol (PEG)/citrate ATPS was used and the composition of the phase forming compounds was fixed according to previously performed unpublished screening experiments. Additionally, different macroporous ceramic and glass particles were screened and the influence of the solid materials, pore and particle sizes were determined. The separation of the dye Patent blue V with the TAPPIR[®]-Technology is compared to classical ATPE and adsorption setups to show the potential of the TAPPIR[®]-Technology.

2. Materials and methods

2.1. Materials

ATPS are prepared with aqueous solutions of tri-sodium citrate dihydrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$, ≥ 99 wt.%), citric acid ($\text{C}_6\text{H}_8\text{O}_7$, ≥ 99 wt.%) from Carl Roth GmbH & Co. KG (Germany) and PEG with an average molecular weight of 4000 g/mol from Merck KGaA (Germany). Phase forming compounds are present in a mass fraction of 12.5 wt.%. The pH of the aqueous salt phase is set between 5.6 and 5.7. Water is received from a Milli-Q Synthesis apparatus with 0.22 μm Millipak express filters from EMD Millipore Corporation (USA).

The reactive dye Cibacron[®] black F-2B is purchased from Kremer Pigmente GmbH & Co. KG (Germany) and the non-reactive dye Patent blue V from Sigma Aldrich Co. LLC (Germany).

VitraPor glass pellets and glass filter plates in different porosities were purchased from ROBU Glasfilter-Geräte GmbH (Germany). The porous ceramic particles Stuttgarter Masse in different sizes was purchased from Pall GmbH (Germany).

2.2. Methods

2.2.1. Analytics

The concentrations of dyes are determined via absorption measurements using the wellplate reader infinite M200 Pro, Tecan Group Ltd. (Switzerland). For both dyes the absorption maximum is determined and the resulting wavelength is used for the measurements. The measurements are performed at least as triplicates.

2.2.2. Experimental procedure of the TAPPIR[®]-Technology

The procedure for single-stage batch mode experiments of the TAPPIR[®]-Technology consists of the steps schematically shown in Fig. 1. All experiments are performed on the rocking platform Rocky RT-MG/R (LTF Labortechnik, Germany) at 10 cycles/min to enhance the mass transport and to ensure a constant mixing for all experiments. Furthermore, all experiments are performed as triplicates in a climate chamber at 21–23 °C to increase reproducibility. The porous solids are washed with water and dried at 100 °C in a drying cabinet.

2.2.3. Impregnation evaluation

All impregnated solids are prepared according to Fig. 1. For all experiments, the reactive dye Cibacron[®] black F-2B (200 mg/L aqueous PEG phase) is added to the ATPS. The impregnation possibility and stability is evaluated. For a first qualitative evaluation of the impregnation possibility, it is checked whether the aqueous PEG phase can be soaked into the pores and stays inside the pores while the particles are surrounded by air. The next step is the investigation of the impregnation stability while suspending the impregnated particles in aqueous citrate phase. Visually it is controlled if the immobilized phase stays inside the pores or leaches. The experiments are carried out on a rocking platform Rocky RT-MG/R (LTF Labortechnik, Germany) at 10 cycles/min and 21–23 °C for 16 h. In case of leaching, droplets of dyed aqueous PEG phase rise to the surface of the aqueous salt phase due to the phase's lower density. As a last qualitative step of the impregnation determination the pore penetration of aqueous PEG phase within the cross-section of the particles is investigated. Therefore, the impregnated solids are mechanically destroyed and the inside of the solids visually analyzed for phase uptake. The solids, which are possible to impregnate are subsequently evaluated according to their handling conditions during preparation and impregnation as well as the solids' properties like form or size in regard to the experimental TAPPIR[®]-setup. With the selected solids the quantitative evaluation of the impregnation stability and the impregnation efficiency is performed.

The quantitative evaluation of the impregnation stability is determined via the distinct leaching of immobilized PEG phase. It is monitored via the volumetric leaching factor L_v , which is defined as the ratio of the leached (V_{leach}) to the initially impregnated (V_{imp}) aqueous PEG phase volume according to Eq. (1). In case of leaching, droplets of dyed aqueous PEG phase rise to the surface of the aqueous salt phase due to the phase's lower density. By shaking, an emulsion is created and via the concentration measurement of Cibacron[®] black F-2B, the volume of leached aqueous PEG phase is calculated.

$$L_v = \frac{V_{\text{leach}}}{V_{\text{imp}}} \times 100\% \quad (1)$$

The lower the volumetric leaching factor, the more stable is the impregnation of the immobilized aqueous PEG phase. For all porous solids the same procedure is carried out. Additionally, one set of impregnated solids is suspended in the bulk citrate phase and another set in water as negative control. In case of the negative control, the immobilized PEG phase and the bulk water do not form a biphasic system. Thus, the immobilized phase is washed out of the pores resulting in complete leaching. Both suspensions are mixed at 10 rpm for 16 h to achieve equilibrium. Samples from the bulk phases of both sets are taken and intensively mixed to achieve a fine emulsion of bulk phase and eventually leached impregnated phase. Samples are diluted with water to guarantee one-phasic samples. The concentration of dye in these samples is determined via absorption measurements. The dye concentration is then used to determine the volume of leached immobilized aqueous PEG phase, whereas the negative control determines the maximum volume leached. All experiments are performed on the rocking platform Rocky RT-MG/R (LTF Labortechnik, Germany) at 10 cycles/min to enhance the mass transport and to ensure a constant mixing for all experiments. Furthermore, all experiments are performed as triplicates in a climate chamber at 21–23 °C to increase reproducibility.

As the last step of the impregnation evaluation, the quantitative impregnation efficiency is analyzed by the ratio of initially impregnated phase volume (V_{imp}) and the total volume of the solid

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