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Review

Continuous aqueous two-phase systems devices for the recovery of biological products

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ABSTRACT

Aqueous two-phase systems (ATPS) have proved to be a suitable technique for the recovery of biological products. Although ATPS have been in the field of primary recovery and purification of products for several years, the majority of the studies exploiting ATPS are usually based on batch mode operation. Reports on the potential of using continuous ATPS are not common. This review attempts to present a practical analysis of selected devices employed for ATPS continuous processing, from the conventional column contactors to novel designed mixer-settler units. A critical analysis of operational and design parameters that impact the system performance is presented. Current trends on the implementation of continuous ATPS approaches are discussed, together with the major challenges faced for the generic adoption of the technique. Conclusions are drawn on the major contribution of previous studies in the field to provide a better understanding of the technique for the newcomers.

Keywords: Aqueous two-phase system; Continuous extraction; Column contactors; Mixer-settler units

Contents

Abbreviations: AMC, axial mixing coefficient; Bot, bottom; BSA, bovine serum albumin; CS, citrate salts; CTG, cashew nut tree gum; Cy-b5, cytochrome b5; FE, fractionation efficiency; H_D, hold up; H/D, height/diameter ratio; HPI, heavy phase inlet; HPO, heavy phase outlet; HRP, horseradish peroxidase; ID, internal diameter; K_Da, mass transfer coefficient; Kp, partition coefficient; LLE, liquid–liquid extraction; LPI, light phase inlet; LPO, light phase outlet; LYZ, lysozyme; MW, molecular weight; NA, not available; PEG, polyethylene glycol; PF, purification factor; PMMA, polymethylmethacrylate; PRDCs, perforated rotating disk contactors; PTFE, polytetrafluoroethylene; PVC, polyvinyl chloride; PS, phosphate salts; RE, recovery efficiency; SE, separation efficiency; SS, sulfate salts; TLL, tie line length; WPI, whey protein isolate.

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1. Introduction

It is known that chromatography is usually preferred in most purification processes. However, some process disadvantages, such as low binding capacity and resin costs (Rosa et al., 2011), raise the need for alternative techniques to complement the performance of chromatography-based downstream strategies. Among these alternatives, aqueous two-phase systems (ATPS), a liquid–liquid extraction (LLE) strategy, is now recognized as a potential technique because of its multiple advantages including: biologic compatibility, low interfacial tension, high load capacity and scale up easiness (Raghavarao et al., 2003).

Since its discovery (Beijerinck, 1896) ATPS have been exploited for the recovery of a wide variety of biomolecules – monoclonal antibodies (Azevedo et al., 2009; Rosa et al., 2010), proteins and enzymes (Liu et al., 2011; Platis and Labrou, 2006; Xu et al., 2003)), antibiotics (Bora et al., 2005; Khederlou et al., 2009; Mokhtarani et al., 2008), dyes (Benavides and Rito-Palomares, 2006; Mageste et al., 2009), low molecular weight compounds (Benavides et al., 2008; Willauer et al., 2002), DNA (Luechau et al., 2010; Mashayekhi et al., 2008), hormones (Haraguchi et al., 2004; Persson et al., 2005), cells (Edahiro et al., 2005; Yamada et al., 2004)) and membrane particles (Cao et al., 2006; Everberg et al., 2006), from different sources such as plant (Ibarra-Herrera et al., 2011; Aguilar and Rito-Palomares, 2010), animal (Boland, 2002), and insect (Benavides et al., 2006) cells as well as fermentation broths. Great part of this research has focused on finding the ATPS physicochemical characteristics (composition, viscosity, density, interfacial tension and pH) that better fits the intrinsic properties of the molecule of interest and matrix source (hydrophobicity, molecular weight (MW), isoelectric point) in order to obtain higher yields. Studies on phase separation (Cabezas, 1996) and partitioning phenomena (Huddleston et al., 1991), basic heuristic rules (Benavides and Rito-Palomares, 2008) for ATPS with other recuperation techniques (Aguilar et al., 2006) and pilot scale experiments (Kepka et al., 2003) have been performed. Most of them have been conducted in a batch mode and noteworthy knowledge has been achieved. However, the suitable characteristics of ATPS as a technology for continuous process have been usually overlooked.

In the biotechnology market, ATPS as a continuous or semi-continuous operation would have clear competitive advantages: diminishing process time and costs and increasing process yields (Igarashi et al., 2004a). Many opportunity areas of research within continuous ATPS operation are now present, such as bioaffinity partitioning (Ruiz-Ruiz et al., 2012), fractionation of pegylated proteins (Mayolo-Deloisa et al., 2010), the use of alternative ATPS (alcohol-salt, micellar, and ionic liquid based systems) to recover different biomolecules, the operational models for process optimization, phase recirculation, large scale operation, etc. In order to achieve these, it is important to validate the equipment that outstands for its versatility and thus can potentially be employed for all these purposes. There are few examples in literature of the use of continuous ATPS performing a validation of different devices for the recovery of a model protein starting form a previously selected batch ATPS.

This review describes the main devices employed for ATPS continuous processing, comparing their characteristics from the conventional column contactors to the novel designed mixer-settler units. A critical analysis of defined operational and design parameters that have a significant impact on the performance of equipment for continuous ATPS is presented. Current trends and challenges are discussed in order to present the areas of opportunity for the newcomers in the field.

2. Selected devices for continuous ATPS extraction

For practical purposes, devices employed for continuous ATPS processes have been classified into three main groups: column contactors, mixer-settler units and other contactors. Among the devices used to date, common column contactors are the most studied, probably due to their successful application in the chemical industry.

2.1. *Column contactors*

In the following sections the most common designs in this set of column contactors employed for continuous ATPS, are discussed: spray columns, perforated rotating disk contactors (PRDCs), pulsed cap columns and other columns (packed, sieve plate and vanes agitated columns). All of them consist of a hollow pillar with two inlets for each one of the phases involved and its corresponding outlets. The examples here discussed (Table 1) share common building materials: glass and polymethylmethacrylate (PMMA). The main variable among these columns is the mechanism by which the mass transfer between the phases is promoted (pulsed caps, rotating discs, rotating vanes, spray mechanism, static packing, and static mixer).