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Purification of biomolecules combining ATPS and membrane chromatography

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A B S T R A C T

Upstream processes for production of therapeutic proteins have been innovated and fermentation processes have been adopted for the use of recombinant microorganisms with high expression, but the downstream process is still the bottleneck in the biotechnological manufacturing process. A combined process consisting of aqueous two phase extraction (ATPE) and membrane chromatography is suggested to debottleneck downstream processing. ATPE has a large capacity, but the yield of the target product is from 74% to 97%. For this reason the product of ATPE waste stream is captured by membrane chromatography. In this work the binding capacity for the protein on protein A, ion exchange and hydrophobic exchange membrane chromatography was investigated experimentally with different concentration of polyethylene glycol (PEG), salt and protein. Protein A membrane was loaded with solutions resembling waste streams of ATPE for purifying IgG. For ion exchange and hydrophobic interaction membrane chromatography, the membrane was loaded with bovine serum albumin (BSA). PEG shows no significant effect on stability and capacity of membrane process. Even for small amount of BSA/IgG and high salt concentrations membrane adsorption is applicable. In this work it is demonstrated experimentally that a total product recovery of 99.9% for the purification of monoclonal antibody is possible.

Keywords: Membrane chromatography; Aqueous two-phase extraction; Downstream processing

1. Introduction

Recent developments in biotechnology enable large-scale manufacturing especially in upstream process where biotechnological goods are produced. The subsequent downstream processing, focusing on purification of target products, has become the bottleneck in terms of time consumption and costs because of low throughputs. In some cases downstream process represents up to 60% of the total manufacturing costs (Straathof, 2011). Consequently, it has become compulsory to advance existing methods and develop innovative new unit operations to cope with the high titre in upstream processes. Common method for the initial purification step of biomolecules is conventional chromatography where columns filled with beads are used. The majority of the active surface – area of the binding sites – is located in the inner pores, which are exclusively accessible by diffusive mass transfer. As a result, the mass transfer rate is relatively low,

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limiting the throughput and therefore the performance of the entire manufacturing process. By increasing the titre, larger chromatography columns are needed. Despite limitations of high expenses, batch operation, low throughput and complex scale-up, column chromatography is being so popular on the strength of high resolution in just one selective purification step for bioseparations. Several alternatives to chromatographic separations classified under bulk, field-based and adsorptive separation techniques have been reported aiming to increase the throughput and overcome the aforementioned drawbacks of column chromatography (Przybycien et al., 2004). Among the bulk separations, aqueous two-phase extraction (ATPE) has the potential for high throughput purifications. Even though the resolution is not comparable to column chromatography ATPE represents a high biocompatible and continuous operating purification process, which is easy to scale up (Albertsson, 1970, 1971; Sturesson et al., 1990). ATPE are based on aqueous two-phase system (ATPS) which can occur when two hydrophilic, but incompatible components are dissolved in water. Examples of such ATPS are the aqueous solution of two hydrophilic polymers (polyethylene glycol(PEG)/dextran) or a polymer (PEG) and a salt (e.g. phosphate salt) (Albertsson, 1971). Several polymer/polymer and polymer/salt ATPS have been reported in literature which have been successfully used to purify biomolecules out of a complex cell culture media (Asenjo and Andrews, 2011; Platis and Labrou, 2009; Andrews et al., 1996). A broad industrial application of ATPE has been hindered due to the high complexity and number of factors which often govern protein partitioning (Rosa et al., 2007a; Asenjo et al., 1994; Hubbuch and Kula, 2007). The first application of ATPS for mAb purification was reported by Andrews et al. (1990). In their extraction process they used a functionalized PEG. As this procedure is very cost intensive Andrews et al. presented a different approach in 1996 (Andrews et al., 1996). They purified murine IgG from hybridoma cells with ATPS consisting of PEG 1450, phosphate and water. Since that time, many investigations on mAb purification by aqueous two-phase extraction (ATPE) have been published, mainly applying PEG–phosphate ATPS for purification. In 2007 Rosa et al. (Rosa et al., 2007b) analyzed the purification of IgG from synthetic protein mixtures containing BSA and myoglobin by a PEG–phosphate ATPS. Various PEG molecular weights were investigated. The total extraction yield of native IgG was reported to be 76% with a purity of 99%. In multi-stage experiments, Rosa et al. (2009) reported a yield of 89% and a purity of 75%. Azevedo et al. (2007) extended the work of Rosa et al. and applied PEG–phosphate ATPS for the purification of IgG from synthetic media, Chinese Hamster Ovary (CHO) cell supernatant and hybridoma supernatant. At the given conditions, 88% of IgG from CHO cell supernatant were recovered with a purification factor of 4.3. A major drawback of phosphate-based ATPS is missing environmental sustainability. Therefore Azevedo et al. (2009a) investigated the applicability of PEG/citrate ATPS for the purification of IgG from hybridoma cell supernatant. The extraction step leads to an IgG recovery of 96% and a purity of 76%. Summing up these results show that the yield of mAb in extraction processes lies between 76% and 96%. But by a growing yield the purity decreases. For this reason, in this work a yield of ATPS of 90% is assumed. The goal of this work is to show that a purification of the waste stream is possible. But for the combination with the ATPE a process has to be chosen which allows a high throughput. Such a process is the membrane chromatography MC.

MC represents an interesting, alternative unit operation to conventional bead-based chromatography. Due to larger pore sizes and an open pore structure the binding sites can be accessed convectively. The presence of the ligands on the surface of the pore walls minimizes limitations in mass transport of the adsorbates from the mobile phase to the binding sites. Therefore, the productivity of membrane adsorbers, defined as the amount of protein adsorbed per unit of time and per unit of volume, is much higher than conventional resins (Gebauer et al., 1997). The main disadvantage of membrane adsorption remains the lower binding capacity in comparison with conventional resins and the price of the membrane adsorbers is still high compared to column chromatography (Thömmes and Etzel, 2007; Azevedo et al., 2009b). But in combination with a high-throughput technology as ATPE, the faster binding kinetics is the operative point. Virus clearance and endotoxin removal are the main application fields of MC and another rather important area is the purification of proteins and peptides from diluted solutions (Roper and Lightfoot, 1995). But MC was also used for the purification of fermentation broths (Puthirasigamany et al., 2013). The overall product yield and process capacity of a multistage ATPE can be increased if the product leaving the process with the waste stream (up to 24% of the valuable product) could be purified. Therefore the overall yield can be increased to almost 99.9% applying an approach combining the ATPE and MC. To combine these two processes, it has to be proofed, that the MC can handle the high salt concentrations and high viscosities caused by increasing PEG concentrations. For this reason this work focuses on the influence of this two parameters on adsorption in MC. As target molecules immunoglobulin G (IgG) and bovine serum albumin (BSA) are used; whereas both molecules are captured from a salt rich phase with protein A and phenyl membrane chromatography modules respectively. BSA in polymer rich phase was also isolated with quaternary ammonium membrane chromatography modules. This two-stage approach for the purification of monoclonal antibody employing an ATPE and protein A affinity membrane chromatography has already been successfully patented (Nikbin, 2012).

2. Materials and methods

2.1. *Materials*

In this study polyethylene glycols (PEG) obtained from Merck (Darmstadt, Germany) with molecular weight of 1000, 2000 and 3000 kDa was used. Dipotassium hydrogen phosphate trihydrate was purchased from AppliChem (Darmstadt, Germany). Sodium dihydrogen phosphate dihydrate and sodium chloride was obtained from ROTH (Karlsruhe, Germany). Bovine serum albumin (BSA) was obtained from Sigma–Aldrich. Polyclonal human IgG (Gammanorm®) was purchased from Octapharma AG. Gammanorm also contains IgG_3, which is not binding to protein A. The reagents were all of analytical grade and have been used without further purification. For membrane chromatography experiments three different types of the Sartobind membranes (Sartorius Stedim Biotech GmbH, Göttingen, Germany) were used: Sartobind phenyl nano 3 ml and Sartobind Q SingleStep nano 1 ml and Sartobind Protein A. The properties of the membranes can be found in Table 1. Expect for the affinity membrane, which is a flat sheet module, the membrane modules have a