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Possibilities to intensify and integrate aqueous two-phase extraction for IgG purification

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ABSTRACT

This work focuses on the integration and intensification of ATPE in the downstream process of an Immunoglobulin G1 (IgG1). For the integration a pre-concentrating step prior to Protein A chromatography is suggested. To achieve an IgG1 concentration an aqueous two-phase system (ATPS) consisting of polyethylene glycol 2000 (PEG), phosphate salt and water is applied for the extraction. Applying extreme phase ratios to pure cell supernatant and cell culture, enables for a concentration of IgG1 by factor nine within a single extraction step. Moreover, an almost complete recovery in the product phase was achieved with an IgG1 concentration of 5000 mg/kg in the product phase. A successful scale up by factor 100 shows excellent reproducibility. To intensify ATPE, it is important to improve the IgG1 loading capacity of ATPS. PEG – phosphate ATPS may cause high IgG1 loss because of precipitation. By applying a hyperbranched polyglycerol–phosphate ATPS, the IgG1 loading capacity of ATPS was increased 30-fold in comparison to the PEG–phosphate salt ATPS which showed initial precipitation of IgG1 below a loading of 50 mg/kg.

1. Introduction

Monoclonal antibodies (mAbs) are applied for the therapy of chronic diseases at high doses. Therefore, a need for large-scale production, short time to market and high cost efficiency exists [1,2]. For these purposes, platform processes are applied in industry. Due to the similarities in biochemical properties and adsorbance behavior of mAbs, general platform approaches were developed for mAb purification [2]. Advantages of platform processes include a reduced time to market and investment per mAb as well as reduced scale up and technology transfer complexities [3]. The conventional platform approach for the downstream processing of mAbs [3] mainly comprises an affinity capture step carried out by Protein A chromatography followed by a polishing step, usually two chromatographic steps, for removal of process- and product-related impurities. Although the Protein A chromatography step is highly effective due to its specificity (increase of mAb purity to >98%), it is often declared as the production bottleneck since increasing productivity and increasing titers in the upstream processing exceed the capacity of Protein A chromatography [3–5]. The high price of Protein A resin [6] as well as continuous perfusion technology for mAb production indicate needs to either replace or support the capture step.

As an alternative, aqueous two-phase extraction (ATPE) has been reported for the purification of mAbs and other biological compounds [7–16]. In this case aqueous two-phase systems (ATPS) are used for the extraction. Major advantage of ATPE is the possibility of a continuous application leading to a reduction of process time and cost [17] at increasing yield, selectivity and purity due to multiple partition steps [18–21].

In most cases, ATPE is suggested as the capture step to substitute Protein A chromatography. Multistage extraction approaches published in literature proved the potential of ATPE to successfully purify mAbs from cell supernatant [18,19,22,23]. The approaches combine a sodium chloride (NaCl)-rich extraction followed by a salt-poor back extraction. Finally, a washing step can be carried out to further improve the purity of mAb. Rosa et al. [24] and Eggersgluess et al. [18], both pointed out the competitiveness of an ATPE based downstream process in comparison to a Protein A chromatography based downstream process. Fig. 1 shows two possibilities to integrate ATPE into the platform downstream process of mAbs.

Although multistage ATPE is a promising alternative, only the two above mentioned publications [18,19] discussed the ability to compete with the performance of Protein A chromatography at present state of the art. High raw material costs, depending on the amount and type of phase forming components [25], solution preparation and control of multistage extraction are major drawbacks which need to be overcome. Further, the limited loading

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Nomenclature

Symbols

A	chromatogram area (−)
c	concentration (mg/kg)
CF	concentration factor (−)
K	partition coefficient (−)
m	mass (g)
P	purity (%)
PF	purification factor (−)
w	weight fraction (g/g)
Y	yield (%)

Greek letters

Φ	phase ratio (−)

Subscripts

BP	bottom phase
CS	cell supernatant
calc.	calculated
exp.	experimental
i	component i
IgG1	Immunoglobulin G1
initial	initial conditions
j	sample/phase j
NaCl	sodium chloride
PEG	polyethylene glycol
phosphate	phosphate
PP	product phase
total	total
TP	top phase

Abbreviations

ATPE	aqueous two-phase extraction
ATPS	aqueous two-phase system
BP	bottom phase
CC	cell culture
CHO	Chinese Hamster Ovary
CS	cell supernatant
HypPG	hyperbranched polyglycerol
IgG1	Immunoglobulin G1
mAb	monoclonal antibody
MP	mixing point
NaCl	sodium chloride
PEG	polyethylene glycol
phosphate	phosphate salt
PP	product phase
TP	top phase

capacity of ATPS to dissolve proteins, especially mAbs, implies a significant weakness. We, therefore, discuss two approaches of ATPE to surpass the drawbacks of multistage ATPE and to overcome the limited loading capacity of conventional ATPS. Fig. 1 shows two possibilities of ATPE integration into the platform downstream process of mAbs.

The first approach describes the integration of ATPE as a preconcentrating and cell removal step prior to Protein A chromatography. ATPS are known to offer the opportunity of concentrating proteins within a single extraction step [26]. A reduction of process streams in the subsequent downstream process is the consequence. Cells and cell debris aggregate at the interface [13] and can be removed from the mAb-rich product phase. High purity is still ensured by Protein A chromatography in that case.

Several publications in literature show, that a reduction of the product-rich phase volume has a positive effect on the concentration of proteins and besides that, on their purification from impurities [10,26,27]. A positive effect of decreasing product phase volume can only be achieved for extreme IgG1 partitioning, which is strongly depending on the amount of added NaCl weight fraction. The main drawback remains the solubility of mAbs at high concentrations. If the solubility of IgG1 can be increased this could be an interesting application.

The second approach deals with the intensification of ATPE to improve the IgG1 loading capacity, which describes the potential of ATPS to dissolve IgG1 without yield loss. Precipitation of proteins, especially mAbs, has been reported as a major drawback of ATPS [10,28,29]. This leads on the one hand to significant yield losses of the mAb, while on the other hand the loading capacity of ATPS is limited, requiring a high amount of ATPS to dissolve small amounts of cell supernatant. Furthermore, low concentrations of phase forming components imply a trade-off between mAb solubility and ATPS stability, since operations have to be accomplished close to the critical point.

A modification of ATPS is therefore necessary to improve protein solubility and necessary to promote the integration of ATPE

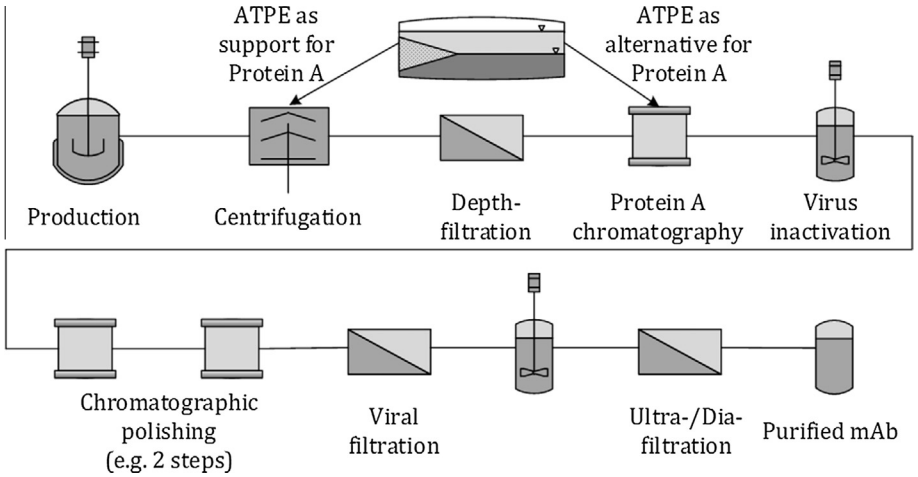

Fig. 1. Possible integration of ATPE into the conventional platform approach for the downstream processing of mAbs.