**Optimization of batch aqueous two-phase extraction and centrifugal partition chromatography in purification of monoclonal antibodies**

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**Highlights**

* Aqueous Two-Phase extraction (ATPE) was applied for purification of a monoclonalantibody.
* ATPS containing PEG and ammonium sulfate was used.
* The efficiency of a batch single-stage and multi-stage ATPE (CPC) was compared.

**1. Introduction**

The most expensive step in downstream processing of monoclonal antibodies is protein A chromatography. Therefore, to reduce the cost of the chromatographic step, an alternative prepurification method can be used. In this work, a method based on coupling aqueous two-phase extraction (ATPE) and centrifugal partition chromatography (CPC), which is a multistage-extraction process, was developed. The subject of the study was a CHO cell-culture supernatant (Harvest), which contained a monoclonal antibody IgG1. ATPE was used for clarification, concentration and capture of the target product and partial separation of impurities in one or two stages.

Alternatively, CPC, comprising several hundreds of extraction stages, was applied. The bottom phase (BP-1) produced in the first ATPE-stage (Fig. 1) was further processed in a CPC system (Fig. 2). Fractions collected from the ATPE (BP-1 and BP-2) and CPC (F-CPC) stages were analyzed by protein A chromatography (AC) and size exclusion chromatography (SEC) for the antibody concentration and purity.

An optimization of the process was performed subject to purity of the target product, the residence time (or flow rate of the mobile phase) and possibility of recycling of the ATPS-phases.

**2. Methods**

ATPE and CPC were performed in systems containing polyethylene glycol (PEG, 3350 Da) and ammonium sulfate dissolved in water. The CPC mobile phase was the bottom phase from ATPE, whereas the CPC stationary phase was the ATPE-upper phase, the rotor speed was 1800 rpm, the rotor volume was 100 mL.

The *SEC purity* was calculated as the ratio of the peak area recorded for the IgG monomer and the sum of the area of IgG peak and peaks of all impurities.

The *level of impurities* in the factions was determined from the AC analysis as the area of the flow-through peak (FT) divided by the area of the FT-peak received for BP-1.



**Figure 1**. Single-stage batch extraction for antibody capture and precipitation of impurities



**Figure 2**. Example of a chromatogram from CPC purification of the ATPE-bottom phase BP-1. F-CPC – fraction of the target protein

**3. Results and discussion**

Impurities with low solubility were precipitated and removed in the first ATPE-stage, in which extraction was accompanied with precipitation of impurities (Fig.1). The bottom phase (BP-1), contained the target product (IgG1), and a high amount of impurities. That amount was reduced by ca. 40% after purification in the second ATPE-stage (see BP-2 in Table 1). Processing of BP-1 by CPC allowed us to further reduce the level of impurities by more than 80%. The fractions of the target product collected after two ATPE-stages and after CPC were analyzed by SEC. They contained low molecular weight impurities and very low amount of IgG-aggregates (<1%).

The CPC stationary and mobile phases (without the target product) were reused in the first ATPE-stage preceding CPC. In case of batch ATPE-extraction stages only the upper phase (e.g. BP-2) was recycled. The recycling conditions were optimized to reduce the solvent consumption.

**Table 1**. Purity of the target product fractions. The level of impurities was referred to BP-1 (assigned as 100%), BP-2 is the bottom phase fraction after the second ATPE-stage, F-CPC is the fraction collected after CPC.

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| --- | --- | --- | --- |
| **Fractions** | **BP-1** | **BP-2** | **F-CPC** |
| **Level**  **of impurities** | 100% | 58% | 12% |
| **SEC purity** | 29% | 42% | 78% |
| **IgG recovery** | 99% | 99% | 82% |

**4. Conclusions**

* High recovery of the antibody was achieved due to selective extraction of the target product into the ATPE-bottom phase
* Significant reduction of impurities in CPC step was observed in comparison to two-stage ATPE.
* Recycling of phases in both ATPE and CPC operations allows reducing solvent consumption and the total cost of the operation.
* ATPE and CPC fractions can be further directly processed by HIC process due to high kosmotropic salt concentration in post-extraction solutions. Such sequential operations have a potential to substitute protein A chromatography.

We would like to acknowledge Polpharma Biologics for providing the biological material.