Optimization of an Extraction System for Purification of Biomolecules

Applying (additional) Excipients as Tool for improving Biomolecule Stability

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The production of high-value biomolecules such as biopharmaceuticals has seen a dramatic increase in (industrial) attention within the last decades. One promising initial purification step is the aqueous two-phase extraction (ATPE) using an aqueous two-phase system (ATPS). However, phase-formers that are used to generate the ATPS often reduce the conformational and colloidal stability of the biomolecule. Within this work, we overcome this drawback by using excipient, selected based on methods derived from (bio)pharmaceutical formulation development. It is shown, that selecting L-arginine as excipient for the ATPE of Immunoglobulin G (IgG), the IgG precipitation is significantly reduced (25 wt% to 1.2 wt% [wt/wt]), whilst simultaneously increasing the extraction yield to 90 wt%.

Process development for high-value biomolecules has mainly focused on the upstream rather than the downstream processing. This leads to the fact that the specific costs for the downstream processing can account for up to 80 % of the total production costs. With up to 90 % of the costs for the downstream processing, chromatographic steps are the main cost drivers. An alternative, cost-efficient purification technology, offering different operation modes (batch or continuous) is the ATPE using an ATPS.

To rapidly select a suitable ATPS for a target biomolecule, a physical sound approach based on investigations on the ATPS phase behavior and the conformational (unfolding temperature) and colloidal stability (second osmotic virial coefficient B_{22}) of the biomolecule has been developed within this work [1]. The approach identified an optimized ATPS for the ATPE of IgG using the phase formers sodium glutamate (NaGlu) and polyethylene glycol 2000 (PEG2000). However, as shown in Figure 1a, the colloidal stability is still decreased (negative B_{22}-values), resulting in an IgG precipitation of 25.2 wt% within ATPS 1 (Figure 1b) for high phase former concentrations. Under knowledge of the phase equilibria, optimization of the process window by decreasing phase former concentration enables a decrease in IgG precipitation by 22.1 wt% (to 3.1 wt% [ATPS 2]). To further increase the ATPS extraction performance, excipients such as amino acids or sugars were investigated applying concepts from (bio)pharmaceutical formulation development recently [2]. As presented in Figure 1a, the addition of L-arginine (L-Arg) as excipient can drastically increase the colloidal stability of IgG (increasing B_{22}-values), and compensate the negative effect of the phase formers applied. Due to the improved stability of IgG, the yield was further increased to 90 wt%, simultaneously decreasing the precipitation to 1.2 wt%. Prospectively, the optimization of ATPS using suitable excipients will support the integration of ATPE in downstream processing of biomolecules.

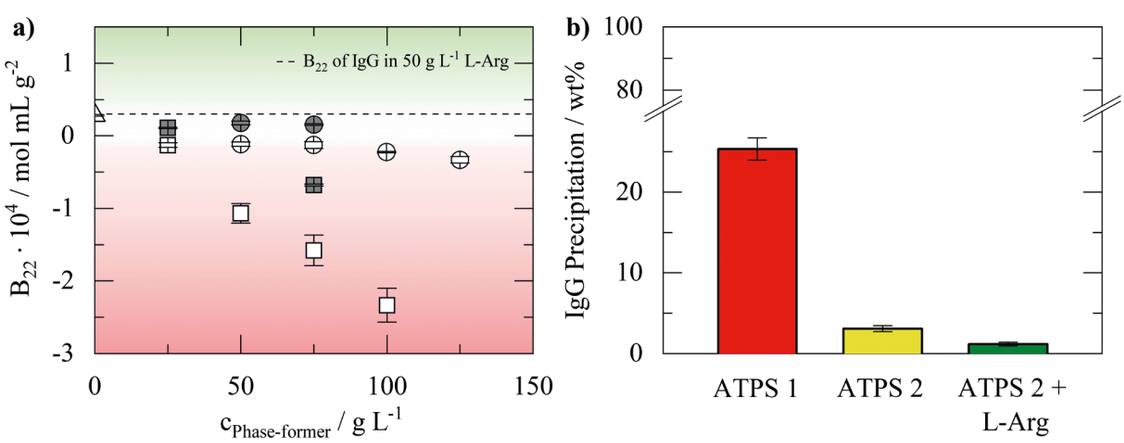

Figure 1: a) B_{22}-values of IgG in presence of: (1) L-Arg in 50 mM PBS (white triangle at 0 g L^{-1} and dashed line). (2) An increasing concentration of one phase former in 50 mM PBS (open symbols: squares = PEG2000, circles = NaGlu). (3) 50 g L^{-1} L-Arg and one phase-former in 50 mM PBS (filled, gray symbols). b) Precipitation of IgG (Feed = 0.02 wt%) after ATPE using different ATPS (ATPS 1: 16.5 wt% NaGlu, 16.5 wt% PEG2000 and 8 wt% NaCl; ATPS 2: 15 wt% NaGlu, 15 wt% PEG2000 and 8 wt% NaCl; ATPS 2 + L-Arg: 15 wt% NaGlu, 15 wt% PEG2000, 8 wt% NaCl and 5 wt% L-Arg). All measurements were performed at 298.15 K, 1 bar and pH 7. Error bars show the standard deviation resulting from a duplicate measurement.

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Publications:
[1] M. Wessner, M. Nowaczyk, C. Brandenbusch, J. Mol. Liq. 314, 113655 (2020).
[2] M. Schleinitz, L. Nolte, C. Brandenbusch, J. Mol. Liq. 298, 112011 (2020).