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Clarification of a monoclonal antibody with cationic polyelectrolytes: Analysis of influencing parameters

Julia Sieberz, Elif Cinar, Kerstin Wohlgemuth*, Gerhard Schembecker

Technische Universität Dortmund, Laboratory of Plant and Process Design, Dortmund, Germany

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ABSTRACT

Precipitation with polyelectrolytes is a promising alternative to conventional methods for purification of monoclonal antibodies (mAb). This study focuses on clarification of a monoclonal antibody by precipitation of the model impurity bovine serum albumin (BSA) with cationic polyelectrolytes. Different types of cationic polyelectrolytes with different functional amine groups and molecular weights are screened for their capability to precipitate BSA in mixture with mAb. Due to the high BSA depletion and mAb recovery achieved, two cationic polyelectrolytes, polyethyleneimine (PEI) and polyallylamine (PAA) with a molecular weight of 65 kDa were selected as most suitable precipitation agents. Additionally, influences of different parameters (polyelectrolyte, BSA and NaCl concentration and pH value) were analyzed by the use of design of experiments (DoE). Herewith, a fast identification of influencing factors and factor interaction was achieved. With DoE, two regression models for the analysis of BSA depletion in dependence of influencing factors were established. This enabled the analysis of the robustness of precipitation as initial clarification step of downstream process of mAb.

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1. Introduction

Since 1986, when the first monoclonal antibody (mAb) was introduced to the market, research on the development of mAbs for medical and biotechnological application is increasing continuously. As the upstream process has been improved successfully by innovative expression systems and optimized fermentation conditions, downstream process (DSP) still remains the bottleneck of the production process.

MAb purification processes typically consist of a series of chromatographic steps like protein A chromatography as well as anion and cation exchange chromatography [1]. Chromatographic steps show some limitations, like a limited throughput and high costs [2]. Consequently, new, efficient and gentler purification methods are in focus of research [3–6]. Precipitation is gaining increasing interest because of advantages like simple handling, inexpensive technical realization and low additive consumption.

Separation of a monoclonal antibody and impurity proteins by polyelectrolytes can be realized in two different modes. One approach, called capture, is the direct precipitation of the antibody by anionic polyelectrolytes while the impurity proteins stay in supernatant [7–9]. The second approach is the precipitation of impurity proteins with cationic polyelectrolytes (cPEs), leaving the antibody in supernatant [10,11]. This mode is called clarification. Polyelectrolytes consist of macromolecules which cover primarily ionic or ionizable groups [12]. In polar solvents, polyelectrolytes dissociate to a poly-charged backbone and a corresponding number of low molecular counter ions [13]. Their structure strongly depends on different factors, like polyelectrolyte concentration, interaction with the surrounding solvent, chain length of the polymer, strength of the ionizable group and type of counter ion. In case of weak polyelectrolytes their degree of ionization and therefore their structure depends on the pH value. While many research studies focus on polyelectrolytes, their behavior is not completely understood yet [14].

Polyelectrolytes can interact with oppositely charged groups on protein surfaces, forming complexes. The consequence of such interactions is the formation of soluble complexes, coacervates (formation of two liquid phases), or amorphous precipitates [15,16]. The solid precipitate can be separated from supernatant by centrifugation or filtration [17]. In case of clarification, the separated precipitate is withdrawn and the supernatant can be purified further. Due to the small quantity of polyelectrolytes needed for precipitation, it offers a very cost efficient method for the purification of proteins and mAbs especially [15,18–25].

* Corresponding author.
 E-mail address: kerstin.wohlgemuth@bci.tu-dortmund.de (K. Wohlgemuth).

Nomenclature

Variables and parameters

k	number of factors investigated
D	depletion
x	factor level
α	star point factor level

Indices

i	component i
PM	precipitation mixture
RP	re-dissolved precipitate
med MW	medium molecular weight
low MW	low molecular weight
S	supernatant

Abbreviations

BSA	bovine serum albumin
Chi	chitosan
CHO	Chinese hamster ovary
cPE	cationic polyelectrolyte
DNA	deoxyribonucleic acid
DoE	design of experiments
DSP	downstream process
HCP	host cell proteins
HPLC	high performance liquid chromatography
mAb	monoclonal antibody
MW	molecular weight
PAA 17	polyallylamine of 17 kDa
PAA 65	polyallylamine of 65 kDa
PAACo	poly(acrylamide-*co*-diallyldimethylammonium chloride)
PDADMA	poly(diallyldimethylammonium chloride)
PE	polyelectrolyte
PEI	polyethyleneimine
pI	isoelectric point
PMACo	poly(dimethylamine-*co*-epichlorohydrin-*co*-ethylenediamine)

Several publications about precipitation and coacervation of proteins with cPEs have been published. In most cases, precipitation or coacervation behavior of pure proteins has been analyzed [26–28]. The focus of most of these studies was the analysis of molecular interaction between the polyelectrolyte and the proteins, and not any separation effect. Some authors (e.g. [11,29–31]) analyzed the influence of different factors on the separation of protein mixtures. The research group around Fahrener presented a most comprehensive overview of antibody purification with different cPEs in a series of two papers [10,11]. Precipitation conditions (pH value, PE concentration, molecular weight (MW) and conductivity) were selected by varying one factor at a time.

This study aims to achieve a structured procedure for the analysis of the purification yield and selectivity of a monoclonal antibody by cationic polyelectrolytes. Within cationic polyelectrolyte precipitation, high yield is represented by small depletion of the antibody from supernatant and high selectivity by high depletion of the impurity and simultaneously small depletion of mAb. For this purpose, in a first step, different cPEs were screened for their capability to form insoluble complexes with an impurity protein, leaving the monoclonal antibody in supernatant. These polyelectrolytes differ in their molecular weights and functional groups, which might influence the interaction with proteins and the precipitation behavior. Bovine serum albumin (BSA) is used as model impurity. Its properties are well known and it is added as growth factor to Chi-

nese hamster ovary (CHO) cell cultures [32] making it a realistic impurity in mAb purification. Additionally, its isoelectric point and molecular weight are in the typical area of CHO cell culture impurities [33]. On basis of these screening experiments the most suitable polyelectrolytes for antibody purification were selected. In a further step, the influence of different factors and factor interactions were analyzed by design of experiments (DoE). In our previous study, we have found that these factor interactions should not be neglected to gain a deeper understanding on the complex formation and to achieve a fast and efficient optimization of influencing parameters [34]. Design of experiments is a suitable tool to reduce the number of experiments and to determine impact factors as well as factor interactions. This enables a fast optimization of the process conditions in downstream process development [35]. DoE has been applied successfully e.g. for the optimization of aqueous two-phase extraction of antibodies [36,37].

2. Materials and methods

2.1. Materials

Table 1 gives an overview of cPEs used in this study. Five commercially available PEs, poly(diallyldimethylammonium chloride) (PDADMA, M_W < 100 kDa, Sigma–Aldrich, 35 wt.% in water), polyallylamine of two different molecular weights (PAA 17 and PAA 65, M_W 17 kDa and 65 kDa, Sigma–Aldrich, 20 and 10 wt.% in water) polyethyleneimine (PEI, M_W 750 kDa, Sigma–Aldrich, 50 wt.% in water), poly(dimethylamine-*co*-epichlorohydrin-*co*-ethylenediamine) (PMACo, M_W 75 kDa, Sigma–Aldrich) and chitosan of low and medium molecular weight (Chi$_{low\ MW}$ and Chi$_{med\ MW}$, solid powder, Sigma–Aldrich) were selected. All these PEs have already been mentioned in literature. The proteins tested in literature are listed in Table 1. Furthermore, one polyelectrolyte poly(acrylamide-*co*-diallyldimethylammonium chloride) (PAACo, M_W 250 kDa, Sigma–Aldrich) was used, which has not been studied as precipitation agent in literature, until now. This polyelectrolyte was selected, as it contains two different kinds of amine groups. This might lead to a broader pH range over which this polyelectrolyte is charged and forms complexes. All polyelectrolytes were used without further purification.

The monoclonal antibody was kindly provided by Merck Millipore. It has a molecular weight of approximately 145 kDa and an isoelectric point (pI) of 8.5. BSA with a molecular weight of 66 kDa was obtained from Carl Roth. Sodium chloride (NaCl) ($\geq$99.8%) was obtained from VWR, sodium formate ($\geq$99.0%) from Sigma–Aldrich, and hydrochloric acid (HCl) from Applichem (37% HCl with water, high-purity ($\geq$99.9%)). Water, used for experiments or analytics, was ultra-filtered by a Milli-Q Water purification system (Millipore, 0.05 µS).

2.2. Precipitation procedure for PE screening

Stock solutions of mAb, BSA, and different polyelectrolytes were prepared to achieve the desired concentrations in the precipitation mixture (PM). All protein and polyelectrolyte stock solutions, despite chitosan, were adjusted to the starting pH value of 4.0 with 1.0 and 0.1 M HCl and were filled up with water to a defined volume to achieve the desired concentration in the PM. Due to its low solubility, chitosan was dissolved in a solution of 0.1 M acetic acid. Stock solutions of polyelectrolytes and proteins were mixed in a beaker. Stock solutions were prepared in such a way that after mixing them a total volume of 100 mL and the concentration desired of each component was achieved. The pH value of PM was controlled and adjusted to pH 4.0, where necessary. Experiments were performed with starting concentrations of BSA