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Optimization of a preparative multimodal ion exchange step for purification of a potential malaria vaccine

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ABSTRACT

In 2000 the implementation of quality by design (QbD) was introduced by the Food and Drug Administration (FDA) and described in the ICH Q8, Q9 and Q10 guidelines. Since that time, systematic optimization strategies for purification of biopharmaceuticals have gained a more important role in industrial process development. In this investigation, the optimization strategy was carried out by adopting design of experiments (DoE) in small scale experiments. A combination method comprising a desalting and a multimodal ion exchange step was used for the experimental runs via the chromatographic system ÄKTA™ avant. The multimodal resin Capto™ adhere was investigated as an alternative to conventional ion exchange and hydrophobic interaction resins for the intermediate purification of the potential malaria vaccine D1M1. The ligands, used in multimodal chromatography, interact with the target molecule in different ways. The multimodal functionality includes the binding of proteins in spite of the ionic strength of the loading material. The target protein binds at specific salt conditions and can be eluted by a step gradient decreasing the pH value and reducing the ionic strength. It is possible to achieve a maximized purity and recovery of the product because degradation products and other contaminants do not bind at specific salt concentrations at which the product still binds to the ligands.

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1. Introduction

The optimization of manufacturing processes for biopharmaceutical products has increased in importance since the PAT/QbD initiative started in 2004. This approach focuses on the quality of the product being produced and thus on the safety of the patient [1,2]. The QbD approach should lead to an improved product and manufacturing understanding in the research and development of new drugs with minimal time and resource effort [3,4]. As a tool to investigate possible influences on the critical quality attributes (CQAs) of the product and thus to establish a process design space, design of experiments (DoE) can be used optimally. Through multivariate analysis, linear and quadratic relationships, as well as factor interactions, can be identified and quantified [5–7].

The multimodal chromatography is characterized by different interactions of the target protein with ligands of the stationary phase. The combination of electrostatic and hydrophobic interactions enables the adsorption of the target protein over a wide range of ionic strength [8,9]. Johansson et al. [10,11] showed that a hydroxyl group, located in the vicinity of the ionic group, has a positive effect on the binding of proteins at high salt concentrations. Typically, multimodal resins are used for the capture of antibodies [12,13] and proteins [10,11,14].

In this investigation, the multimodal resin Capto™ adhere was investigated as an alternative to conventional ion exchange and hydrophobic interaction resins for an intermediate purification of a potential malaria vaccine. The used sorbents Capto™ adhere [15,16] is a strong anion exchanger coupled with a phenyl group for hydrophobic interactions and a hydroxyl group for hydrogen bonding. The nature of the interaction depends on the specific characteristics of the target protein and the set conditions of the mobile phase during the chromatographic separation.

In this work, the chromatographic purification of the malaria vaccine candidate D1M1 [17–20] was developed and optimized in small scale experiments. The target protein was produced in an integrated production process described by Martens et al. [21]. The aim of the work presented is to develop an intermediate purification step after an ultrafiltration/diafiltration in the preparative integrated bioprocess.

For the optimization procedure, the possible influencing factors [22,23], binding pH value pH_{bC} and sodium chloride concentration C_{NaClbC}, pH value for elution pH_{e2C} and the volumetric flow rates for sample loading F_{VSaC} and for the equilibration, wash and elution steps F_{VSyC} were analyzed in a screening. This was done in order to

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Table 1
Antibodies used for immunological characterization of the target protein D1M1.

Epitope	Antibody	Origin	Reference
AMA1	Monoclonal 4G2dc1	Rat	[10]
$MSP1_{19}$	Monoclonal 12.10	Mouse	[11]
Prodomain	Monoclonal 58F8	Rat	[12]

identify these factors influences on purity P_{P1e2C} and recovery R_{P1C} of the target product. In addition, the appropriate binding mode of the multimodal chromatographic sorbents Capto™ adhere, either ion exchange or hydrophobic interaction, could be identified for the purification of D1M1. The influence of the critical process parameters (CPPs) resulting from the screening via fractional factorial design was quantified in the optimization phase using a central composite circumscribed (CCC) design.

2. Materials and methods

2.1. Biological material and target protein

The biological material that was used was produced by an integrated manufacturing process consisting of a sequential cultivation of *Pichia pastoris* strain KM71H phenotype Mut^S and a subsequent capture via Expanded Bed Adsorption EBA Immobilized Metal chelate Affinity Chromatography (IMAC) (STREAMLINE 50 column and STREAMLINE Chelating media, GE Healthcare, Uppsala, Sweden) described by Martens et al. [21].

The protein of interest D1M1 is a fusion protein of *merozoites surface protein1* MSP1 and *apical membrane antigen1* PfAMA1 described by Faber et al. [17,18]. D1M1 consists of 619 amino acids with a molecular weight of 71.5 kDa and a *pI* of 5.2. In addition, the protein contains two disulfide bonds that are involved in the formation of specific antibody epitopes.

2.2. Analytical methods

The quantification of the total protein concentration was performed using Bradford kit (Bio-Rad Laboratories, Munich, Germany).

The identification of the target protein D1M1 was carried out by a non-reducing SDS-PAGE (Criterion™ TGX ™ Precast Gel, 4–15%, Bio-Rad Laboratories, Munich, Germany) analysis. As a protein marker a Page Ruler Unstained (Thermo Scientific, Karlsruhe, Germany) was used. After Coomassie staining, densitometric analysis using TotalLab Quant Software (Totallab, Newcastle upon Tyne, United Kingdom) was performed.

The antigenicity and the unique identification of immunologically active target protein were detected with Western blot analysis. The proteins of the starting solution were separated using size exclusion chromatography (SEC) via Superdex 75 prep grade (GE Healthcare, Uppsala, Sweden) prior to Western blot analysis. The individual protein fractions were separated on a 12% SDS gel. The antibodies that were used are shown in Table 1. For detecting the first antibody alkaline phosphatase (AP)-conjugated secondary antibodies (antirat or antimouse) were used. By adding the substrate BCIP (5-bromo-4-chloro-3-indolylphosphate)-nitroblue tetrazolium (NBT) insoluble NBT diformazan is formed, which results in a visible purple to blue staining on the blot (BCIP®/NBT solution, premixed, Sigma–Aldrich, Munich, Germany).

2.3. Quality criteria for the purification step

To evaluate the chromatographic purification, the quality criteria purity of immunologically active target protein D1M1 in eluate fraction e2 (Fig. 3) P_{P1e2C} and the recovery of the product R_{P1C} were defined.

The purity, P_{P1kC},

$$P_{P1kC} = \frac{B_{P1kC}}{\sum_{i=1}^{n} B_{ikC}}, \quad (1)$$

of the respective chromatographic step k was determined relative to the total band volume from the target protein band volume B_{P1kC}.

The recovery, R_{P1C},

$$R_{P1C} = \frac{m_{P1e2C}}{m_{P1e1C}} = \frac{c_{Ptote2C} \cdot P_{P1e2C} \cdot V_{e2C}}{c_{Ptote1C} \cdot P_{P1e1C} \cdot V_{e1C}}, \quad (2)$$

results from the ratio of the product mass in the eluate fraction e2 of the ion exchange chromatography m_{P1e2C} to the mass of the intact target product in the eluate fraction e1 (Fig. 3) of the desalting step m_{P1e1C}. The respective masses m_{P1kC} were determined by calculating the total protein concentration by Bradford protein assay c_{PtotkC} (Bio-Rad Laboratories, Munich, Germany) multiplied with the analyzed purity P_{P1kC} and the volume of each V_{kC} eluate fractions e1 and e2.

2.4. Chromatographic materials and setup

A chromatographic system ÄKTA™ avant 150 (GE Healthcare, Uppsala, Sweden) was used. The automation was done with the software Unicorn™ 6.1 (GE Healthcare, Uppsala, Sweden).

Fig. 1 shows the flow diagram of the chromatographic method that was used. In the first step the initial sample Sa (20 ml) was desalted with a HiScale 26/20 column using Sephadex G-25 (CV 107 ml) in the specific binding conditions BBi for multimodal ion exchange chromatography (Capto™ adhere, GE Healthcare, Uppsala, Sweden). The protein fraction (27 ml) of the desalting steps e1 was fractionated through the out valve into a 50 ml centrifuge tube and cooled to 10 °C in the transfer storage. Via the sample inlet valve 22 ml of protein fraction were purified with a Tricorn 10/100 column by Capto™ adhere (CV 9.5 ml).

The binding buffer BB for multimodal ion exchange was a 50 mM Tris buffer (Roth, Karlsruhe, Germany). The pH value was adjusted, using 25% hydrochloric acid (Roth, Karlsruhe, Germany) or 1 M sodium hydroxide solution (Roth, Karlsruhe, Germany). In addition, sodium chloride (Roth, Karlsruhe, Germany) was added. The pH value of the elution buffer BE (100 mM sodium acetate buffer (Roth, Karlsruhe, Germany)) was adjusted with 25% acetic acid (Roth, Karlsruhe, Germany), or 1 M sodium hydroxide solution (Roth, Karlsruhe, Germany).

2.5. The DoE based systematic optimization procedure

The optimization strategy that was used is based on the concept of simultaneous variation of possible influencing factors x_i on the quality criteria y_i of the system considered. Through this approach, a relatively high amount of knowledge is gained compared to the conventional methods of optimization [5]. Based on individual measurement outcomes and application of multiple linear regression – MLR method, the factor(s)-response relationship $y_i = f(x_i)$ was determined in terms of a mathematical model. This model enables computation of numerical values of system responses for an arbitrary combination of factors and thus to predict the outcomes of future experiments [6].

Possible influencing factors were defined on the quality criteria P_{P1e2C} purity and recovery R_{P1C} to optimize the chromatographic process. The ligand N-benzyl-N-methyl ethanolamine of the multimodal ion exchanger Capto™ adhere (GE Healthcare, Uppsala, Sweden) has hydrophobic and ionic interaction properties [15]. For

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