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Ultrafiltration concentration of monoclonal antibody solutions: Development of an optimized method minimizing aggregation

E. Rosenberg[a,*], S. Hepbildikler[a], W. Kuhne[a], G. Winter[b]

[a] Roche Diagnostics GmbH, Biotech Production and Development, Penzberg, Germany
[b] Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics, Ludwig-Maximilians-University, Munich, Germany

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ABSTRACT

For the production of highly concentrated monoclonal antibody intermediate solutions in the course of downstream processing, ultra filtration is the industry standard in manufacturing scale. Key challenges are to achieve high end concentrations and to reduce both process time and aggregate formation, particularly for therapeutic proteins, intended for subcutaneous administration. Therefore, in this study the effects of operational parameters on permeate flux and aggregation in the concentrated solutions were investigated. An optimized ultrafiltration concentration method was developed based on systematic screening of cross-flow rate and transmembrane pressure at different protein concentrations in the retentate. In this method flow and pressure values are adjusted depending on the prevailing retentate concentration. The resulting three stage protocol reduces process time and assures a low aggregate burden compared to concentration processes operated at constant flow and pressure conditions. Flow and pressure profiles were set and recorded in lab scale using an automated tangential flow filtration system which has been shown to adequately reflect process conditions at manufacturing scale. The formation of IgG aggregates was monitored by turbidity measurement, SE-HPLC, light obscuration, dynamic light scattering and a microscopic method. These techniques allow characterization of a wide range of aggregate sizes of soluble and insoluble aggregates that can occur during processing. In addition, FT-IR spectroscopy was performed to investigate secondary structure of the aggregates, revealing different quantities of structurally perturbed protein depending on applied flow and pressure conditions. Finally, the concentrated material derived from the optimized method showed improved processability at sterile filtration which is an important manufacturing step prior to storage.

1. Introduction

There is an unbroken demand for highly concentrated low volume formulations of monoclonal antibodies (mAb) for subcutaneous administration, especially in the field of chronic disease therapy, to improve patient convenience and compliance by offering outpatient treatment [1,2]. Not only these high dose/low volume dosage formulations, but also concentrated intermediate solutions of different purification steps are highly desired in the course of downstream processing (DSP). Low volume batches allow profitable handling, economic storage and increase flexibility of hard-piped GMP manufacturing suites with fixed vessel volumes. To produce highly concentrated mAb solutions in large scale, reliable ultrafiltration (UF) processes are required. The challenge is to achieve high end-concentration, short process time and high quality of the active pharmaceutical ingredient (API) at the same time [1,3].

Through out manufacturing of biopharmaceutics including cell culture, purification, filling and storage, proteins are prone to chemical and physical instability [4]. Protein exposition to air–water interfaces, adsorption to hydrophobic or hydrophilic surfaces or mechanical stress may facilitate aggregation and precipitation [5,6]. Shaking is known to create hydrophobic interfaces where protein accumulates, exposes hydrophobic amino acid residues and initiate aggregation to minimize thermodynamically unfavorable interactions between water and hydrophobic protein patches [7,8]. Only a small expansion of the protein surface in the native state rather than a complete unfolding seems to be necessary to trigger aggregation [9,10] and these structurally altered molecules are considered as precursors for aggregation [11].

Hence, every step of the manufacturing process has to be evaluated for the potential formation of protein aggregates and aggregate precursors in order to diminish the risk of adverse effects in patients [12] or loss in efficacy [13] and to increase API stability during storage.

During UF the protein faces a combination of physical stress principles like pumping, flow induced shear stress or extensive contact to membrane surfaces. In addition, the protein reaches

* Corresponding author. Tel.: +49 8856 605849; fax: +49 8856 6018920.
E-mail address: eva.rosenberg@roche.com (E. Rosenberg).

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extremely high concentrations [14] in the gel and polarization boundary layer near the membrane which is considered to support aggregation. Meireles et al. studied the effects of different pump heads and observed an increase in turbidity of an albumin preparation with pumping time at room temperature by using a screw pump [15]. Likewise, it was observed that the use of a peristaltic pump enhances aggregate formation [16]. Increased aggregation of albumin on UF membranes by using a stirring cell was reported for high solvent velocity at high protein concentration [17]. Other studies pronounced the enhanced deposition of protein on the membrane surface due to concentration-dependent protein–membrane and protein–protein interactions [18–20].

Concentrated gel and polarization boundary layers near the membrane are leading to decreased permeate flux [21–25]. Therefore, a lower level of protein aggregates should reduce additional membrane clogging and hence longer process time. Especially when protein concentration exceeds approximately 50 mg/ml the viscosity will exponentially increase affecting permeate flux adversely as well [26,27]. On the one hand, the feed flow going tangentially to the membrane surface helps to remove deposited material from the membrane surface if enhanced back diffusion and lift velocity are sufficient to offset opposing sedimentation and permeation drag forces [28,29]. On the other hand, high shear rates in the flow channel potentially provoke aggregates which can be transported to the retentate solution and adversely affect bulk quality.

In this study the influence of applied shear stress during the UF concentration process on aggregation, permeate flux (J) and concentration time was investigated using the proportional correlation between the pressure drop Δp ($p_i - p_o$) and the shear stress τ_w in the flow channel of the membrane module. This correlation has been presented by Ahrer et al. [30]: in the following equation:

$$\tau_w = \frac{d_H(p_i - p_o)}{4L} \quad (1)$$

d_H is the hydraulic diameter of the flow channel, p_i is the inlet pressure, p_o is the outlet pressure and L is the length of the membrane [31]. The hydraulic diameter d_H which behaves proportionally to τ_w was kept constant for all UF experiments by using the same cassette module for each experiment. The hydraulic diameter d_H can be calculated by the presented equation where a is the width and b is the height of the flow channel [32]:

$$d_H = 4\frac{ab}{2(a+b)} \quad (2)$$

The second approach in the study presented here additionally considers that increased concentration during the UF process is detrimental to permeate flux. In order to reflect this the optimal values of cross-flow rate and transmembrane pressure (TMP) at different protein concentrations in the retentate were systematically determined. The aim was to develop an optimized UF method offering improved permeate flux depending on the prevailing concentration in the retentate and concomitantly reducing concentration time and aggregation in the concentrated mAb solutions.

Size exclusion chromatography, dynamic light scattering, light obscuration, turbidity measurement and a microscopic method based on filtration and staining were applied to allow the characterization of a wide range of sizes of soluble and insoluble aggregates [33,34]. Finally processability in terms of sterile filtration was studied which is an important manufacturing step to ensure the quality of the API. Further the secondary structure of the aggregates and the protein deposited on the membrane was investigated by attenuated total reflection (ATR) FT-IR spectroscopy to clarify, if perturbation in secondary structure is involved due to adsorption to the membrane surface or rather to high flow velocity during the tangential flow filtration (TFF) process.

2. Materials and methods

2.1. Material

Concentrated mAb intermediate solutions were prepared from a filtered pool of a purified chimeric human Fc (IgG4)/rat Fab antibody in citrate buffer pH 5.5 (IgG A). In addition two chimeric human Fc (IgG1)/rat Fab antibodies (IgG B and IgG C) in histidine buffer pH 6.0 were taken to generalize and confirm our analytical findings (Table 3). All mAbs were provided by Roche Diagnostics GmbH (Penzberg, Germany). Before UF processing the solutions of 5 mg/ml protein concentration were filtered through a 0.2 μm membrane cartridge (Sartorius, Goettingen, Germany). All chemicals and reagents used were at least analytical grade.

2.2. Ultrafiltration concentration experiments

For the preparation of concentrated mAb solutions the automated TFF system ÄKTAcrossflow (GE Healthcare, Uppsala, Sweden) was used [35]. Before recovery, one dead volume was used to flush the system and the membrane and to dilute to a target concentration of about 100 mg/ml. A Sartocon Slice flat sheet cassette with a Hydrosart membrane of regenerated cellulose, a nominal molecular weight cut-off of 30 kDa and a membrane area of 0.02 m^2 was provided by Sartorius (Goettingen, Germany). Total membrane loading was about 400 g/m^2 for each experiment. After concentration the membrane module was cleaned with 1 M sodium hydroxide. The normalized flux rate for water (NWF) was determined after every cleaning cycle and compared to the obtained value before initial use. The cassette was only applied for the next experiment, if the NWF decline in (l/m^2/h)/1 bar at 20 °C was below 10% of the initial value to ensure sufficient cleaning and comparable membrane properties.

2.3. Turbidity measurements

Turbidity was determined as photometric absorbance of the undiluted concentrate at 350 nm, where no intrinsic chromophores of the mAb absorb (UV–Vis spectrophotometer Evolution 500, Thermo Fisher Scientific, Waltham, USA) [36]. The samples were mixed before measuring in order to avoid a decrease in absorbance due to the settlement of large particles [37].

2.4. Size exclusion high pressure liquid chromatography

Experiments were conducted with a TSK 3000 SWXL column (Tosoh Biosep, Stuttgart, Germany) on a Summit HPLC-system (Dionex, Idstein, Germany). The elution peaks were monitored at 280 nm by the UV diode array detector UVD170U from Dionex (Idstein, Germany). Isocratic chromatography was conducted at room temperature using an aqueous buffer composed of 200 mM potassium phosphate and 250 mM potassium chloride at pH 7.0 and a flow rate of 0.5 ml/min. Each sample contained 50 μg mAb load per injection.

2.5. Light obscuration particle counting

Light obscuration (LO) was used to monitor the formation of particles in a range of 1–200 μm similar to the method <788> Particulate Matter of Injection in the United States Pharmacopoeia and the European Pharmacopoeia method 2.9.1. [38,39]. The particle counter SVSS-C (PAMAS Partikelmess- und Analysesysteme,