Sterile Filtration of Highly Concentrated Protein Formulations: Impact of Protein Concentration, Formulation Composition, and Filter Material

ANDREA ALLMENDINGER,[1,2] ROBERT MUELLER,[1] JOERG HUWYLER,[2] HANNS-CHRISTIAN MAHLER,[1] STEFAN FISCHER[1]

[1]Late Stage Pharmaceutical and Processing Development, Pharmaceutical Development & Supplies, Pharma Technical Development Biologics EU, F. Hoffmann-La Roche Ltd., Basel 4070, Switzerland

[2]Division of Pharmaceutical Technology, Department of Pharmaceutical Sciences, University of Basel, Basel 4056, Switzerland

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ABSTRACT: Differences in filtration behavior of concentrated protein formulations were observed during aseptic drug product manufacturing of biologics dependent on formulation composition. The present study investigates filtration forces of monoclonal antibody formulations in a small-scale set-up using polyvinylidene difluoride (PVDF) or polyethersulfone (PES) filters. Different factors like formulation composition and protein concentration related to differences in viscosity, as well as different filtration rates were evaluated. The present study showed that filtration behavior was influenced by the presence or absence of a surfactant in the formulation, which defines the interaction between filter membrane and surface active formulation components. This can lead to a change in filter resistance (PES filter) independent on the buffer system used. Filtration behavior was additionally defined by rheological non-Newtonian flow behavior. The data showed that high shear rates resulting from small pore sizes and filtration pressure up to 1.0 bar led to shear-thinning behavior for highly concentrated protein formulations. Differences in non-Newtonian behavior were attributed to ionic strength related to differences in repulsive and attractive interactions. The present study showed that the interplay of formulation composition, filter material, and filtration rate can explain differences in filtration behavior/filtration flux observed for highly concentrated protein formulations thus guiding filter selection. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:3319–3329, 2015

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INTRODUCTION

Highly concentrated protein formulations for subcutaneous injection are desirable for chronic indications requiring a frequent dosing regimen. They may also offer the possibility for either self-administration by the patient or home-treatment by a healthcare professional.[1–3] Especially the rheological behavior of these concentrated solutions have a major impact on processing during product manufacture (e.g., aseptic filling and filtration[4–6]) and parenteral administration[7–9] of the product. For filtration of parenteral products, hydrophilic polyvinylidene difluoride (PVDF) and polyethersulfone (PES) filters are widely used during the fill-finish process.[6,10,11] The filtration process can be influenced by different parameters including solution viscosity and formulation properties besides filter area, filtration pressure, pore size, and surface properties of the filter material.[6,11,12]

To protect the protein from interfacial stress occurring during manufacture (e.g., filtration, freeze/thawing) and storage (e.g., glass as primary packaging, shaking stress), protein formulations often require the addition of stabilizers like surfactants.[13–21] Currently, most commonly used surfactants of marketed protein therapeutics are polysorbate (PS) 20 or 80.[10,11,22,23] These nonionic surfactants bind with a higher affinity to interfaces than proteins, such as to the air–liquid or ice–liquid interface, thus preventing interfacial protein adsorption and protein aggregation.[20,24] It was recently reported that PS 80 significantly adsorbs to PES sterilizing-grade filters.[11] Furthermore, Zhou *et al.*[10] have recently shown that PS 20 also adsorbs to filter material (PES and PVDF) suggesting a non-specific hydrophobic binding mechanism. The authors reported that PES filters were adsorbing PS 20 to a much greater extent than the PVDF membranes. Both articles suggested pre-conditioning of the filters for saturation of the binding sites with formulation buffer or protein formulation, the relevance of filter flushes (waste) to ensure product homogeneity, or the evaluation of more suitable and compatible filter membranes.

Little is known about the influence of the formulation composition (e.g., surfactants, isotonizers, viscosity reducers, and buffer systems) on filtration behavior of highly concentrated protein solutions. In own observations, differences in filtration behavior of highly concentrated protein formulations were found during sterile filtration of monoclonal antibody solutions dependent on the composition of the formulation (data not shown).

The present study aimed to investigate filtration forces of different monoclonal antibody formulations during filtration in a standardized lab-scale set-up. Different filter materials, that is, hydrophilic PVDF and PES filters, were tested with a

Correspondence to: Stefan Fischer (Telephone: +41-61-68-79-118; E-mail: stefan.fischer.sf2@roche.com)

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nominal pore size of 0.2 μm. Filtration behavior of two different monoclonal antibody formulations (mAb 1 and mAb2) was investigated in particular in dependence on protein concentration and formulation composition (buffer systems, excipients like the addition of sucrose and arginine–HCl, absence/presence of surfactant). The influence of filtration rate on filtration forces was further investigated and differences between the two tested filter materials were linked to filter resistance and pore size distribution.

MATERIALS AND METHODS

Materials

Monoclonal Antibody Formulations

The purified monoclonal antibodies mAb1 (IgG_1, pI 8.4; estimated M_w 145.5 kD) and mAb2 (IgG_1, pI 9.4; estimated M_w 148 kD) were provided by F. Hoffmann-La Roche Ltd. (Basel, Switzerland).

mAb1 was formulated in an arginine succinate buffer (Ajinomoto, Louvain-la-Neuve, Belgium; Merck, Darmstadt, Germany) at pH 6.5 with addition of methionine (Ajinomoto) and 0.06% (m/v) PS 20 (Croda, Edison, New Jersey) at a protein concentration between 20 and 180 mg/mL. For investigation of different formulation properties, mAb1 was additionally formulated at a protein concentration of 20 and 150 mg/mL at pH 6.5 in either a 20 mM histidine–HCl buffer (Ajinomoto) or a 20 mM sodium citrate buffer (Jungbunzlauer, Basel, Switzerland) with addition of 0.06% (m/v) PS 20 and either (A) no excipient, (B) 150 mM arginine–HCl (Ajinomoto), or (C) 200 mM sucrose (Ferro Pfanstiehl, Waukegan, Illinois).

mAb2 was formulated at protein concentrations between 20 and 180 mg/mL in a histidine–HCl buffer (pH 6.0) with addition of arginine–HCl (Ajinomoto) and 0.02% (m/v) PS 80 (Croda). Surfactant concentrations were always above the theoretical critical micelle concentration (without protein)[24] for all tested solutions, which were 0.006% and 0.001% (m/v) for PS 20 and 80, respectively.

Surrogate Solutions

Glycerol solutions were used as surrogate for viscous solutions. Aqueous glycerol solutions were prepared by dilution of 99.5% (m/v) glycerol (Acros Organics, Morris Planes, New Jersey) with either water for injection, 20 mM histidine–HCl buffer, or 20 mM sodium citrate buffer in concentrations between 0% and 60% (m/v, pH 6.0 and 6.5) with addition of 0.06% (m/v) PS 20.

Aqueous 0.001 M potassium chloride solution (Metrohm, Zofingen, Switzerland) was prepared at a pH of 6.5 with and without addition of 0.06% (m/v) PS 20. The pH was adjusted by addition of 0.1 N sodium hydroxide solution (Merck).

Filtration Equipment

Syringe filters with a hydrophilic PVDF or a PES filter membrane from the same supplier were used for the filtration experiments in combination with 5 mL plastic syringes with luerlok™ tip (BD, Franklin Lakes, New Jersey). The syringe filters had a nominal pore size diameter of 0.2 μm and a nominal filter area of 3.9 cm^2.

METHODS

Small-Scale Filtration

Filtration experiments were performed using a standardized small-scale set-up on a TA.HD*plus* Texture analyzer (Stable Micro Systems, Surrey, UK). A plastic syringe was filled with the sample under consideration and all air bubbles were removed. A syringe filter was mounted on the syringe and placed on the texture analyzer with the syringe pointing to the bottom. A constant speed was applied to the plunger and the restoring force was measured during filtration of the sample. Filtration forces can be easily converted to filtration pressure by knowledge of filtration area. The experiments were performed as triplicates at room temperature (23 ± 1°C). Before each filtration, the filter was washed/equilibrated with at least 5 mL of water for injection and subsequently with 5 mL of placebo in order to saturate the filters with surfactant as suggested by Mahler *et al.* and Zhou *et al.*[10,11] Data analysis was performed using the Exponent Stable Micro Systems' software (Stable Micro Systems). The filtration force was determined as average value from the plateau of the force-travel distance graph. A representative example for data analysis is shown in Supporting Figure S1 of the supporting information.

Characterization of Samples

Viscosity Measurement

Dynamic viscosity was measured by plate/cone rheometry at 20°C as duplicates at a shear rate of 2×10^3 s^{-1} as recently described by Allmendinger *et al.*[25]

Light Obscuration

Sub-visible particles were counted by light obscuration as described by Kiese *et al.*[21] using a low-volume method.

Dynamic Light Scattering

The protein–protein interaction (PPI) parameter A_2 was determined for the protein samples by dynamic light scattering (DLS) as described by Lehermayr *et al.*[26] The measuring time was adjusted to 5 s and 20 consecutive measurements were performed per dilution ($N = 3$). This method measures A_2 at low protein concentrations (1–10 mg/mL) as there are currently only limited methods available to measure PPIs at high concentrations.[26,27] However, A_2 is considered to be a good qualitative predictive factor for PPIs in highly concentrated protein formulations as previously suggested by Yadav *et al.*[28]

Size-Exclusion Chromatography

Monomer, high-molecular, and low-molecular weight species were analyzed for the protein samples by size-exclusion chromatography (SEC) according to Kiese *et al.*[21] The monomer content was found >98% for all samples.

Filter Characterization

Filter Resistance

Filter resistance β (m^{-1}) was determined by filtration force experiments ($N = 3$): The filtration process can be described by the modified Darcy equation[12] if cake formation is neglected:

$$\Delta p = \frac{F + F_{\text{Friction}}}{A} = \frac{Q}{A} \cdot \eta \cdot \beta + \frac{F_{\text{Friction}}}{A} \quad (1)$$