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High concentration tangential flow ultrafiltration of stable monoclonal antibody solutions with low viscosities

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ABSTRACT

During production of concentrated monoclonal antibody formulations by tangential flow ultrafiltration (TFF), high viscosities and aggregation often cause extensive membrane fouling, flux decay and low product yields. To address these challenges, the co-solutes histidine or imidazole were added at high concentrations from 250 to 320 mM to reduce the viscosity by up to ten-fold relative to conventional low co-solute formulations, to as low as 40 cP at 250 mg/mL. At high mAb concentrations of up to 280 mg/mL, the transmembrane flux was increased threefold by adding high concentrations of co-solutes that also lowered the viscosity. Furthermore, the co-solutes also increased the mAb gel point concentration c_g by up to 100 mg/mL mAb and thus enhanced concentration polarization-driven back-diffusion of the mAb at the membrane wall, which led to increased fluxes. The low viscosity and hollow fiber filter modules with straight flow paths enabled more uniform TMP and wall shear stress τ_w profiles, which mitigated the reversible flux decay that results from an axial decline in the local TMP. The concentrated mAb was stable by SEC before and after extended storage at 4 °C and 37 °C.

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1. Introduction

For subcutaneous (subQ) delivery of monoclonal antibodies (mAb) and other protein therapeutics, the desired dosage often requires protein concentrations of 150 mg/mL or higher given the small injection volume of 1.5 mL [1]. At these concentrations, attractive short-ranged interactions between proteins may produce aggregation [2] and/or high viscosities [3,4] above the desired limit of 20–50 cP for subQ injection [5]. Even though tangential flow filtration (TFF) is used commonly for manufacturing concentrated proteins, relatively few publications have reported results for concentrations above 150 mg/mL. Here, low fluxes in TFF and concentration polarization resulting from high viscosities [3,4,6] may result in protein gelation, membrane fouling and protein aggregation [7]. Thus, novel concepts would be highly beneficial for weakening protein interactions to reduce viscosity and aggregation, both to improve TFF ultrafiltration and to advance subcutaneous injection at high concentrations.

Several studies of TFF have optimized the transmembrane pressure (TMP), cross-flow rates and shear stress to reach concentrations of 150 to 200 mg/mL [6,8–12]. These studies have attempted to minimize protein aggregation during filtration [6,8,13] and to avoid large viscosity increases and the associated flux decay [6,8,14–17] and large axial pressure drops ΔP [3,12], both of which limit the maximum achievable mAb concentration. The large pressure drops may exceed pump capacity [3,4] and increase axial variation in TMP, with undesirably high TMP values at the influent port [12,15,16] and back-filtration near the effluent port where the TMP is negative [12]. The TMP is typically optimized based on the transition point between the pressure-dependent and pressure-independent regions of the flux-TMP profile, which is also known as the 'knee-point TMP'. To maximize the membrane flux while minimizing the risk of protein gelation, the TMP should be maintained near this optimal value [6]. As protein concentration increases, it was found that the optimal TMP decreases [6]. The low optimal TMP is problematic since high TMPs would be desirable to overcome losses in protein fluxes at high concentrations resulting from concentration polarization and fouling. In an alternative approach, single-pass TFF has been used to reach a final mAb concentration of 225 mg/mL by eliminating the recirculation loop to minimize mAb exposure to high shear stresses in the pump head [13].

In TFF, fouling and concentration polarization may be mitigated by applying an appropriate wall shear stress τ_w or shear rate γ_w to sweep protein molecules near the wall back into the bulk flow [14,18]. If the shear stress is too large, it may cause protein denaturation and aggregation [19], especially in the presence of air-solution and solution-solid interfaces [20,21]. For proteins, flat sheet cassettes (filter modules) are typically used for TFF due to

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facile scalability and high fluxes [6,8–10,13,22,23]. However, given the serpentine flow path, a high local τ_w is required in the stagnation zones in the 90-degree bends to mitigate protein fouling and concentration polarization, but this may cause protein denaturation and aggregation. For example, an average τ_w of 200–300 Pa is recommended for ultrafiltration of concentrated mAb solutions at typical feed flow rates of 300–400 LMH (liters per square meter per hour) with the flat sheet geometry [6,8]. In contrast, more uniform flow in the hollow fiber geometry may avoid the possibility of high local values of τ_w to minimize shear-induced protein aggregation, particularly at high concentrations. Recent advancement in the design of asymmetric hollow fiber membranes make them more attractive for protein separations given improved fluxes [14,24].

The design of mAb formulations with lower viscosities at high concentrations would be highly beneficial for advancing ultrafiltration in TFF. In TFF, lower viscosities would enable higher fluxes, lower axial ΔP, and better control of the TMP for lower values of τ_w. The solution viscosity exhibits an exponential dependence on the mAb concentration, as described by the Ross-Minton equation [4,25] (Eq. (1)),

$$\eta = \eta_0 \exp\left(\frac{c[\eta]}{1-\frac{k}{\nu}c[\eta]}\right) \tag{1}$$

where η is the solution viscosity, η_0 is the solvent viscosity, c is the mAb concentration, $[\eta]$ is the intrinsic viscosity, k is the crowding factor and ν is the Simha shape factor. To first order, the intrinsic viscosity $[\eta]$ is an effective specific volume of the mAb molecule at infinite dilution [4,26], whereby the viscosity increases with $[\eta]$. Furthermore, the effect of pair-wise interparticle interactions on η may be captured by $[\eta]$, as described in a virial expansion of viscosity as a function of mAb concentration [4]. The level of the exponential increase in viscosity may be ameliorated by weakening attractive intermolecular interactions at higher concentrations in order to lower $[\eta]$. Even when protein-protein interactions are repulsive at dilute conditions, they may become attractive at high concentrations [2,4] due to anisotropic short-ranged electrostatic and hydrophobic interactions [27] at small protein separation distances < 5 nm [28]. The anisotropic nature of these interactions arises from the heterogeneous distribution of charged and hydrophobic residues on the protein surface [29–31].

Recently, strategies to lower the solution viscosity and improve mAb stability by the addition of high concentrations (150–1000 mM) of co-solutes are gaining attention. In some cases, electrostatic interactions can be modulated by the addition of salts to screen the protein charge and minimize attractive local anisotropic interactions [4,11,32–35]. Furthermore, hydrophobic salts have been hypothesized to adsorb on hydrophobic sites and further screen hydrophobic interactions, resulting in large viscosity reductions [36,37]. The amino acids arginine [5,28,38–43] and histidine [44] in the protonated form have been shown to produce significant viscosity reductions at high co-solute concentrations without adversely impacting the mAb stability. The two are hypothesized to screen both electrostatic and hydrophobic interactions via a similar binding mechanism [39,45,46]. Alternatively, we introduced a concept of adding a high concentration of a nonionic crowding agent, for example the disaccharide trehalose [47,48] to provide a depletion attraction force [49,50] to osmotically compress proteins to attempt to raise the stability. According to a free energy model, the compression generates nanoclusters of primary colloidal charged spheres, whereby the protein may adopt a more stable conformation through a self-crowding mechanism [51,52]. Despite the benefits of co-solutes on protein viscosity and stability, to our knowledge, protein solutions with elevated concentrations of co-solutes have not been formed by TFF.

Herein we utilize high concentrations of histidine or imidazole with trehalose as co-solutes to form stable solutions of a human IgG1 mAb by TFF with viscosities as low as 70 and 40 cP at 280 and 255 mg/mL, respectively. Histidine at low concentrations is a common pharmaceutical buffer that may preferentially bind to and shield interaction-prone mAb residues to mitigate protein network formation (reversible aggregates) and possibly reduce the viscosity significantly. In control experiments with low co-solute concentrations, the solutions gelled and were ~10 times more viscous than the formulations with high concentrations of co-solute, resulting in poor transmembrane fluxes. The low viscosities of the solutions and the choice of the hollow fiber geometry are shown to provide for low axial ΔP, resulting in a relatively uniform and small wall shear stress and more uniform TMP. The high concentrations of co-solute also enhance concentration polarization-driven back-diffusion of the mAb near the membrane wall at high mAb concentration by increasing the mAb gel point concentration c_g. Because these factors mitigate flux decay from stagnation at the membrane wall and from axial decline in the local TMP, it became possible to achieve relatively low losses in membrane flux (low permeation resistance). A secondary objective was to show that the TFF process is well suited for forming concentrated protein solutions with high co-solute levels and low viscosities to complement previous techniques [11,36,37,41–43,47,48]. The mAb solutions were diluted and studied by SEC before and after extended storage at 4 °C to show that the formation of irreversible aggregates was minimal.

2. Materials and methods

2.1. Materials

The IgG1 mAb used in this study was provided by AbbVie as a concentrated solution at 130 mg/mL in a proprietary buffer containing 10 mM histidine, 4% mannitol and 0.1% Tween-80 at pH 5.8 (referred to as the "freezing buffer"). The mAb solution was aliquoted into 5 ml sub-samples stored in 5-mL cryogenic vials (Corning Incorporated, Corning, NY) and frozen using a dry ice-ethanol freezing mixture for extended storage at −80 °C. α-trehalose dihydrate (Tre) was purchased from Ferro Pfanstiehl Laboratories Inc., Waukegan, IL. All other chemicals (L-histidine (His), imidazole (Im), citric acid monohydrate (CitrA), hydrochloric acid (HCl), and o-phosphoric acid (PhosA)) were purchased from Thermo Fisher Scientific, Fair Lawn, NJ. Disposable 0.22 µm polyethersulfone (PES) bottle top and 13 mm syringe sterile filters were obtained from Celltreat Scientific Products, Shirley, MA (product codes 229717 and 229746). Disposable 50 kDa PES MidiKros hollow fiber filter modules with a length of 20 cm and an ID of 0.5 mm (36 fibers and 115 cm^2 area, part no. D02-E050-05-N) or 1.0 mm (12 fibers and 75 cm^2 area, part no. D02-E050-10-N from Spectrum Labs, Rancho Dominguez, CA) were utilized for TFF. Amicon Ultra-15 Ultracel – 30 K centrifugal filters were purchased from Merck Millipore Ltd. Ireland.

2.2. Diafiltration and ultrafiltration to 250 mg/mL by TFF

Buffers were prepared at the desired co-solute composition and sterile filtered with the Celltreat bottle top PES filters and then degassed under vacuum for 30 min. The frozen mAb stock (25 mL in 5 vials) was thawed in a 4 °C water bath and diluted with an equal volume (25 mL) of the buffer, resulting in a mAb concentration of 65 mg/mL. The diluted mAb solution was gently mixed in a 50-mL centrifuge tube, which served as the retentate reservoir during the TFF experiments. In two of the experiments, the buffer exchange was done at a lower concentration of

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