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Protein Adsorption to In-Line Filters of Intravenous Administration Sets

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ABSTRACT

Ensuring compatibility of administered therapeutic proteins with intravenous administration sets is an important regulatory requirement. A low-dose recovery during administration of low protein concentrations is among the commonly observed incompatibilities, and it is mainly due to adsorption to in-line filters. To better understand this phenomenon, we studied the adsorption of 4 different therapeutic proteins (2 IgG1s, 1 IgG4, and 1 Fc fusion protein) diluted to 0.01 mg/mL in 5% glucose (B. Braun EcoFlac; B. Braun Melsungen AG, Melsungen, Germany) or 0.9% sodium chloride (NaCl; Freeflex; Fresenius Kabi, Friedberg, Germany) solutions to 8 in-line filters (5 positively charged and 3 neutral filters made of different polymers and by different suppliers). The results show certain patterns of protein adsorption, which depend to a large extent on the dilution solution and filter material, and to a much lower extent on the proteins' biophysical properties. Investigation of the filter membranes' zeta potential showed a correlation between the observed adsorption pattern in 5% glucose solution and the filter's surface charge, with higher protein adsorption for the strongly negatively charged membranes. In 0.9% NaCl solution, the surface charges are masked, leading to different adsorption patterns. These results contribute to the general understanding of the protein adsorption to IV infusion filters and allow the design of more efficient compatibility studies.

Introduction

Intravenous (IV) infusion is a common route for administration of protein-based therapeutic drugs. Accordingly, pharmaceutical development activities include evaluating the physicochemical compatibility of the drug with the IV bags, diluents, and administration sets, simulating the administration conditions at the recommended storage temperature for the recommended storage time and at the extremes of concentration.[1,2] This is according to the regulatory guidelines, and the generated data are commonly provided as a part of the regulatory filings.[3]

These compatibility studies are usually designed to evaluate the product's critical quality attributes, such as the delivered dose, bioactivity, protein aggregation, and particle formulation.[4] For low protein doses, administration using IV sets may involve significant dilution to low protein concentrations (<<0.5 mg/mL).[5] At such concentrations, low recovery of the administered dose is among the most common manifestations of incompatibility and is due to protein adsorption to any of the components of the administration set.[5] This can be critical in phase 1 clinical trials, where the clinical study protocol involves evaluation of ascending dose levels to assess the safety of the new biologic entity. In this case, the first administered doses can be extremely low, increasing the risk of protein adsorption and low recovery, which could lead to erroneous clinical outcomes.

Although different components of the IV administration set could potentially contribute to protein adsorption, one of the main sites of protein adsorption is the in-line filter. The latter is usually used to reduce the risk of severe complications during IV administration[6] by removing particulate contamination, precipitates, bacteria, fungi, and air.[7] However, in-line filters usually have a high specific surface area, and accordingly a high adsorption potential, with several publications reporting on adsorption of chemotherapeutics, antibiotics, antifungals, and small polypeptides to in-line filters,[8-12] resulting in concerns regarding appropriate dosing. There is, however, a scarcity of publications regarding adsorption of therapeutic proteins to in-line filters and a lack of understanding of the factors governing it.[12]

In this study, we performed a systematic investigation of protein adsorption to in-line filters, where the adsorption of 4 different proteins to 8 different filters was investigated in different dilution

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media. In addition, the physicochemical properties of the protein and membranes were evaluated, and an explanation for the protein adsorption observations is proposed.

Materials and Methods

Materials

Four proteins were selected for this study, 2 IgG1 molecules, 1 IgG4, and 1 Fc fusion protein, with original protein concentrations of the drug product ranging between 25 and 150 mg/mL.

The tested filters are listed in Table 1, and their nominal surface area ranges between 9 and 13 cm^2. The dilution solution was either 0.9% sodium chloride (NaCl) (Freeflex; Fresenius Kabi, Friedberg, Germany) or 5% glucose (B. Braun EcoFlac; B. Braun Melsungen AG, Melsungen, Germany). Aprotonin ultrapure was from Affymetrix (Santa Clara, CA). All other chemicals were of analytical grade and used as received.

Protein Characterization

The proteins' biophysical properties were evaluated as part of the standard developability assessment program,[13] which includes—among other things—evaluating protein hydrophobicity, self-interaction propensity, and isolectric point (pI).

Protein Hydrophobicity

Protein hydrophobicity was evaluated by hydrophobic interaction chromatography (HIC) using Tricorn 5/100 column (from GE Healthcare) packed with PPG-600M (from Tosoh Biosciences) and Äkta Explorer 100 LC System (from GE Healthcare). The protein solution was diluted to 1 mg/mL, and 500 μL was injected. The protein solution was eluted using a gradient of ammonium sulfate solution from 1.5 to 0 M. The elution time was converted to molar concentration of ammonium sulfate. The higher the concentration of ammonium sulfate, the lower the protein hydrophobicity.

Self-Interaction

Protein self-interaction was measured by static light scattering (SLS) (method adapted from Bajaj et al.[14]). The measurement used an Agilent LC 1200 equipped with a UV detector and a multiangle light scattering (MALS) detector (miniDawn Treos; Wyatt Technology Corporation). A serial dilution for the protein was prepared in the mobile phase (20 mM of histidine buffer; pH, 6.0), with concentrations ranging between 0.2 and 1.4 mg/mL. About 900 μL of each dilution was injected at a flow rate of 0.4 mL/min through the UV detector (which measures absorption at 280 nm) and the MALS detector. By plotting $Kc/R\theta$ as a function of concentration, the slope was used for calculating B22.

Isoelectric Point

The pI is calculated using pK values from Grimsley et al.[15]

Protein Adsorption to Filters

Each protein solution was diluted using either 0.9% NaCl or 5% glucose solution to a concentration of 0.01 mg/mL. About 50 mL of the solution was filled into 50 mL of luer lock syringe (B. Braun) and incubated for 2 h at room temperature. After that, the solution was infused at a rate of 100 mL/h using a syringe infusion pump (B. Braun Perfusor Compact S) through the infusion line (Original Perfusor Leitung, 150 cm; B. Braun) and one of the in line-filters mentioned in Table 1. Samples were collected after 5, 10, 20, 30, and 40 mL into Nalgene polyethylene terephthalate glycol-modified container containing 0.1% aprotinin solution to prevent adsorption to the container wall. Protein concentration and recovery were determined by ultra–high-pressure size exclusion chromatography (SEC) carried out on Agilent 1290 system with 3-mm flow cell and UV detection. The system is equipped with a Waters ultra performance liquid chromatography BEH200 SEC column (Waters 186005225; 1.7 μm, 4.6 × 150 mm). The column was equilibrated with the mobile phase (50 mM of sodium phosphate solution and 400 mM of sodium perchlorate; pH, 6.0) for 20 min at 40°C. Twenty microliters of the protein solution was injected onto the column and eluted isocratically at a flow rate of 0.4 mL/min with the mobile phase, with a runtime of 6.5 min. Protein elution was monitored at 210 nm. For determination of protein concentration recovery, the ratio of the protein concentration before and after infusion was calculated. The cumulative dose was calculated as the infused volume multiplied by the measured concentration and is depicted as percentage of the recovered cumulative dose relative to the nominal value.

Membrane Characterization

Zeta Potential Measurements

Streaming potential and streaming current measurements, respectively, were performed with the SurPASS (Anton Paar GmbH, Graz, Austria) using the adjustable gap cell. For each measurement, a pair of membrane sample was fixed on the sample holders (with a cross section of 20 × 10 mm) using double-sided adhesive tape. The sample holders were inserted in the adjustable gap cell such that the surfaces of the sample were exactly facing each other. A gap of approximately 100 μm was adjusted between the sample surfaces. Before starting the measurement, the samples were carefully rinsed with the measuring electrolyte. A solution of 5% glucose and 1 mmol/L potassium chloride is used as the background electrolyte, and the pH of this aqueous solution is adjusted with 0.05 mol/L hydrochloride and 0.05 mol/L sodium oxide, respectively. The measurement of streaming potential and streaming current is performed alternatively in both flow directions. The dependence of streaming potential on the applied differential pressure is strictly linear with a coefficient of linear regression better than $R^2 = 0.99$. The flow behavior of the electrolyte passing through the gap between sample surfaces as volume flow rate as a function of the

Table 1
List of Filters Used for the Study

Manufacturer	Brand Name	Reference Number	Filter Material
BD	Alaris Extension Set	MFX1826	Positively charged PES
Codan	Codan I.V. star 10	76.3400	Positively charged PES
B. Braun	Intrapur Plus Infusion filter 0.2 μm	4099800	Positively charged PES
Rowemed	Rowefil 120	A-2356	Positively charged PA
Pall	Posidyne ELD	ELD96LL	Positively charged PA
Hospira	LifeShield Macrobore Extension Set	12689-28	PES
B. Braun	Sterifix	4099303	PES
Terumo	Terufusion	TF-SW231H	PSU