SARTORIUS

Application Note

April 23, 2020

Keywords or phrases:
Syringe Filter, Protein Adsorption, mAb, RFP, RuBisCo, Design of Experiments (DoE)

How to Increase Recovery at Critical Protein Samples: Impact of Syringe Filter Membrane, Volume and pH

A. Croon, J. F. Buyel
Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Forckenbeckstraße 6, 52074 Aachen, Germany

Corresponding author
Tel.: +49 241 6085 13162
E-Mail address: johannes.buyel@ime.fraunhofer.de

Abstract

Protein loss during sample preparation can be an obstacle to reliable product quantitation in biological, biotechnological and biopharmaceutical settings. We compared four membranes typically used as part of syringe filters for sample preparation. In a design of experiments approach we quantified the recovery of four model proteins under different sample conditions and found that membranes composed of cellulose acetate or polyethersulfone adsorbed on average less than 5% of protein analyte. Even when only 0.5 mL sample with 0.01 g L^{-1} protein was filtered, the recovery was ~90% with these membranes. In contrast, nylon or polyvinylidene difluoride-based membranes exhibited adsorption of more than 30% of product under these conditions. Furthermore, adsorption was dependent on sample properties like pH which can facilitate a fine tuning of the sample conditions to improve product recovery during preparation.

Find out more: sartorius.com/en/products/lab-filtration-purification/syringe-filters

Introduction

Biopharmaceutical samples are often prepared from feedstocks containing insoluble particles like cell debris or protein aggregates and therefore require a solid-liquid separation before analysis to protect analytical instruments. Because separation by centrifugation requires a difference in density between solid and liquid phase, sample filtration can be advantageous and membrane filters offer absolute particle retention. However, filter membranes can adsorb analytes like proteins and thereby distort the results of the subsequent analyses. It is therefore important to select filter membranes with a minimal tendency to protein adsorption. But the latter does not only depend on the membrane type, yet is also affected by the sample and protein properties, like pH and surface charge respectively, as well as the specific handling steps including sample volume per unit filter area. Identifying conditions suitable to achieve minimal analyte loss can thus be a complex multi parameter problem with a work load that would be prohibitively high, especially for early development and screening approaches. We have therefore selected four typical syringe filter membranes and quantified the recovery of four model proteins including two different antibodies under various sample conditions representative for many biological, biotechnological and biopharmaceutical applications. The design of experiments (DoE) approach we used may provide guidance as to which conditions and membranes can help to minimize analyte loss during sample preparation.

Materials and Methods

Four model proteins were used to study protein adsorption to filter membranes (Table 1).

A split-plot I-optimal design with 120 runs containing four numerical and two categorical factors (Table 2) was set up to investigate protein binding to different membranes of syringe filters by a mixed linear-quadratic model. The numerical factor levels were selected based on typical sample conditions, for example in-process-controls during biopharmaceutical production. Proteins were dissolved in phosphate buffer (10 mmol L^{-1}, pH 5.5 or pH 7.5) containing 140 mmol L^{-1} (15 mS cm^{-1}) or 550 mmol L^{-1} (50 mS cm^{-1}) of sodium chloride according to the DoE approach. Sample preparation was carried out in glass containers and protein solutions were loaded to membrane filters using polypropylene syringes. Filtrates were collected in glass containers and filtration was performed at 22° C.

Table 1: Model proteins used for filter membrane testing

Protein name [-]	Protein type [-]	Molecular mass (monomer) [kDa]	Isoelectric point (pI) [-]	Oligomeric state	Purity [-]
DsRed	Red fluorescent protein (RFP)	27.15	7.4	4	0.84
Adalimumab	Monoclonal antibody (mAb1)	145.4	8.4	1[c]	>0.97
M12	Monoclonal antibody (mAb2)	144.8	7.9	1[c]	>0.97
RuBisCO[a]	Enzyme	52.9/20.3[b]	6.6	16[d]	0.92

a. Ribulose-1,5-bisphosphate carboxylase/oxygenase; b. values for large and small subunit respectively; c. composed of two heavy and two covalently linked heavy and light chains; d. composed of 8 small and 8 large subunits that are non-covalently attached.

Results and Discussion

RFP was diluted in 0.9% m/v sodium chloride and quantified by fluorescence spectroscopy with excitation at 559 nm and emission at 585 nm in black 96-well plates with a 7 mm measurement height and 50 flashes per sample using an EnSpire (Perkin Elmer) multimode plate reader. RuBisCO containing 10-µL samples were analyzed at 220 nm by ultra-high performance size exclusion chromatography (UHPSEC) using an Ultimate 3000 (Thermo Fischer Scientific). Proteins were separated isocratically on an Acquity UPLC Protein BEH SEC Column, 20 nm, 1.7 µm, 4.6 × 150 mm with 50 mmol L^{-1} sodium dihydrogen phosphate, 250 mmol L^{-1} sodium chloride, pH 6.8 at a column temperature of 30° C and a flow rate of 0.2 mL min^{-1}.

Monoclonal antibody samples of M12 and Adalimumab were analyzed by surface plasmon resonance (SPR) spectroscopy using a Biacore T200 (GE Healthcare). Samples were diluted and analyzed in 0.01 mol L^{-1} HEPES, 0.15 mol L^{-1} sodium chloride, 3 mmol L^{-1} EDTA and 0.005% v/v polysorbate-20 and loaded to a Protein A functionalized chip surface at 22° C with 0.03 mL min^{-1} and a contact time of 180 s. Injections of 45 µL 0.03 mol L^{-1} hydrochloric acid were used for surface regeneration.

A statistical experimental design (DoE) was used to quantify the binding of four model proteins to four different types of syringe filter membranes (all with a pore size of 0.2 µm), frequently used for sample preparation, for example in the context of in-process controls. The highest protein recovery of >98% was observed for a cellulose acetate (CA) membrane (Minisart® NML, Table 3) which was insignificantly higher than the average recovery achieved with a polyethersulfon (PES) membrane (Minisart® High Flow) (two-sided t-test with 0.05 alpha level). Also, both membranes exhibited a 3 to 8-fold lower standard deviation compared to a nylon or a polyvinylidene difluoride membrane, indicating that high recoveries were achieved with these membranes even for varying sample conditions and target proteins (Table 2).

When analyzing the DoE, sample volume and especially protein concentration had the strongest effects on protein recovery and the latter increased with higher concentrations and volumes (Figure 1). These observations were in good agreement with a saturation model for protein adsorption to surfaces, for example a Langmuir model. In such a model, a given surface will bind a certain absolute quantity of protein and accordingly the (relative) recovery increases as sample volume and concentration increase. Therefore, large volumes and high concentrations can reduce the percentage of product loss during sample preparation using syringe filters.

Table 2: Summary of the DoE setup used to study protein adsorption to filter membranes

Factor	Unit	Type	Level
Conductivity	mS cm^{-1}	Numeric	15; 50
pH	-	Numeric	5.5; 7.5
Protein concentration	g L^{-1}	Numeric	0.01; 0.10; 1.00
Specific sample volume	mL cm^{-2}	Numeric	0.5; 5.0
Protein	-	Categoric	[see Table 1]
Membrane	-	Categoric	[see Table 3]

a. Ribulose-1,5-bisphosphate carboxylase/oxygenase; b. values for large and small subunit respectively; c. composed of two heavy and two covalently linked heavy and light chains; d. composed of 8 small and 8 large subunits that are non-covalently attached.

Conclusion

The membrane type had a relevant effect as well and membranes composed of CA or PES exhibited substantially less protein adsorption (>95% recovery) compared to counterparts made of nylon or polyvinylidene difluoride (PVDF), especially when exposed to low product concentrations and sample volumes (<75% recovery) (Figure 1). Importantly, the recovery achieved with CA and PES membranes was largely independent of protein, sample conditions and handling, implying that a fine tuning may not be necessary for each new product to be investigated. Therefore, CA or PES-based membranes can help to limit product loss during sample preparation for analysis if a target protein is scarce. The pH-effect was strongly protein specific. For example, no substantial pH effect was observed for mAb1 at pH 5.5 (0.01 g L^{-1}, 0.5 mL cm^{-2}) but a recovery of only ~60% was observed for RuBisCO even when Minisart® NML was used under the same conditions. However, the low recovery of RuBisCO was linked to a known low-pH instability of the protein and therefore unlikely an effect of membrane adsorption.

Whereas the conductivity did not exhibit a significant influence on recovery within the parameter space investigated in this study, a salinity below 15 mS cm^{-1} or above 50 mS cm^{-1} may cause product losses as it can affect protein solubility and may trigger protein aggregation. The resulting aggregates in turn may interact with the membrane or, depending on their size, can be sterically retained by the latter. Therefore, care should be taken if conditions outside the reported parameter space are used.

Most sample manipulation or preparation is associated with some product loss. However, analytics during process development or monitoring require that such losses are kept to a minimum so that reliable results can be obtained. Minimal product loss during sample preparation can be achieved over a wide range of conditions by selecting an adequate filter membrane. For example, ~90% of product was recovered using Minisart® NML (CA) or Minisart® High Flow (PES) filter membranes even with sample volumes and concentrations as little as 0.5 mL cm^{-2} and 0.01 g L^{-1} respectively. The product recovery may be further improved by fine tuning the sample conditions for an individual product, e.g. by selecting a proper pH value. In contrast, if protein binding is beneficial for sample preparation, nylon-based membranes such as Minisart® NY can be used instead.

Table 3: Properties of 0.2 µm pore size filters and average protein recovery after filtration in dependence of membrane type. RFP, mAb1, mAb2 and RuBisCO samples were in a 5.5–7.5 pH range, conductivities of 15 or 50 mS cm^{-1}, concentrations between 0.01 and 1.00 g L^{-1} and loadings of 0.5 or 5.0 mL sample per cm^2 membrane area

Filter name [-]	Membrane type [-]	Housing material [-]	Filter area [cm^2]	Average recovery [%][a]	n
Minisart® NML	Cellulose acetate (CA)	Methacrylate butadiene styrene (MBS)	6.2	98.4 ± 7.4	15
Minisart® High Flow	Polyethersulfon (PES)	Methacrylate butadiene styrene (MBS)	6.2	98.2 ± 5.3	18
Minisart® NY	Nylon (NY)	Polypropylene (PP)	4.8	59.7 ± 41.4	20
Standard filter	Polyvinylidene difluoride (PVDF)	Polypropylene (PP)	4.2	81.7 ± 27.4	17

a. The variability is indicated as the standard deviation.

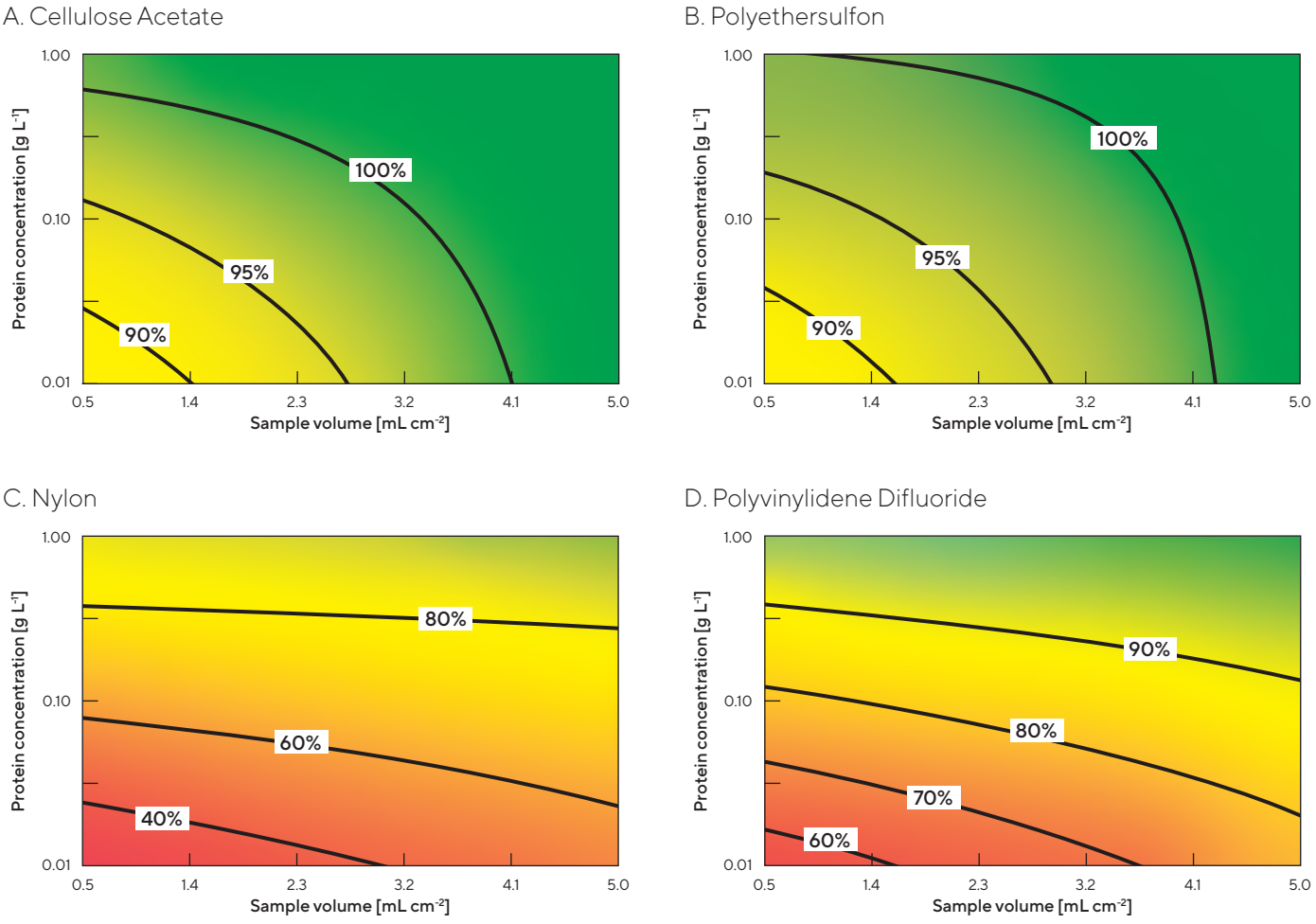

Figure 1: Average protein recovery with membrane-based syringe filters. Recovery was averaged over proteins RFP, mAb1, mAb2 and RuBisCO for a conductivity of 32.5 mS cm^{-1} at pH 6.5 using cellulose acetate (A), polyethersulfon (B), nylon (C) and polyvinylidene difluoride (D) membranes.

Acknowledgements

The authors acknowledge Klaus Schöne for providing the syringe filters and a monoclonal antibody used in this study.

.

References

1. Interferon alfa-2 concentrated solution. *European Pharmacopoeia.* 8.3: p. 2502-2505.

Sales and Service Contacts

For further contacts, visit
www.sartorius.com

Germany
Sartorius Lab Instruments
GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

Version 1: 04 | 2020