BioProcess™ INTERNATIONAL

BIOPROCESS TECHNICAL

Accounting for the Donnan Effect in Diafiltration Optimization for High-Concentration UFDF Applications

Alexandra Steele and Joshua Arias

The biopharmaceutical industry is targeting high-concentration protein formulations to enable subcutaneous administrations. Such administration can provide better patient convenience than intravenous administration. One challenge associated with high-concentration formulations is increased electrostatic interaction between proteins and excipients. That is a result of increased protein-charge density at high protein concentrations. Such interactions can create an offset between excipient levels in final products and diafiltration buffers in ultrafiltration processes. The effect of such electrostatic interactions in a membrane process is known as the *Donnan effect*.

PRODUCT FOCUS: HIGH-CONCENTRATION BIOLOGICS

PROCESS FOCUS: FORMULATION, FILL AND FINISH

WHO SHOULD READ: PROCESS DEVELOPMENT AND MANUFACTURING, FORMULATION, FILTRATION OPERATORS

KEYWORDS: EXCIPIENTS, AGGLOMERATION, BUFFER REMOVAL, DIAFILTRATION

LEVEL: INTERMEDIATE

The Donnan effect on excipient levels has received significant attention in recent years. Theoretical modeling has been developed to predict excipient and pH changes as a result of the Donnan effect in monoclonal antibody (MAb) processes. One model based on the Poisson–Boltzmann equation provided good prediction of excipient levels in the final retentate pool (**1**). A second model developed by Bolton et al. demonstrated to be predictive for basic MAb and acidic Fc-fusion proteins (**2**). The latter study also included several mitigation strategies to achieve target levels of excipients at the end of an ultrafiltration–diafiltration (UFDF) process. Both publications provide tools for understanding the influence of the Donnan effect on target formulation excipients. By contrast, our study focuses on the influence of the Donnan effect on removal of starting buffer excipients during diafiltration.

A typical final-formulation UFDF step will target eight to 10 diavolumes. For an ideal process, in which excipients pass freely through the membrane (the retention value $R = 0$), 10 diavolumes provide 99.995% removal of starting excipients. That equates to a "complete" exchange (Figure 1).

The Donnan effect, however, can influence that removal. We performed several test runs to demonstrate how the Donnan effect changes the removal efficiency of positively and negatively charged excipients. We conducted diafiltration test runs using a MAb at two different concentrations to additionally assess the influence of MAb concentration on excipient removal efficiency.

MATERIALS AND METHODS

Protein: We used SAN-300, a MAb provided by Santarus Inc., for the diafiltration studies. It is a glycosylated IgG1 monoclonal antibody directed against VLA1 (very late antigen-1, $\alpha 1\beta 1$ integrin). The protein is expressed by a Chinese hamster ovary (CHO) cell line and purified using a standard three-column MAb purification process. SAN-300 protein has pI >8 with a molecular weight >140 kDa.

Excipients: We studied three different excipients in subsequent experiments. Two were negatively charged organic-acid buffers (referred to here as E_A^- and E_B^-). The third excipient was positively charged (referred to here as E^+).

UFDF Procedure: We perfomed all tests at room temperature (21 °C) using EMD Millipore Ultracel

(regenerated cellulose) 30-kD membranes in 88-cm^2 Pellicon 3 devices installed in a Pellicon Mini cassette holder. We ran diafiltrations at a transmembrane pressure (TMP) of 20 psig. The retentate was continuously stirred and recirculated through the system using a peristaltic pump. We performed initial concentration steps as needed to achieve the desired SAN-300 test concentration. Feed flow rate was 4.5 L/min/m^2 for the runs. Table 1 provides a summary of the test matrix.

Measurement of Protein Concentration: Spectrophotometric methods determined protein concentration using the extinction coefficient at 280 nm. We diluted the reference standard and sample to a specified concentration and then measured at A_{280}, A_{320}, and A_{360}. We corrected the A_{280} reading for background absorbance at A_{320} and A_{360}. We then calculated protein concentration using the corrected A_{280} dilution factor and extinction coefficient.

Excipient Assays: The positively charged excipient (E^+) levels were determined using a capillary zone electrophoresis (CZE) method that uses a fused silica capillary (ID = 50 µm) with an enhanced detection cell, a borate electrolyte, and direct ultraviolet (UV) detection at 195 nm. We diluted the samples one hundredfold before analysis.

We measured the negatively charged excipients E_A^- and E_B^- using a special electrolyte for indirect UV detection of excipients, including a modifier to remove electroosmotic flow. We used a fused silica capillary (ID = 75 µm) and performed indirect detection at 200 nm. For both methods, we calculated concentration levels using the standard addition method.

***R* Value:** We calculated the apparent retention of the excipients using Equation 1 (see Equations box). *N* is the number of diavolumes.

Figure 1: Excipient removal for an ideal process

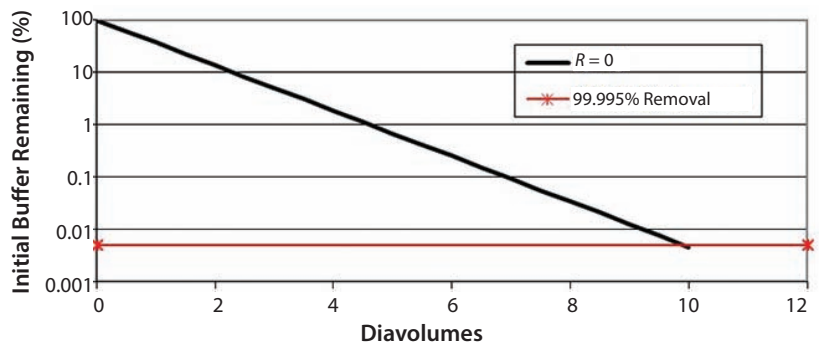
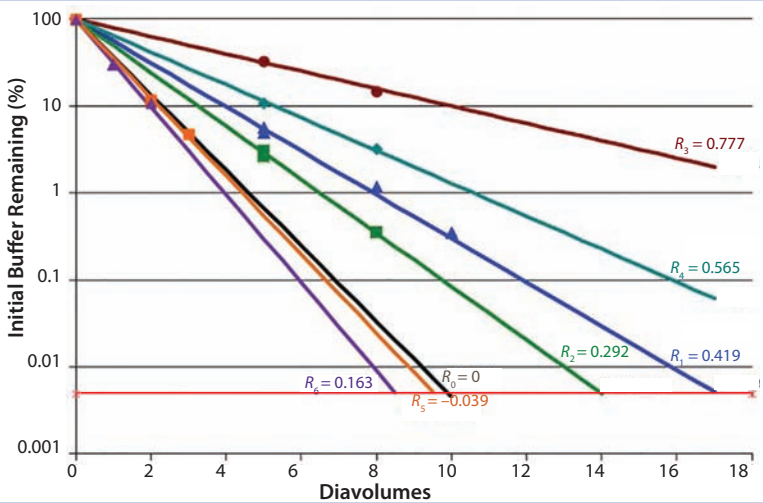


Figure 2: Percentage of initial buffer remaining and diavolumes; R_n corresponds to run "*n*" as listed in Table 1.



Results

We tested three starting/diafiltration excipient combinations for removal efficiency at 55 g/L and 120 g/L MAb concentration values. A total of six diafiltration runs were performed.

The first combination consisted of a positively charged MAb, a negatively charged starting excipient (E_A^-), and a negatively charged diafiltration excipient E_B^-. Figure 2 shows the results as Runs 1 and 3. In that figure, the black line plot shows removal efficiency for a system in which excipients pass freely through the membrane with no effect from charge interactions (99.995% removal in 10 diavolumes). Removal efficiencies for E_A^- at 55g/L (Run 1) and 120 g/L (Run 3) were comparable to solutes with apparent retention values of R_{app} = 0.419 and R_{app} = 0.770,

Table 1: Experiment test matrix

Run	MAb Charge	Starting Buffer Charge	DF Buffer Charge	Concentration (g/L)
1	+	–	–	55
2	+	–	+	55
3	+	–	–	120
4	+	–	+	120
5	+	+	–	55
6	+	+	–	120

Equations:

Equation 1

$$\text{Retention} = \ln\left(\frac{\frac{\%\text{Remaining}}{100}}{N}\right) + 1$$

Equation 2

$$C_{optimum} = \frac{C_{gel}}{e}$$

Equation 3

$$\text{area} * \text{time} \propto \frac{1}{(1-R)(C_b)(\ln C_{gel}/C_b)}$$

Equation 4

$$R = d * C_b$$

Equation 5

$$\frac{C_{gel}}{C_b} = \exp\left(\frac{d * C_b - 1}{2d * C_b - 1}\right)$$

Table 2: Experiment results for E_A^- removal using E_B^- as diafiltration

Test	Concentration (g/L)	Starting Excipient	DF Excipient	Apparent Retention	Percent Remaining at 10 DV	Diavolumes for 99.995% Removal
Ideal ($R = 0$)	N/A	N/A	N/A	0.000	0.005	10
1	55	E_A^-	E_B^-	0.419	0.704	17
3	120	E_A^-	E_B^-	0.777	10.026	43

Table 3: Experiment results for E_A^- removal using E^+ as diafiltration

Test	Concentration (g/L)	Starting Excipient	DF Excipient	Apparent Retention	Percent Remaining at 10 DV	Diavolumes for 99.995% Removal
Ideal (Excipient passes freely)	N/A	N/A	N/A	0.000	0.005	10
2	55	E_A^-	E^+	0.084	0.084	14
4	120	E_A^-	E^+	1.290	1.290	23

Table 4: Experiment results for E^+ removal using E_B^- as diafiltration

Test	Concentration (g/L)	Starting Excipient	DF Excipient	Apparent Retention	Percent Remaining at 10 DV	Diavolumes for 99.995% Removal
Ideal ($R = 0$)	N/A	N/A	N/A	0.000	0.005	10
5	55	E^+	E_B^-	−0.039	0.003	9.5
6	120	E^+	E_B^-	−0.163	0.001	8.5

Table 5: Comparison of 99.966% removal using E_A^- /E^+ data

T	55 g/L		120 g/L	
	Area	Diavolumes	Area	Diavolumes
"Pass freely" (assumes $R = 0$)	7.3 m^2	8.0	3.6 m^2	8.0
Donnan effect (R 0)	10.1 m^2 (R_{app} = 0.292)	11.3	13.5 m^2 (R_{app} = 0.565)	18.4

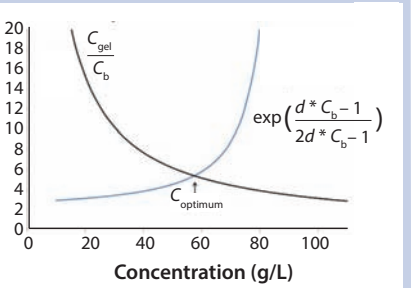
*Note the Donnan effect membrane area values were calculated using calculated average flux values from "8 diavolume" test runs. It is estimated that the Donnan effect area values are about 2–5% lower than reported.

respectively. As Table 2 shows, when those results are compared with an excipient that passes freely through the membrane, seven additional diavolumes at 55 g/L protein concentration or 33 additional diavolumes at 120 g/L protein concentration would be needed to achieve a 99.995% removal.

We obtained similar results from the second combination, which consisted of a positively charged MAb, a negatively charged starting excipient E_A^-, and a positively charged diafiltration excipient E^+, which corresponds to Figure 2, Runs 2 and 4. The negatively charged starting excipient E_A^- behaved as a partially retained solute with an apparent retention of R_{app} = 0.292 at the 55-g/L test condition. At the 120-g/L test condition, apparent retention increased to R_{app} = 0.419. For that case, a 99.995% removal would require 14 diavolumes at 55-g/L protein concentration or 23 diavolumes at 120-g/L protein concentration — compared with 10 diavolumes for an excipient that passes freely through the membrane (Table 3).

The third combination consisted of a positively charged MAb, a positively charged starting excipient E^+, and a negatively charged diafiltration excipient E_B^-. Starting excipient and MAb are both positively charged (Figure 2, Run 5 and Run 6). By contrast with the previous test results, positively charged E^+ excipient exhibited enhanced removal efficiency. It provided 99.995% removal at <10 diavolumes. Removal efficiency was further enhanced at 120 g/L concentration (Table 4).

Figure 3: Optimum diafiltration concentration



Discussion

Results showed that the removal efficiency of charged excipients during a diafiltration step was influenced by the Donnan effect. Excipients that had a charge that was opposite of the charge of the protein experienced electrostatic attractive forces, thereby partially retaining the excipient. That was demonstrated by the negatively charged excipient E_A^- and the positively charged MAb protein. By contrast, excipients with the same charge as the protein (e.g., positive–positive) experienced repulsive forces. For those cases, the excipient experienced enhanced removal comparable with a solute that passes freely through a membrane. Tests with E^+ excipient and positively charged MAb demonstrated that result.

Protein concentration also influenced removal efficiency. At higher concentrations, protein charge density increased, which in turn increased electrostatic interactions between protein and excipient (increased attractive or repulsive forces). That occurred with the diafiltration of E_A^- excipient using E_B^- diafiltration excipient. Diafiltering at 120 g/L protein concentration required 43 diavolumes, whereas 17 diavolumes at 55 g/L protein concentration provided a 99.995% removal, a 2.5× difference.

Because one objective of a final UFDF step is to provide a buffer exchange, process development scientists often target eight to 10 diavolumes under the assumption that process excipients pass freely through the membrane. Furthermore, optimum diafiltration concentration is typically

based on hydraulic considerations (e.g., $C_{optimum} = C_{gel}/e$) (3).

Our data demonstrate that the number of diavolumes required to achieve excipient removal varies as a function of electrostatic interactions with diafiltration concentration. For excipients with a charge opposite to that of the protein, a diafiltration concentration below the C_{gel}/e optimum provided improved removal and lowered overall membrane area requirement.

To illustrate, we calculated membrane area requirements for diafiltration of the E_A^-/ E^+ excipient combination (E_A^- removal using E^+ diafiltration excipient) (Table 3). Calculations were based on a starting volume of 100 L at 55 g/L MAb, a diafiltration time of three hours, and a 99.966% E_A^- removal (Table 5).

Table 5 shows that by assuming that E_A^- passes freely through the membrane, diafiltration at 120 g/L would require 51% less membrane area than needed for diafiltration at 55 g/L. By our analysis, a 120 g/L concentration would be a more optimal diafiltration concentration. If apparent retention due to the Donnan effect is considered, however, the number of diavolumes required to achieve 99.966% removal would be 11.3 and 18.4 for the 55 g/L and 120 g/L MAb concentrations, respectively. In this case, diafiltration at 55 g/L would require 39% less membrane area than would diafiltration at 120 g/L to achieve 99.966% removal. By our analysis, a 55 g/L concentration would be the optimum diafiltration concentration.

As described previously, the optimum concentration for diafiltration is generally determined from Equation 2, in which C_{gel}/e represents the concentration at which the process area × time (m^2h) value is minimized. This dependence of the optimum concentration on the C_{gel} value is determined on the assumption that excipients pass freely through the membrane ($R = 0$).

But because process area × time proportionality is as shown in Equation 3 (in which R is the excipient retention and C_b is the concentration of product in the bulk solution), an expression for optimum diafiltration concentration that includes excipient retention can be determined. For example, if excipient retention is proportional to electrostatic forces (e.g., protein concentration) as suggested by our data, then Equation 4 is reached (in which d is a proportionality constant).

Equation 5 can be used to determine optimum concentration, in which optimum concentration for diafiltration is the C_b value that satisfies the equation. Figure 3 shows the left side and right side of the optimum concentration equation for the diafiltration process described in Table 3. For the E_A^-/E^+ buffer combination, we determined the d value from the C_b and apparent retention values shown in Table 3. For this buffer combination with C_{gel} = 307 g/L and d = 0.005, we calculated $C_{optimum}$ = 57 g/L. This example shows how an expression for excipient retention as a function of C_b can be used to determine optimum concentration for diafiltration that considers both hydraulic performance and electrostatic interactions.

Recommendations

The Donnan effect influences removal of charged excipients during diafiltration of a high-concentration protein solution. Removal efficiency will be enhanced for excipients with the same charge as the protein and decreased for excipients with a charge opposite the protein. Removal of oppositely charged excipients is analogous to that of partially retained solutes.

Process development scientists should consider excipient removal when designing diafiltration steps. The required number of diavolumes to achieve excipient removal will depend on the extent of the Donnan effect, which is a function of protein concentration, protein charge, and excipient charge.

In certain cases, achieving a 99.995% removal may be impractical (e.g., high filter-area requirements). For such cases, process development scientists should identify acceptable removal targets. Proper development of the UF step can minimize process time and area while taking into account initial excipient removal, final excipient concentrations, and final protein concentration.

Acknowledgments

We acknowledge Christian Wolf (Merck KGaA, Darmstadt, Germany) and Jana Boden and Ingo Haumann (ICA, Langen, Germany) for analytical method development support at Merck KGaA's Site Operations Analytical Chemistry Chromatography and Mass Spectrometry site and Herb Lutz (EMD Millipore) for guidance on an expression for optimum diafiltration concentration.

References

1 Miao F, et al. Theoretical Analysis of Excipient Concentrations During the Final Ultrafiltration/Diafiltration Step of Therapeutic Antibody. *Biotechnol. Prog.* 25(4) 2009: 964–972.

2 Bolton GR, et al. Effect of Protein and Solution Properties on the Donnan Effect During the Ultrafiltration of Proteins. *Biotechnol. Prog.* 27(1) 2011: 140–152.

3 Ng P, Lundblad J, Mitra G. Optimization of Solute Separation by Diafiltration. *Separation Sci.* 11(5) 1976: 499–502.

***Alexandra Steele** and corresponding author **Joshua Arias** are bioprocess engineers at the Biomanufacturing Sciences Network unit of EMD Millipore Corp., 900 Middlesex Turnpike Rd., Billerica, MA 01821; 1-781-533-2459; joshua.arias@emdmillipore.com.*

Eprinted and posted with permission to EMD Millipore from *BioProcess International.* January © 2014 Informa.