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TECHNOLOGY REVIEW

Simulation of Process Performance on Contaminant Removal Using Constant and Non-Constant Volume Diafiltration Modeling

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Abstract

Constant volume diafiltration (CVD) is commonly used in the biopharmaceutical industry for impurity removal or buffer exchange. The number of diavolumes is usually determined empirically or by theoretical analysis to achieve the target degree of impurity removal. There is, however, a lack of conclusive information about the effect of contaminant removal in variable volume diafiltration (VVD). VVD can occur when the diafiltration control mode is not functioning as intended.

In this study, a mathematical model has been proposed to predict removal efficiency during VVD. Experiments were performed to compare the results to model calculations. A dilute concentration of bovine serum albumin solution was used as the feed solution to study variable volume effects. Study results demonstrate that the contaminant removal rate is higher when the diafiltration flow rate is lower than the permeate flow rate (flux).

The simple analytical expression for contaminant removal presented in this article, developed and supported by the experimental results, serves as a guide for the optimization and troubleshooting of industrial diafiltration processes. The expression verifies the diafiltration process performance when it deviates from the traditional constant volume approach, with the assumption that the contaminant has no interactions with the product.

Introduction

Diafiltration (DF) is a process of removing microsolutes such as salts and solvents from the initial volume using a semipermeable membrane. During the diafiltration process, the product of interest (usually molecules larger than the membrane molecular weight cut-off) is retained while the smaller molecules are washed out by the addition of new buffer. The main objectives of diafiltration in downstream purification processes are to exchange buffer, remove impurities, or to bring the product into the final formulation. There are two major modes of diafiltration control: discontinuous (batch) and continuous.[1–6]

Discontinuous diafiltration is a dilution and concentration process. Initially, a relatively large volume of diafiltration buffer (similar to the retentate volume) is added to the feed tank. The concentrate is then brought to a certain retentate volume. Finally, another volume of buffer is added until either the desired total volume of buffer has been reached or the contaminant removal objective is met. This mode of control is also known as non-constant volume diafiltration (non-CVD).

Continuous diafiltration, on the other hand, is performed by using a secondary pump to add the diafiltration buffer to the recycle tank while the permeate is being removed at the same rate. This is the most common diafiltration method in the biopharmaceutical industry because the protein concentration remains constant and contaminant removal is more efficient. Continuous diafiltration occurs by either maintaining a constant tank level or by measuring the permeate flow rate to control the addition of diafiltration buffer using a control valve or a diafiltration pump. The measure of continuous diafiltration is also based on the total volume (flow rate multiplied by time) introduced to the recycle tank compared to the initial retentate volume (start of diafiltration). Thus, continuous diafiltration is also referred to as constant volume diafiltration (CVD).

Because constant volume control is necessary, any malfunction of level or flow control in or around the tangential flow filtration (TFF) system could lead to a deviation from CVD. The deviation may lead to a variable volume diafiltration (VVD) process where both the volume and protein concentrations change throughout the process.[7]

This article compares the rate of contaminant removal between CVD and VVD (or non-CVD). A model which accurately predicts the purification efficiency of the TFF process when non-CVD is occurring is also presented.

Theory

Constant Volume Diafiltration

In a typical ultrafiltration/diafiltration (UF/DF) process, the ultrafiltration membrane selected is fully retentive for the protein or molecule of interest and permeable to the impurities or contaminants to be eliminated.

The observed sieving for a specific component is defined as ($S = C_p/C_b$) and membrane retention or rejection is defined as:[2,5,8]

$$R = (1 - S) = \left(1 - \frac{C_p}{C_b}\right) \qquad (1)$$

where C_p is the concentration in the permeate and C_b is the product bulk concentration.

For CVD, permeate flow is at the same rate as the addition of diafiltration buffer to the retentate tank. The relationship between retentate concentration and diavolumes, N (sum of buffer volumes added/fixed retentate volume), for different membrane sieving characteristics, is calculated as:[1,2,8,9]

$$C = C_0 \exp[-N_D S] \ = \frac{C}{C_0} = \exp[-N_D S] \qquad (2)$$

where C_0 is the initial contaminant concentration ($t = 0$), C is the contaminant concentration at any time, and N_D is the number of diavolumes (or volumes of diafiltration).

The rate of contaminant in the permeate (Y^P_{CVD}) given by:

$$Y^P_{CVD} = \frac{V_0 C_0 - VC}{V_0 C_0} = 1 - \frac{VC}{V_0 C_0} = 1 - \frac{C}{C_0} \qquad (3)$$

where V_0 is the initial volume ($t = 0$), V is the final volume, and $V_0 = V$ at CVD working conditions.

Replacing C/C_0 with equation 2 gives:

$$Y^P_{CVD} = 1 - \exp[-N_D S] \qquad (4)$$

Assuming the contaminant sieving is 1 (no retention of contaminant) for the selected membrane, the equation can be rearranged to provide the contaminant removal to the permeate:

$$Y^P_{CVD} = 1 - \exp(-N) \qquad (5)$$

Non-Constant or Variable Volume Diafiltration

In the case of non-constant diafiltration, the permeate flow rate is different from diafiltration flow or the rate of addition of diafiltration buffer to the recycle tank.

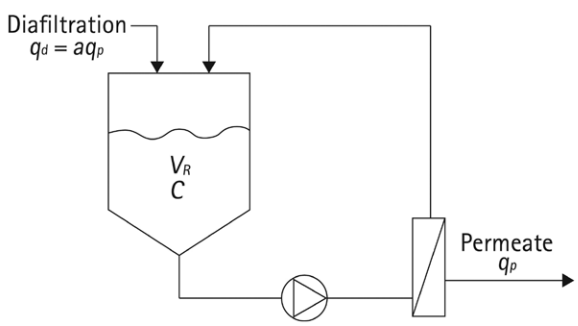

FIGURE 1. Diafiltration strategy with non-CVD ratio ($a = q_d/q_p$) as control parameter.

As illustrated in **Figure 1**, q_p and q_d are permeate and diafiltration flow, respectively. In non-CVD applications, the flow of buffer addition (q_d) to the recycle tank varies with the permeate flow rate (q_p).

The value "a" represents deviation from CVD and can be expressed as the ratio of diafiltration rate to permeate rate [$a = (q_d/q_p)$].

V_0 is the initial retentate volume (before diafiltration), V is the retentate volume at time t (where t is the diafiltration time), N is the number of diavolumes ($N = q_p t/V_0$, assuming retentate volume is constant), and C is the contaminant concentration at time t.

The following equation expresses the volume changes based on mass balance:

$$\frac{dV}{dt} = q_d - q_p = aq_p - q_p \qquad (6)$$

Solving from V_0 at time $= 0$ up to V at time t:

$$V = V_0 \ + q_p(a-1)t \qquad (7)$$

$$\frac{V}{V_0} = 1 + \frac{q_p(a-1)t}{V_0} \qquad (8)$$

where $N = (q_p t/V_0)$:

$$\frac{V}{V_0} = 1 + N(a-1) \qquad (9)$$

When $a = 1$, the diafiltration rate is equal to the permeate removal rate and diafiltration is run at CVD. When $0<a<1$, there is partial concentration/diafiltration, and when $a>1$, there is partial diafiltration/dilution, as shown in **Figure 2** (on the following page).

For equation 9, $V/V_0>0$ when $a>1$.

Limitations exist for a values $0<a<1$. In fact, $V/V_0=0$ when $a=0.5$ and $N=2$, or $a=0.7$ and $N\leq 4$.

Moreover, when $a<1$, the protein solution becomes more concentrated in the retentate as the retentate volume (V) decreases, and flux would drop to 0 long before $V/V_0=0$. By applying mass balance calculation on the protein of interest, assuming complete retention, $V/V_0 = C_0/C$, it is unlikely for V/V_0 to be 0 as C/C_0 would become infinitive (∞). Therefore, taking a reasonable C/C_0 value, for instance, diafiltration at 50 mg/mL and max protein concentration of 250 mg/mL ($C/C_0 = 5$), from a practical perspective, V/V_0 will typically not be <0.2.

In non-CVD, the rate of contaminant removal is described by:

$$Y^P_{non\text{-}CVD} = 1 - [1 + N(a-1)]^{\frac{R-1}{(a-1)}} \qquad (a \neq 1) \qquad (10)$$

where a and R are constant during the diafiltration process. In equation 10, R is the retention coefficient of the retained species (see appendix for definition and derivation).

In cases where $R=1$ (full retention of contaminants), $Y^P_{non\text{-}CVD}=0$. At the other extreme, when $R=0$ (full passage of contaminants):

$$Y^P_{non\text{-}CVD} = 1 - [1 + N(a-1)]^{\frac{1}{(1-a)}} \qquad (11)$$

Hence, the rate of contaminant removal can be predicted using a simple mathematical model. However, the actual sieving of contaminants may be lower than expected due to ion-protein interaction or binding phenomenon in the diafiltration process. A small-scale experiment was conducted to verify the consistency of the mathematical model against actual data.[8]

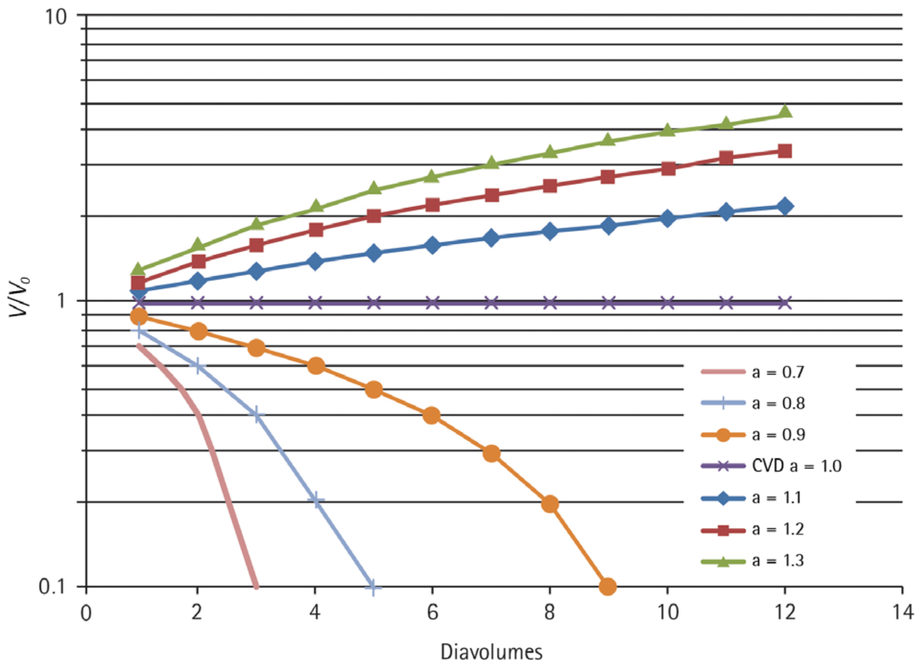

FIGURE 2. Volume ratio (V/V_0) versus diavolumes at different diafiltration ratios, $a = q_d/q_P$.

Test Method

Experiments were performed using Pellicon® 2 Mini (0.1 m²) 10 kD, C-screen cassette with 1 L of bovine serum albumin (BSA) (10 g/L) in 10 mM PBS solution (buffer A conductivity = 15.4 mS/cm). The experimental feed flow rate was 5 L/min/m², and the transmembrane pressure (TMP) range was 10–15 psi. Permeate flow rate was measured and the diafiltration pump speed was adjusted to the desired rate, as shown in **Table 1**. Deionized water (buffer B conductivity <1 µS/cm) was used to wash out the PBS solution.

During the experiment, a secondary permeate pump was used to control the permeate flow rate to ensure there was no fluctuation of flow over time. Permeate samples were taken for conductivity measurement, with the system in total recycle mode, after every 500 mL of permeate removal. The experiment was stopped when the conductivity of permeate sample reached the conductivity of buffer B, indicating that buffer A had been completely washed out by buffer B.

TABLE 1. Permeate flow rates.

Scenario	Condition	DF Rate [$a = (q_d/q_p)$]
A	Constant volume diafiltration	1.0
B	Partial concentration/diafiltration	0.8
C	Partial concentration/diafiltration	0.9
D	Partial diafiltration/dilution	1.1
E	Partial diafiltration/dilution	1.2

Results

The yield (of contaminant) for each sample reading was calculated as:

$$Y = 1 - \left[\frac{(instant\ conductivity - initial\ buffer\ B\ conductivity)}{(initial\ buffer\ A\ conductivity - initial\ buffer\ B\ conductivity)}\right] \quad (12)$$

A graph of yield versus N (the number of diavolumes) was plotted and compared with theoretical calculated values. **Figure 3** shows that the non-CVD experiment data overlapped meaningfully with theoretical permeate yield data. The small deviations in the experimental data could be due to sampling or measurement error.

The experimental data verifies the validity of the mathematical model. Therefore, the model can be used to generate different graphs of diavolumes versus the log of purification level as C/C_0 when membrane retention of the contaminant (R) is 0 or the sieving value (S) is 1, indicating full passage.

By using **Figure 4**, a prediction can be made of the purification level attained in a diafiltration process where non-CVD occurs. This prediction is valid when $R = 0$ (or $S = 1$). It can also be used as a troubleshooting exercise to identify the contributing factors to poor purification during a diafiltration process that deviates from the normal constant volume approach.

In CVD mode, $a = 1$. Based on **Figure 4**, and considering the red line when $a = 1$, five diafiltration volumes are needed to achieve 99% contaminant removal (purity). In a non-CVD situation, the number of diafiltration volumes required increases with an increase in a to achieve the same purity level.

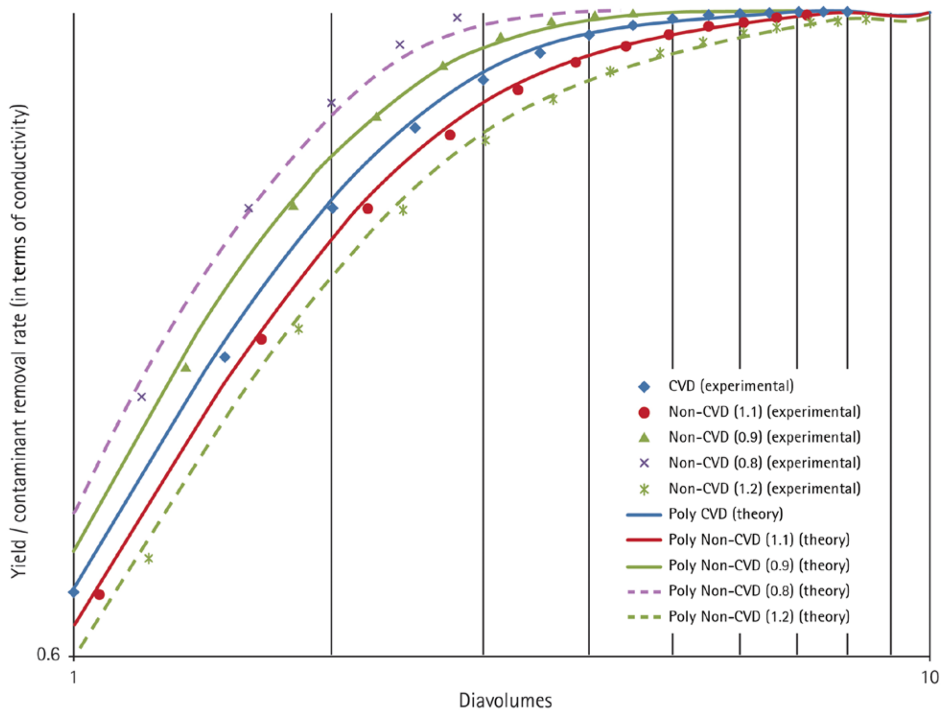

FIGURE 3. Contaminant removal rate comparisons between experimental data (coloured bullets) and theoretical calculations (coloured continuous lines).

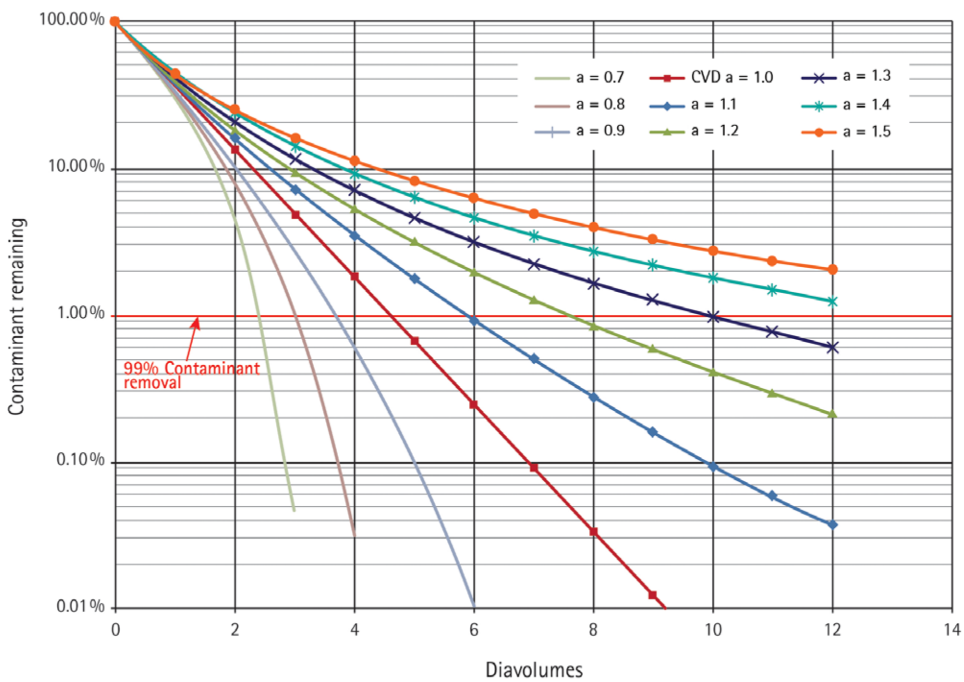

FIGURE 4. Calculated contaminant removal rate versus diavolumes at $R = 0$.

The effectiveness of contaminant removal can also be impacted by R. Equation 10 may be used to study the effect of contaminant retention on diafiltration volume requirements for a given purity and non-CVD situation (*i.e.*, $a \neq 1$). **Figures 5** and **6** demonstrate the impact of contaminant retention on diafiltration volume requirements for a given purification factor for $a = 0.8$ and $a = 1.2$, respectively. They also indicate that diafiltration volumes need to be increased in order to achieve the same purity level when the contaminant retention increases.

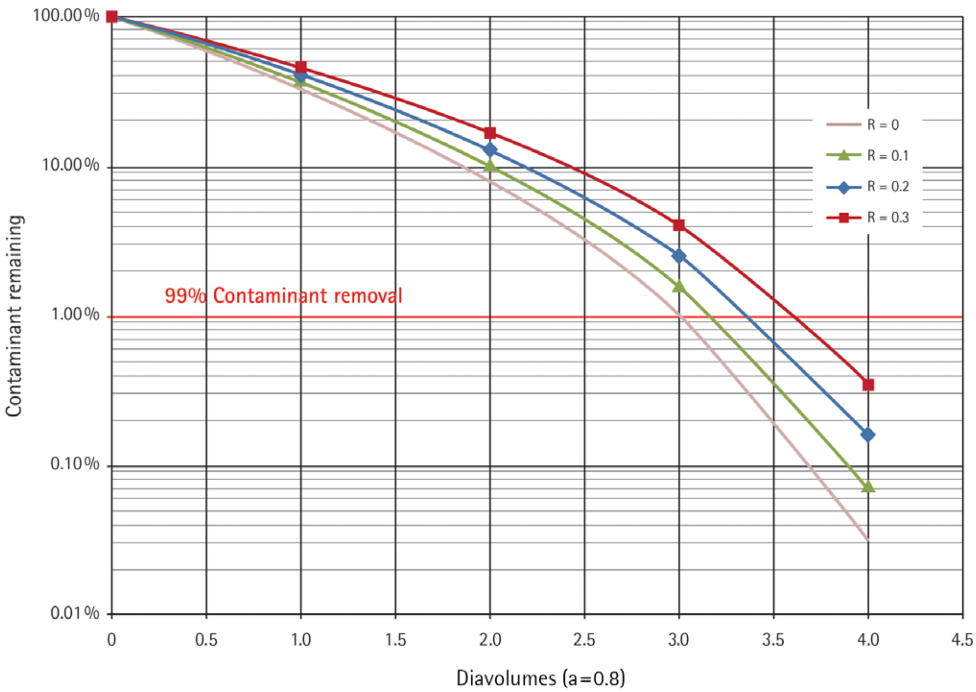

FIGURE 5. Contaminant removal rate versus diavolumes at $a = 0.8$.

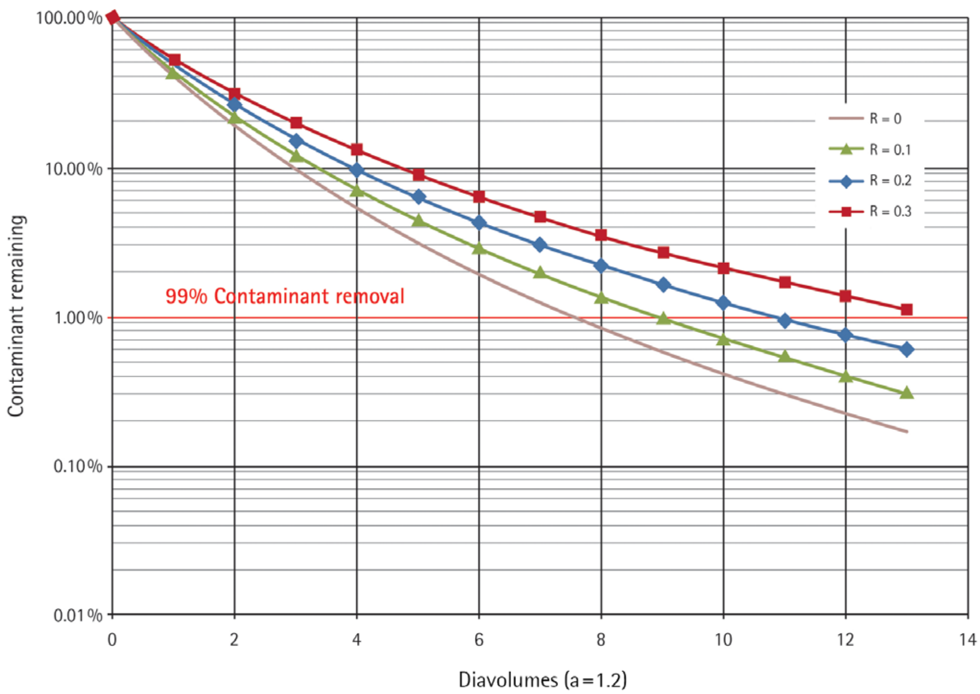

FIGURE 6. Contaminant removal rate versus diavolumes at $a = 1.2$.

Summary

This article provides an effective method to analyze and predict the impact of non-CVD conditions or situations on diafiltration effectiveness in biopharmaceutical processing. The contaminant removal rate was compared between CVD and non-CVD, and experimental data corroborated well with the model demonstrating that the contaminant removal rate can be predicted using the CVD and non-CVD mathematical models.

It was shown that the effectiveness of the contaminant removal can be affected by membrane retention (R) of the contaminant and the ratio of buffer addition rate to buffer removal rate (a). Contaminant removal effectiveness decreases when the buffer addition to the recycle tank or membrane retention increases. Hence, more diafiltration volumes would be required to achieve target formulation.

CVD is the most commonly applied methodology for buffer exchange or contaminant removal in biopharmaceutical processing. However, situations leading to non-CVD may occur because of failure or malfunctioning of control techniques (*e.g.*, level or flow control) that are used to establish a CVD condition. In the event that such a situation arises, the analysis in the current article helps the reader to understand and evaluate the potential impact of the extent of a non-CVD condition (a) on the contaminant removal accomplished under these conditions. This analysis may also help users conduct a risk analysis to determine the potential impact and/or severity of a deviation from a CVD condition.

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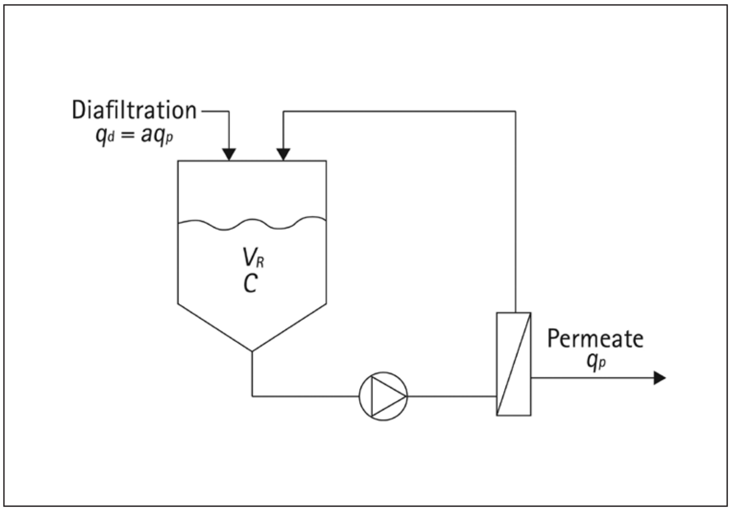
Appendix: Theoretical Calculation

Assume that there is constant passage (initial sieving) of contaminants at all times, and the process is not buffer-dependent.



q_p = permeate rate
q_d = diafiltration rate
a = q_d/q_p
V = retentate volume at time t
V_0 = initial retentate volume before diafiltration
t = time
N = $q_d t/V_0$ (total diavolume/initial retentate volume)
N_D = number of diavolumes
C = contaminant concentration at time t
C_0 = contaminant concentration at time zero ($t=0$)
S = sieving coefficient ($S = C_p/C$)
R = retention ($R=1-S$)
Y = yields

Input – Output = Accumulation:

$$\frac{dV}{dt} = q_d - q_p = aq_p - q_p = q_p\,(a-1) \tag{13}$$

Solving from V_0 at $t=0$ up to V at t:

$$V_t = V_0 + q_p(a-1)t \tag{14}$$

Component:

$$\frac{d(VC)}{dt} = 0 - C_p q_p \tag{15}$$

Where $C_p = SC$:

$$-SC = C\frac{dV}{dt} + V\frac{dC}{dt} \tag{16}$$

Substitute dV/dt from equation 1:

$$-SC = Cq_p\,(a-1) + V\frac{dC}{dt} \tag{17}$$

$$\frac{dC}{dt} = \frac{-Cq_p\,(S+a-1)}{V} \tag{18}$$

Because:

$$R = 1 - S \tag{19}$$

$$\frac{dC}{dt} = \frac{-Cq_p\,(a-R)}{V} \tag{20}$$

or:

$$\frac{dC}{C} = \frac{-q_p\,(a-R)}{V}dt \tag{21}$$

Substitute V from equation 2:

$$\frac{dC}{C} = \frac{-q_p\,(a-R)}{V_0 + q_p(a-1)t}dt \tag{22}$$

Substitute:

$$m = V_0 + q_p(a-1)t \tag{23}$$

$$dm = q_p(a-1)dt \tag{24}$$

$$dt = \frac{dm}{q_p(a-1)} \tag{25}$$

Rearrange equation 22:

$$\frac{dC}{C} = \frac{-q_p\,(a-R)\,dm}{q_p(a-1)m} = \frac{-(a-R)\,dm}{(a-1)m} \tag{26}$$

Integrate from $t=0$ (when $m=V_0$ and $C=C_0$) and t (when $m=V_0+q_p\,(a-1)t$ and $C=C$):

$$\ln\frac{C}{C_0} = \frac{-(a-R)}{(a-1)}\ln\left[\frac{V_0 + q_p(a-1)t}{V_0}\right] \tag{27}$$

Because $N = q_p t/V_{Ri}$:

$$\ln\frac{C}{C_0} = \frac{-(a-R)}{(a-1)}\ln\left[\frac{V_0}{V_0} + N(a-1)\right] \tag{28}$$

$$\ln\frac{C}{C_0} = \frac{-(a-R)}{(a-1)}\ln[1 + N(a-1)] \tag{29}$$

$$\frac{C}{C_0} = [1 + N(a-1)]^{\frac{R-a}{(a-1)}} \tag{30}$$

Having defined $Y^P_{non\text{-}CVD}$ = *non-CVD % contaminant removal*:

$$Y^P_{non\text{-}CVD} = 1 - \left(\frac{V}{V_0}\right)\frac{C}{C_0} \tag{31}$$

Replacing C/C_0 with equation 30:

$$Y^P_{non\text{-}CVD} = 1 - [1+N(a-1)][1+N(a-1)]^{\frac{R-a}{(a-1)}} \quad (a \neq 1) \tag{32}$$

Simplifying:

$$Y^P_{non\text{-}CVD} = 1 - [1 + N(a-1)]^{\frac{R-1}{(a-1)}} \quad (a \neq 1) \tag{33}$$

In case of full component passage ($R=0$):

$$Y^P_{non\text{-}CVD} = 1 - [1 + N(a-1)]^{\frac{1}{1-a}} \quad (a \neq 1) \tag{34}$$

Continued on the following page ⟶

Appendix: Theoretical Calculation

Theoretical Calculated Yield for $R=0$ (full passage of contaminants):					
	$a=$	**0.8**	**0.9**	**1.1**	**1.2**
N	**Yield in Permeate, CVD**	**Yield in Permeate, Non-CVD**			
1	0.6321	0.6723	0.6513	0.6145	0.5981
2	0.8647	0.9222	0.8926	0.8385	0.8141
3	0.9502	0.9898	0.9718	0.9275	0.9046
4	0.9817	0.9997	0.9940	0.9654	0.9471
5	0.9933	1.0000	0.9990	0.9827	0.9688
6	0.9975		0.9999	0.9909	0.9806
7	0.9991		1.0000	0.9950	0.9874
8	0.9997			0.9972	0.9916
9	0.9999			0.9984	0.9942
10	1.0000			0.9990	0.9959
11	1.0000			0.9994	0.9970
12	1.0000			0.9996	0.9978
13	1.0000			0.9998	0.9983
14	1.0000			0.9998	0.9987
15	1.0000			0.9999	0.9990
16	1.0000			0.9999	0.9992
17	1.0000			1.0000	0.9994
	$Y_{CVD}^{P} = 1 - \exp(-N)$	$Y_{non\text{-}CVD}^{P} = 1 - [1 + N(a-1)]^{\frac{1}{(1-a)}}$			

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