Modélisation d'une étape de filtration tangentielle pour optimiser le développement d'un procédé de biomédicament

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Résumé

La filtration à flux tangentielle (TFF), parfois appelée filtration à flux croisés, est une technique de séparation couramment utilisée dans les applications biopharmaceutiques. Dans la filtration tangentielle, une membrane sert à retenir sélectivement certains composés en fonction de leur taille. Les processus membranaires sont utilisés pour stériliser, pour récolter la biomasse après la fermentation ou pour séparer ou concentrer des produits. Le système de flux tangentiel se caractérise par une vitesse de circulation élevée entraînée par la pression, tangentielle à la surface de la membrane afin de réduire l'encrassement et la formation de gâteau, qui ont un impact considérable sur la résistance du fluide à travers la membrane. Les composés qui traversent la membrane forment le flux de perméat, tandis que ceux qui sont retenus forment le flux de rétentat.
Le traitement en aval d'un bioprocédé (le downstream process) implique généralement une ou plusieurs étapes d'ultrafiltration/diafiltration (UF/DF), l'une des applications les plus largement utilisées de la TFF. Une étape d'UF/DF se compose d'une première séquence de diminution du volume (ultrafiltration) à quantité de composé d'intérêt fixe, puis d'une étape à volume constant afin de remplacer un tampon par un autre. L'objectif d'une étape UF/DF est principalement de réduire les volumes de lots et d'échanger les tampons avant la formulation finale.
Les modèles computationnels basés sur une description mécanistique des phénomènes et des relations empiriques peuvent être utilisés pour accélérer le développement des procédés et faciliter l'exploration de l'espace de conception (design space) tel que défini par les autorités de santé (ICH Guideline Q8 – European Medicine Agency). La modélisation de la filtration tangentielle repose sur le bilan des concentrations des composants dans le réservoir, dans le perméat et dans le rétentat pour chaque composé. Ce bilan prend en compte la quantité de composé qui sort dans le perméat et la quantité qui entre en ajoutant un tampon. Ce bilan est adapté aux étapes d'ultrafiltration et de diafiltration. Le flux de perméat est modélisé comme une fonction de la pression transmembranaire (TMP) et de la résistance en utilisant la loi de Darcy. La résistance au flux à travers la membrane pour l'étape de la concentration est décrite comme une fonction du volume alimenté cumulatif envoyé à la membrane, de la pression transmembranaire et du débit d'alimentation. La résistance au flux à travers la membrane pour l'étape de la diafiltration est décrite comme une fonction de la masse des impuretés dans le rétentat et du volume traversant la membrane pendant l'étape de concentration. La partition de chaque composant entre le rétentat et le perméat est modélisée à travers le coefficient de rétention. Cette modélisation est réalisée dans le logiciel gPROMS FormulatedProducts, développé par Siemens.
Dans le cadre du développement d'une étape UF/DF pour un nouveau biomédicament, des expériences ont été réalisées afin de recueillir des données réelles permettant de calibrer le type de modèle mécanistique décrit ci-dessus. Des paramètres mesurables, dans ce cas le débit de perméat, la pression transmembranaire et les concentrations de composés d'intérêts sont utilisés pour calibrer le modèle de membrane et estimer les paramètres non mesurables des fonctions empiriques des résistances pour les étapes d'ultrafiltration et de diafiltration. Ensuite, le modèle a été validé avec d'autres jeux d'expériences indépendants. Une analyse globale du système est réalisée afin d'explorer et de déterminer les paramètres de fonctionnement optimaux. Cette analyse repose sur un nombre important de simulations faisant varier les paramètres procédés. L'impact de la TMP, du débit d'alimentation et du volume final après la phase de concentration sur la durée du processus est étudié. L'approche par modélisation de la TFF permet une meilleure compréhension du module, le réglage des paramètres de fonctionnement, l'optimisation de la durée d'étape, aide à définir l'espace de conception pour l'approche QbD (Quality by Design), le passage à l'échelle et à limiter le nombre de tests.

Modeling a tangential filtration step to optimize the development of a biopharmaceutical process

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Abstract

Tangential Flow Filtration (TFF), sometimes called cross flow filtration, is a separation technique commonly used in biopharmaceutical applications. In tangential filtration, a membrane is used to selectively retain certain compounds according to their size. Membrane processes are used to sterilize, harvest biomass after fermentation, or separate or concentrate products. The tangential flow system is characterized by a high circulation speed driven by pressure, tangential on the surface of the membrane to reduce fouling and cake formation, which have a considerable impact on the resistance through the membrane. Compounds that pass through the membrane form the permeate flow, while those that are retained form the retentate flow.
The downstream processing of a bioprocess usually involves one or more ultrafiltration/diafiltration (UF/DF) steps, one of the most widely used applications of TFF. A UF/DF step consists of a first volume reduction sequence (ultrafiltration) at a constant quantity of compound of interest, then a step at constant volume to replace one buffer with another. The objective of a UF/DF step is mainly to reduce batch volumes and exchange buffers before the final formulation.
Computational models based on a mechanistic description of phenomena and empirical relationships can be used to accelerate process development and facilitate the exploration of design space as defined by health authorities (ICH Guideline Q8 – European Medicine Agency).
Tangential filtration modelling is based on the assessment of concentrations of components in the tank, permeate, and retentate for each compound. This assessment considers the amount of compound that comes out in the permeate and the amount that enters by adding a buffer. This assessment is adapted to the ultrafiltration and diafiltration steps.
The permeate flow is modeled as a function of transmembrane pressure (TMP) and resistance using Darcy's law. The resistance to flow through the membrane for the concentration step is described as a function of the cumulative volume sent to the membrane, transmembrane pressure, and feed rate. The resistance to flow through the membrane for the diafiltration step is described as a function of the mass of impurities in the retentate and the volume passing through the membrane during the concentration step. The partition of each component between the retentate and the permeate is modeled through the retention coefficient. This modelling is carried out in the gPROMS Formulated Products software, developed by Siemens.
As part of the development of a UF/DF step for a new biopharmaceutical, experiments were carried out to collect real data to calibrate the type of mechanistic model described above. Measurable parameters, in this case permeate flow, transmembrane pressure, and concentrations of compounds of interest are used to calibrate the membrane model and estimate the non-measurable parameters of empirical resistance functions for the ultrafiltration and diafiltration steps. Then, the model was validated with other independent experiment sets.
A global system analysis is performed to explore and determine optimal operating parameters. This analysis is based on a significant number of simulations that vary the process parameters. The impact of TMP, feed rate and final volume after the concentration phase on the duration of the process is studied. The TFF modelling approach allows to better understand the module, to optimize the operating parameters, to help to define the design space for the QbD (Quality by Design) approach, to scale the process and to limit the number of physical experiments.

Article

Introduction

A membrane acts as a physical barrier through which pure solvent can pass while other molecules or particles are retained. In the case of ultrafiltration (UF), this semi-permeability is primarily due to the relative sizes of the solute/particles and the membrane pores. UF is typically done in a crossflow mode, also known as tangential flow filtration (TFF), rather than in dead-end mode. The reason for using crossflow mode in UF is to minimize cake formation, i.e., the accumulation of a layer of solids on the membrane surface, which leads to a reduction in flow through the membrane. It is important to note that cake formation does not normally occur in UF. This process exhibits a phenomenon referred to as concentration polarization. This involves the formation of a gradient in solute concentration, with the highest concentration near the membrane and the lowest in the bulk flow, as described by par Foley, G in 2013. Ultrafiltration, along with the related technique of diafiltration, is used widely in industry (Lutz, H et al. in 2015; Elich T, et al. 2019). Membrane-based TFF unit operations are used for concentration and purifications of solutions of macromolecules of all kinds, especially proteins. The downstream processing of bioprocess usually involves one or more ultrafiltration/diafiltration (UF/DF) steps. A UF/DF step considered here consists of a first volume reduction sequence (ultrafiltration) at a fixed quantity of the compound of interest, followed by a step at constant volume to replace one buffer with another. The main goal of a UF/DF step is to reduce batch volumes and exchange buffers prior to the final formulation.
Computational models of TFF can be used to accelerate process development and facilitate the exploration of design space as defined by health authorities (ICH Guideline Q8 – European Medicine Agency).
In the literature, numerous articles have been published on the modeling of UF systems, such as Fuchs, M et al. in 2023; Thakur, G et al. in 2021; Marcos B. et al., 2009. These articles include phenomenological (such as gel-polarization, osmotic pressure, resistance-in-series, and fouling models) and non-phenomenological models, as well as updates on traditional models or the development of new ones. The article of Quezada et al., in 2021 provides an extensive review and comparison between the two types of models. A more detailed classification of models can be found in the article Bahadır Saltık et al. 2017.
In this article, we develop a mathematical model that combine a mechanistic understanding of phenomena consisting of mass balance relation with Darcy's Law, with empirical relationships of resistance terms, which is described in the following section.

Model description

In a TFF unit operation, a pump is used to generate flow of the feed stream tangentially along the surface of the membrane. An applied pressure during each pass of fluid over the surface of the membrane forces a portion of the fluid through the membrane to the filtrate side. Particles that are too large to pass through the membrane pores are retained on the retentate side. The scheme of a simple TFF system is shown in Figure 1.

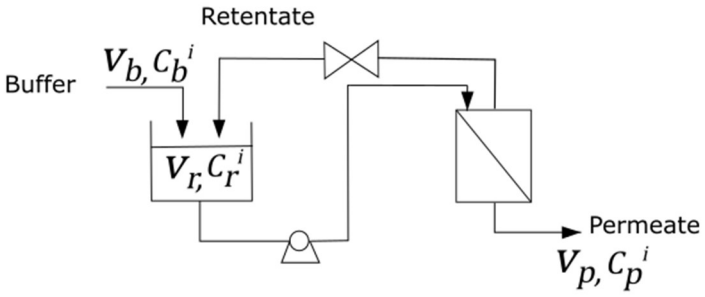


Figure 1. TFF scheme.

Model assumptions:

- The membrane handles dilute aqueous solutions.
- The contribution of the osmotic pressure to the transmembrane pressure is negligible in comparison to the operating pressures.

- Due to the high flow rates, the concentration of components is uniform across all modules of a membrane stage.

Equations

Modelling of tangential filtration is based on mass balance of components in the tank, in the permeate and the retentate for each component *i*.

$$\frac{d(C_r^i V_r)}{dt} = C_b^i \frac{dV_b}{dt} - C_p^i \frac{dV_p}{dt} \quad (1)$$

$$C_r^i \frac{dV_r}{dt} + V_r \frac{dC_r^i}{dt} = C_b^i \frac{dV_b}{dt} - C_p^i \frac{dV_p}{dt} \quad (2)$$

where $C_r^i, C_p^i, C_{b,}^i V_r, V_p, V_b$ are the concentration and volume of the retentate, permeate, and feed.
During the concentration phase, the solution is fed to a membrane where ultrafiltration takes place and the retentate is returned to the retentate tank while the permeate is removed. Consequently, the volume of feed solution decreases over time and the concentration of the protein increases. We have $\frac{dV_r}{dt} = -J\,(t)S$. During the diafiltration phase, buffer is added to the retentate tank at the same rate that the permeate is removed. The total volume of retentate remains constant throughout the process, we have $\frac{dV_r}{dt} = 0$.

The flow rate of liquid crossing the membrane to the permeate side is calculated by:

$$\frac{dV_p}{dt} = J\,(t)S \quad (3)$$

where J ($m^3/(m^2.s)$) is the permeate flux across the membrane and S (m^2) surface area of the membrane. The permeate flux, J, is then given by the following equation as per Darcy's Law for fluid flow across a membrane

$$J = \frac{P_{TMP}}{Resistance} \quad (4)$$

where $P_{TMP}$is the transmembrane pressure. The average transmemebrane pressure is calculated by

$$P_{TMP} = \frac{P_{feed} + P_{retentate}}{2} - P_{permeate} \quad (5)$$

The resistance to flux across the membrane is caused mainly by four factors [3][4]: (i) the intrinsic membrane resistance, capturing the physical restriction to flow caused by the membrane material; (ii) the concentration polarization, representing the effect of the high concentration of retained components near the membrane surface on mass transfer to the boundary layer; (iii) the gel/cake formation, representing the accumulation of retained components on the membrane surface reducing diffusion to the membrane; and (iv) the fouling, representing the effect of pore blockage over time by retained components. The factors affecting the resistance of the membrane are lumped into two broader components: the static membrane resistance and the dynamic membrane resistance (Bahadir S, in 2018).

$$R_{total} = R_{static} + R_{dynamic} \quad (6)$$

The static membrane resistance captures the membrane's intrinsic resistance and is a function of the transmembrane pressure and concentration of retained components.

$$R_{static\_concentration} = f(C_{protein}, TMP) \quad (7)$$

The dynamic resistance considers the concentration polarization, the cake formation and the fouling phenomena and is described as a function of membrane flux, transmembrane pressure, and concentration of retained components.

$$\frac{dR_{dynamic\_concentration}}{dt} = f(C_{protein}, TMP, flux) \quad (8)$$

In our model we do not consider the dynamic resistance. This choice is discussed in the Calibration section.

The selectivity of the membrane is described through a retention fraction (R), which in our model is considered constant.

$$R = 1 - \frac{C_p}{C_r} \quad (9)$$

This assumes that the protein of interest is fully retained, with a retention factor equal to 1, while for other substances the retention factor is equal to 0.

Model calibration and validation.

First, we comment on the static term resistance. Within the gPROMS, the user can select one of the three available empirical functions or can define its own custom model if other relevant factors need to be added. All three static resistance models were tested, but the current resistance expressions were not suitable for our purpose as we had to customize the resistance function to fit the experimental membrane flux. The analysis and identification of possible factors affecting the resistance in our process were carried out. The effect of buffer concentration, TMP, feed flow rate, as well as the cumulative feed volume sent to the cassette, were identified as the most important factors affecting resistance. In our model, we designed a customized function for each of the concentration and diafiltration stages. Resistance for the concentration stage is a function of the cumulative feed volume sent to the cassette, transmembrane pressure, and feed flow with three parameters to calibrate:

$$R_{static\_concentration} = f(FeedFlow, TMP, V;\ a_{0,}a_{1,}a_{2,}) \quad (10)$$

Resistance for the diafiltration stage is a function of the mass of impurities in the retentate and of the volume going through the membrane during the concentration stage with three other parameters to calibrate.

$$R_{dynamic\_concentration} = f(M_{impurities}, V;\ a_{0,}a_{1,}a_{2,}) \quad (11)$$

The membrane resistance terms in TFF model are calibrated using the permeate mass flow data, collected throughout the duration of the process. We conducted 10 experiments to collect datasets across a range of operating TMPs to ensure representativeness of the data. To calibrate the model, we needed data covering the range of concentrations and transmembrane pressures we are interested in exploring. Five runs were used for the model calibration and three for its validation.

Two experiments were not used during the calibration process. In these, no increase in the permeate flow rate was observed during the diafiltration phase due to less viscous buffer. These experiments were conducted at low feed flux and high TMP, which is the worst combination, leading to pore blockage. We considered that it was not necessarily to include them in the parameter estimation and to describe them with the dynamic resistance expression that assumes a drop in flux for that combination of conditions. Therefore, experiments with high TMP and low feed flow rate are out of model validation domain. It was decided to start modelling with the remainder of experiments and to include those extreme cases if needed.

To calculate the retention factor, the concentrations of the product and of the relevant impurities were measured at several time points; all the protein was retained without any leaking into to the permeate.
If the retention is known, measuring the total mass leaving the system (permeate flow rate or permeation flux) is enough for the model to calculate, through mass balance, the concentration in the system. Since concentration measurements are usually made offline and not directly integrated in the process, once the retention is known, the calculated concentration can be assumed to match the value that would be obtained if measured experimentally. If concentration measurements are available, they can be used to validate the model's predictions and make sure that there are no hidden phenomena causing deviations (such as adsorption to the membrane or leaking to the permeate). If the retention is unknown, then concentration measurements are required for calibration and to allow the accurate calculation of the fraction of material in the permeate and retentate. In theory, having the initial and final concentrations should be enough to identify deviations to the mass balance. However, having measurements throughout the process is useful to ensure that the model is calibrated with the right values or to identify and describe potential deviations.

Measurable parameters, in this case permeate flow, transmembrane pressure, and concentrations of compounds of interest, were used to calibrate the membrane model and to estimate the non-measurable parameters of empirical resistance functions for the ultrafiltration and diafiltration steps. gPROMS contains model calibration capabilities that allows finding these parameters to fit the model to experimental permeate flux data. Model calibration in gPROMS is based on the Maximum Likelihood formulation which provides simultaneous estimation of parameters in both the physical model of the process and the variance model of the measuring instruments, which comprises information associated with the variance of the error of the measurement produced by the sensor. The error bar for each experimental point represents the standard deviation. The standard deviation of the measurement errors is estimated for runs repeated several times under the same conditions and is applied to the other runs. Plots corresponding to model calibration and validation are shown in Figure 2.

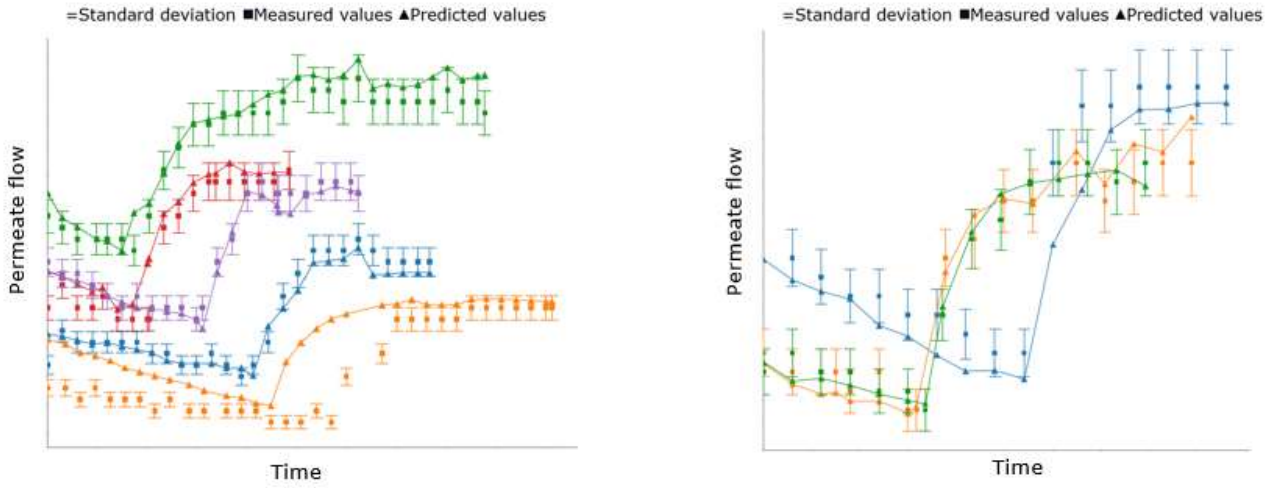


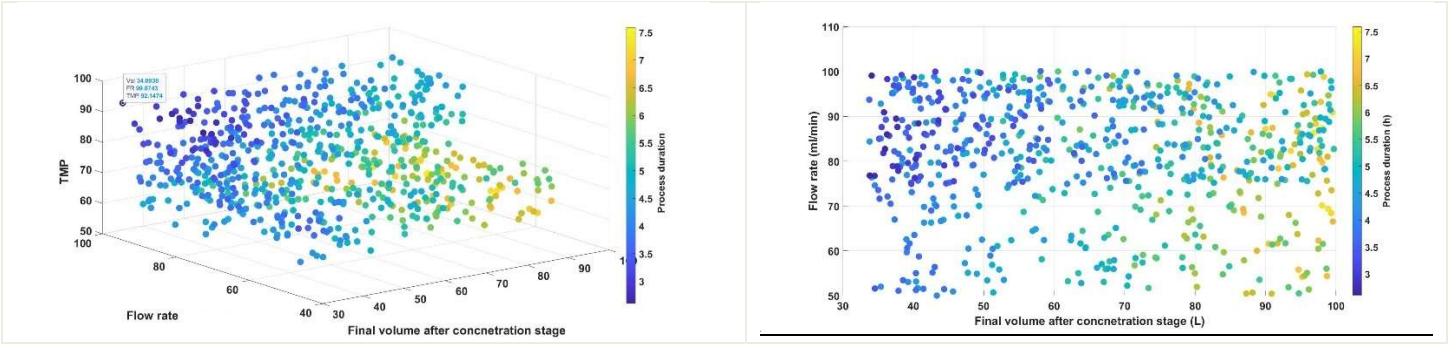
Figure 2. On the left-hand side, the model is calibrated using five different experiments. On the right-hand side, the model is validated using three other sets of experiments. The values and units are omitted.

Results

Global system analysis

Since the resistance terms are modelled empirically, it is important to emphasize that the model's use is limited to the region of operating conditions utilized to calibrate the model.

We use the Global System Analysis (GSA) within gPROMS to investigate the global behavior of the system. TMP, flow feed, and concentration factor across the range of values of interest were analyzed for their impact on the process duration using GSA. Volume load and diavolume number were held constant. Process parameters corresponding to the lowest process duration were identified and corresponded to the use of both the highest TMP and concentration.



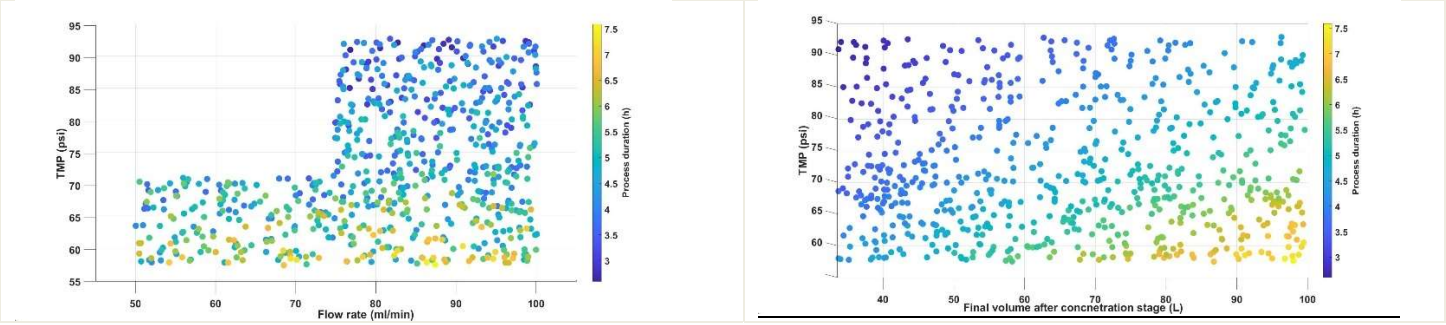


Figure 3. Impact of TMP, flow feed and Final volume after concentration phase on the process duration. All values are expressed in percentage compared to a nominal reference.

Conclusions/Discussions

A mechanistic model of TFF, combined with empirical functions to describe membrane resistance, was constructed to aid in the development of a UF/DF step for a new biopharmaceutical. To estimate the non-measurable parameters of empirical resistance functions for the ultrafiltration and diafiltration steps, experiments were carried out to collect data. The model was then validated using separate sets of independent experiments.

A global system analysis was performed to explore and identify optimal operating parameters. This analysis relied on a significant number of simulations with variations in process parameters values. The study investigated the impact of TMP, feed rate, and final volume after the concentration phase on the duration of the process.

The main benefits of TFF modelling and GSA are the following:

- Optimization of operations: GSA allows determination of the best operating parameters to reduce time and buffer consumption.
- Reduced time and cost: GSA on each unitary step allows a decrease in buffer and time for the TFF step.
- Enhanced process understanding: GSA permits a clearer comprehension of the modules.
- Scale-up and tech transfer: Further use of GSA for industrialization is planned. The current modeling was applied to research and development (R&D) scale.

Acknowledgements: The authors would like to acknowledge Pedro Matos Pereira, Juan Marin Gallego and Edward Close from Siemens for gPROMS modelling assistance and Didier Caudron from Sanofi for fruitful discussions.

This work was funded by Sanofi. The authors are Sanofi employees and may own shares and/or stock options in the company.

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