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Research Article

A shortcut method for evaluation of protein deposition onto the membrane surface in crossflow ultrafiltration

In this study, a procedure for quantifying the surface deposition of proteins in cross-flow ultrafiltration has been developed. The procedure consists of determining the protein adsorption behavior onto the membrane surface from a few dynamic measurements performed in a nonfiltration and a filtration mode, and evaluating the concentration polarization (CP) layer thickness based on the adsorption data. To predict the interdependence between the protein adsorption and CP, a simplified mathematical model has been formulated. The model was used to assess the protein adsorption and thus yield reduction in the ultrafiltration process at different protein concentration in the solution. As a case study, ultrafiltration of aqueous solutions of BSA and lysozyme (LYZ) was examined on a polyethersulfone membrane with the molecular weight cutoff of 10 or 100 kDa. The protein concentration in the solutions varied within a relatively low concentration range, i.e. below 10 mg mL^{-1} , characteristic for solvent exchange between sequential operations of protein purification by chromatography and extraction. Both proteins markedly differed in the mechanism of surface deposition; for BSA hydrophobic interactions were suggested to be dominant, whereas in case of LYZ electrostatic interactions contributed the most to the deposition mechanism. The effect of additives of the protein solutions, i.e. inorganic salts, PEG, and urea depended on the adsorption mechanism and was also specific for each protein. Nevertheless, the proposed procedure performed well in the evaluation of surface deposition and yield reduction, regardless of the protein type and its solvent environment.

Keywords: BSA / Concentration polarization / Lysozyme / Protein adsorption / Ultrafiltration

Received: November 8, 2015; *revised:* July 29, 2016; *accepted:* September 8, 2016

DOI: 10.1002/elsc.201500159

1 Introduction

Purification of proteins with a series combination of various downstream processing operations based on electrostatic, hydrophobic, or affinity interactions requires exchange of solvents between each subsequent stage [1]. The composition of effluents of downstream processes depends on the underlying separation mechanism. Therefore, the presence of solution components, which facilitate the realization of one operation, can be detrimental for effectiveness of another. Hence, ultrafiltration is often

applied as an effective method that provides fast adjusting the process environment by exchange of solution additives.

However, the ultrafiltration performance is limited by the phenomenon of protein deposition onto the membrane surface, which is attributed to fouling and concentration polarization (CP). Membrane fouling corresponds to the formation of a compact layer of protein molecules deposited on the membrane surface or in membrane pores, whereas the CP layer is of a loose structure and it spans from the surface to the end of the boundary layer [2]. The development of the CP layer is caused by increase in the solute concentration in the vicinity of the membrane surface due to different membrane permeability for various components of the bulk solution [3, 4]. The resulting permeate flux decline can be explained by the boundary layer theory. The difference between the mass transport rate in the bulk phase, where the solute motion is governed by convection, and in the boundary layer, in which molecular diffusion dominates, generates the concentration gradient against to the convective flow. This imposes the

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Abbreviations: CP, concentration polarization; LYZ, lysozyme; TMP, transmembrane pressure

transport of solutes in the CP layer back to the bulk solution [4]. Moreover, CP causes an increase of the osmotic pressure gradient in the membrane, which reduces the driving force for the permeate transport [5]. The CP layer thickness can be altered by ultrafiltration hydrodynamics; increase in the crossflow velocity reduces the boundary layer thickness; hence, it diminishes the protein deposition [6–8]. The fouling phenomenon causes blocking the membrane pores with retained protein molecules. It arises from adsorption onto the membrane surface and gel formation [3]. Adsorption is attributed to interactions between proteins and membrane, and protein–protein interactions between the molecules deposited on the surface [11–15]. The gel formation occurs as an effect of exceeding the solubility concentration, which initiates the protein aggregation and fouling [9, 10]. It can happen in highly concentrated protein solutions with the extent of the CP deposition.

The phenomenon of protein deposition due to the CP layer formation and fouling in ultra- or microfiltration membranes has been analyzed in a number of publications [16–20]. The interplay of fouling and CP in protein ultrafiltration has also been discussed and quantified using dynamic models based on simple boundary layer theory [16, 21–24], or on a more advanced computational fluid dynamic approach (e.g. [15, 25–29]). In all the above-mentioned approaches, the contribution of fouling to overall protein deposition was evaluated by fitting underlying kinetic and thermodynamic model parameters to the experimental flux profiles, without support of adsorption data. This can be attributed to the emphasis of that research on the correctness of flux profile predictions, whereas the extent of yield reduction due to membrane fouling has not been predicted and verified. In turn, in a number of studies focused on the quantification of the protein adsorption onto membrane surface, the interdependence between CP and fouling has not been taken into account (e.g. [30–34]).

The latter issue is considered in this study, which is focused on the analysis and quantification of the protein deposition onto membrane surface in crossflow ultrafiltration as a result of the combined effect of CP and fouling. For this purpose, we employed a dynamic method to measure the adsorption isotherm, and a simplified mathematical model to examine the interdependence between the CP layer thickness and protein adsorption. The model was formulated based on the results of ultrafiltration experiments performed under specified hydrodynamic conditions.

The procedure developed cannot be adopted for highly concentrated protein solutions, for which gel is formed onto the membrane surface. Nevertheless, a number of downstream operations are realized at relatively low protein concentrations, e.g. extraction and chromatography and their sequential combinations, which requires additional solvent exchange operations.

Two proteins differing in the adsorption behavior were selected for a case study: BSA and lysozyme (LYZ). The protein solutions were subjected to crossflow ultrafiltration using a polyethersulfone membrane, which is the commonly used membrane type in protein ultrafiltration. The process was performed under various operating conditions, including the protein concentration, feed flowrate, and transmembrane pressure (TMP). Various constituents of aqueous protein solutions were considered such as: inorganic salts, i.e. sodium chloride and

ammonium sulfate, or urea and PEG, which are frequently employed in downstream protein processing using chromatography or extraction.

1.1 Mathematical modeling

1.1.1 Pore-flow model

This model supposes a constant concentration of the solute and the solvent over the membrane. The permeate transport through membrane is driven by the pressure difference over the porous membrane, which can be described by the Darcy's law:

$$J_p = \frac{\Delta p}{R \eta} \quad (1)$$

where J_p is the permeate flux, Δp is the pressure difference over the membrane (TMP), η is viscosity of the solution, and R is the membrane resistance that couples characteristic membrane parameters.

Deviations from Eq. (1) can be caused by protein deposition onto the membrane surface due to CP, adsorption, and gel layer formation. These factors induce additional resistances on the feed side to the transport across the membrane. Moreover, CP causes an increase of the osmotic pressure, Π , at the membrane surface, which reduces the driving force for the permeate transport [11]:

$$J_p = \frac{\Delta p - \Pi}{(R + R_\Sigma) \eta} \quad (2)$$

where R_Σ includes all additional resistances: $R_\Sigma = R_{\text{ads}} + R_{\text{gel}} + R_{\text{CP}}$, caused by adsorption (R_{ads}), gel layer (R_{gel}), or CP layer (R_{CP}) formation. If the protein concentration is markedly below the solubility limit, the contribution of gel layer resistance can be neglected.

1.1.2 Steady state model of the ultrafiltration module

To quantify protein deposition onto the membrane surface, a simplified steady state model of the ultrafiltration module was formulated, in which the following assumptions have been made:

- (i) Protein is rejected from the membrane pores, as it is required for solvent exchange operations; therefore, the protein transport through the membrane is negligible.
- (ii) Variation of the permeate flowrate in longitudinal direction is negligible.
- (iii) CP layer thickness is constant over the axial coordinate.
- (iv) Due to a low protein concentration in solutions, the protein solubility limit is not exceeded and gel is not formed.

1.1.2.1 Steady state mass transport in the CP layer. The mass balance equation for the protein in the CP layer, in the direction of the mass transfer perpendicular to the membrane surface, can be represented as follows [11]:

$$w_p \frac{\partial C_{\text{CP}}}{\partial y} = \frac{\partial}{\partial y} \left(D_m \frac{\partial C_{\text{CP}}}{\partial y} \right) \quad (3)$$

where y is the space coordinate perpendicular to the membrane surface; C_{CP} is the protein concentration in the CP layer; D_m is the diffusion coefficient of the protein in the layer; and w_p is the

steady state linear permeate velocity (equivalent to the steady state permeate flux).

The boundary conditions for Eq. (3) can be expressed as follows:

$$y = 0, \quad C_{CP} = C_b \quad (4)$$

$$y = \delta, \quad w_p C_\delta = D_m \frac{\partial C_\delta}{\partial y} \quad (5)$$

where δ is the CP layer thickness; C_b is the bulk protein concentration; and C_δ is the protein concentration at the membrane surface.

1.1.2.2 Steady state mass transport in the bulk phase. The mass transport of the protein in the bulk phase in the axial direction can be written as follows:

$$w_x \frac{\partial C_b}{\partial x} + C_b \frac{\partial w_x}{\partial x} = D_{L,a} \frac{\partial^2 C_b}{\partial x^2} \quad (6)$$

where x is the axial coordinate; w_x is the linear retentate velocity in the longitudinal direction; and $D_{L,a}$ is the apparent dispersion coefficient that accounts for indirectly all possible departures of the flow distribution from the plug flow.

The differential change in the mass flux of the mobile phase (solvent) is related to the corresponding change of the permeate flux:

$$d(\rho_x Q_x) = -d(\rho_p Q_p) \quad (7)$$

where Q_x is the flowrate in axial direction (retentate flowrate), which varies in the axial direction; Q_p is the permeate flowrate, which, according to the assumption we made, is constant in axial direction; and ρ_x and ρ_p are the densities of retentate and permeate, respectively.

The protein solution density is concentration independent in a relatively wide concentration range [15, 35]; therefore, a constant density for the mass transfer simulations can be assumed. This results in Eq. (8):

$$s h dw_x = -s w_p dx. \quad (8)$$

Hence,

$$\frac{\partial w_x}{\partial x} = -\frac{w_p}{h} \quad (9)$$

where s and h are the width and height of the membrane module channel, respectively.

The boundary conditions can be written as follows:

(i) at the channel inlet, for $x = 0$:

$$w_F (C_F - C_b) = D_{L,a} \frac{\partial C_b}{\partial x} \quad (10)$$

where w_F is the linear velocity of the feed stream and C_F is the feed concentration;

(i) at the channel outlet, for $x = l$:

$$\frac{\partial C_b}{\partial x} = 0 \quad (11)$$

where l is the membrane length.

The integration of Eq. (3) provides the axial profile of the protein concentration at the membrane surface:

$$C_\delta(x) = C_b(x) \exp\left(\frac{w_p \delta}{D_m}\right) \quad (12)$$

where $C_b(x)$ is the axial distribution of the bulk protein concentration, which can be obtained from the solution of the set of Eqs. (6)–(11). If a plug flow can be assumed, i.e. $D_{L,a} = 0$, the steady state distribution of the protein concentration in the axial direction can be calculated by combining Eqs. (6) and (9), which results in the following equation:

$$C_b(x) = \frac{C_F w_F}{w_F - \frac{w_p}{h} x}. \quad (13)$$

The protein concentration in the adsorbed phase, q^* , is related to C_δ by the isotherm equation. In case of a convex curvature of isotherm shape, the Langmuir isotherm can be used:

$$q^* = \frac{q^\infty K_S C_\delta}{1 + K_S C_\delta} \quad (14)$$

where q^∞ is the saturation capacity and K_S is the equilibrium constant.

For a concave curve of the adsorption isotherm, which is often reported for proteins on hydrophobic surfaces [36], the BET isotherm can be used. The BET equation for liquid–solid adsorption equilibrium can be expressed as follows [37, 38]:

$$q^* = \frac{q^\infty K_S C_\delta}{(1 - K_L C_\delta)(1 - K_L C_\delta + K_S C_\delta)} \quad (15)$$

where K_S and K_L are the equilibrium constants characterizing interactions between the adsorbent surface and the protein and between the adsorbed molecules of proteins in adjacent layers, respectively.

The total amount adsorbed onto the membrane surface can be calculated as follows:

$$q_{\text{tot}}^* = s \int_0^l q^*(x) dx. \quad (16)$$

For the average concentration in the adsorbed phase it holds:

$$\bar{q}_{\text{tot}}^* = \frac{q_{\text{tot}}^*}{A}. \quad (17)$$

2 Materials and methods

2.1 Instruments

The crossflow ultrafiltration experiments were performed using Minim II System, i.e. a benchtop Tangential Flow Filtration system with a peristaltic pump, three pressure gauges, and a temperature gauge (Pall Life Sciences, Poland). The system was equipped with a tangential flow filtration capsule that contained a low protein binding polyethersulfone ultrafiltration membrane, Omega, with the pore size of 10 or 100 kDa cutoffs. The maximum volume of the solution was 1 L, and the minimum volume was about 15 mL.

Figure 1. Schematic drawing of the experimental setup. 1 = Tangential flow filtration capsule; 2 = feed reservoir; 3 = peristaltic pump; 4 = temperature gauge; 5 = pressure gauge; 6 = TMP screw clamp; F = feed; P = permeate; R = retentate.

The membrane capsule comprised two parallel membranes with the total surface area $A = 0.007 \text{ m}^2$. Two channels for the fluid phase had dimensions as follows: length $l = 0.167 \text{ m}$, width $s = 0.021 \text{ m}$, height $h = 2.5 \times 10^{-4} \text{ m}$. A simplified scheme of the system is shown in Fig. 1.

To analyze the protein concentration, we used an ÄKTA purifier system (GE Healthcare Life Sciences, Poland) equipped with a conductometric and UV detector. The injector was a rheodyne sampling valve with a sample loop of 0.1 mL.

To analyze the aggregate fraction in the BSA commercial product, an HPLC chromatographic system (Hitachi, Merck, Germany), equipped with a size-exclusion column Super SW 3000 TSK Gel (Tosoh Bioscience LLC, Tokyo, Japan) $\text{id} = 0.4 \text{ cm}$, $L = 30 \text{ cm}$, was used.

2.2 Chemicals

The protein and chemicals were purchased as follows: albumin from bovine serum lyophilized powder BSA (Sigma, Poland) with the purity $>96\%$, with a molecular weight of 66.5 kDa, $\text{pI} 4.7$ (negatively charged at pH 7), LYZ from chicken egg white, LYZ (Sigma, Poland), with the purity $> 90\%$, with a molecular weight of 14.3 kDa, $\text{pI} 11.3$ (positively charged at pH 7), poly(ethylene glycol), PEG with an average molecular weight of 3015–3685 Da (Aldrich, Poland), ammonium sulfate pure p.a. (Chempur, Poland), sodium chloride p.a. NaCl (POCH, Poland), disodium hydrogen phosphate (POCH).

2.3 Procedures

2.3.1 Determination of membrane permeability in crossflow ultrafiltration

Aqueous solutions of additives: inorganic salts, i.e. ammonium sulfate 3–15% w/v ($\text{g mL}^{-1} \times 100\%$), sodium chloride 3–15% w/v, urea 2.4–24% w/v, and PEG 0.3–15% w/v, were prepared using phosphate buffer at pH 7 in Milli-Q (Millipore, USA) water, and filtered through $0.22 \mu\text{m}$ cellulose membrane filter before use. The permeate flowrate in the crossflow ultrafiltration of solutions free of proteins was measured at steady state in function of TMP, which ranged from 0.7 to 3.5 bar.

The membrane permeability in solutions of proteins with the concentration $0.25\text{--}8 \text{ mg mL}^{-1}$ was measured at TMP ranged from 1 to 3 bars. The maximum of the salt concentration in the protein solutions was limited by the protein solubility.

2.3.2 Determination of protein deposition onto the membrane surface

The proteins were dissolved in phosphate buffer at pH 7 with or without additives, the protein concentration in the solutions ranged from 0.25 to 8 mg mL^{-1} . Prior to the adsorption measurements, the liquid contained in the membrane module and connecting capillaries was removed with a syringe. Next, the protein solution was placed into the feed tank and let to circulate in the membrane module in two different modes: (i) nonfiltration mode with full recycle of retentate at $\text{TMP} = 0$, at the permeate valve closed; or (ii) filtration mode with full recycle of both permeate and retentate at various TMP values.

When steady state was established, all of the liquid was removed from the system and recycled back into the feed tank. A sample was acquired from the feed tank for the concentration analysis. To exclude adsorption of proteins onto internal surfaces of the experimental set up, the protein solution was let to circulate in the module bypassing membrane, and then subjected to the concentration analysis.

The concentration analysis was performed using a UV detector. A pulse of samples acquired was injected into the detector using the ÄKTA injection system and eluted. The mobile phase used for eluting the pulse was the same as the sample solvent. The detector signal was recorded at 280 wavelength, converted into the concentration unit using the detector calibration curve, and integrated to calculate the protein concentration. The initial concentration of the feed solution was determined in the same manner.

To minimize errors in the determination of the concentration changes in the feed tank, the volume of the feed solution subjected to ultrafiltration was small (25 or 50 mL) compared to the maximum module capacity (1 L). Hence, a large ratio of the membrane surface area to the solution volume was achieved, for which the concentration changes in the feed tank due to the protein deposition were sufficiently high to ensure good detection sensitivity and reproducibility of the measurements. All measurements were repeated two or three times.

To account for dilution of the feed stream with the residual liquid adhered to the internal surfaces of the module, a blank solution free of the protein was prepared, which contained NaCl salt (3% w/w), and let to circulate in the module in the same way, as described in this section for the protein solutions. The difference in the salt concentration before and after the operation was calculated based on the conductivity measurements in the ÅKTA system. Since the salt ions were not retained onto the membrane surface, the difference determined could be related to the system dead volumes and feed stream dilution.

The protein deposition was also measured at varying solution volume, i.e. without recycling the permeate flux. For that purpose, solutions of BSA with the initial volume of 350 mL, and concentrations about 0.5 mg mL^{-1} , were filtered out to obtain about 20 mL of the concentrated retentate. The permeate flowrate was measured at different time points. The initial and final protein concentrations in the feed tank were analyzed using the ÅKTA system, according to the procedure described in this section. Those experiments were performed for the membrane that had a cutoff of 10 kDa.

After each experiment, the membrane was regenerated with 0.3 M solution of NaOH at 40°C for 40 min. Next, the membrane was rinsed with deionized water and subsequently with 0.06 M NaOH solution, which was used for the membrane storage.

2.3.3 Measurements of the adsorption kinetics

The adsorption kinetics was measured using the same dynamic method as that employed for the isotherm determination, i.e. with full recycle of the retentate flux at $\text{TMP} = 0$. The samples of the solution were acquired from the feed tank over different time intervals and subjected to the concentration analysis until steady state was reached.

2.3.4 Numerical tool

To solve the mass balance equation in the bulk phase Eqs. (6)–(12), a finite difference method was used (see e.g. [39]). The accuracy of numerical solutions was verified by comparing the predictions at $D_{\text{L,a}} = 0$ to the corresponding analytical solutions given by Eqs. (12) and (13). Since two symmetrical membranes in the module worked in parallel, the calculations were performed for a single membrane at the feed flowrate reduced by half.

3 Results and discussion

3.1 Adsorption onto the membrane surface

3.1.1 Determination of the isotherm equation in nonfiltration mode

The adsorption measurements were performed with the dynamic method, in nonfiltration mode, in which retentate freely circulated over the membrane at $\text{TMP} = 0$ (Section 2.3.2). The amount of the protein deposited onto the membrane surface was calculated from the protein mass deficiency measured in the feed tank at steady state, according to the following equation:

$$\bar{q}_{\text{tot}}^* = \frac{V_F (C_0 - C_{\text{eq}})}{A} \quad (18)$$

where C_0 and C_{eq} are the initial and equilibrium (steady state) protein concentrations in the feed tank, respectively. The dilution of the feed concentration with the residual liquid adhered in the dead volumes of the system, i.e. 0.5 mL at the permeate valve closed ($\text{TMP} = 0$), and 5.5 mL at the permeate valve open ($\text{TMP} > 0$), was accounted for in the calculation.

The measurements were repeated for the protein solution that circulated in the module bypassing the membrane (Section 2.3.2), which was aimed to check the possibility of protein adsorption onto internal surfaces of the experimental set up. The concentration analysis of the solution proved that the protein was not adsorbed on surfaces other than membrane.

The adsorption measurements were performed for various protein concentrations ranged from 0.5 to 8 mg mL^{-1} . Duplicate or triplicate measurements were performed for each concentration. The error bars depicted in Fig. 2A and B illustrate the measurement accuracy. The percentage analysis error was smaller for higher protein concentrations.

Additionally, a few experiments were repeated for the same protein concentration but at different solution volumes in the feed tank, i.e. 25 or 50 mL. We did not observe substantial differences in the results obtained, though, due to higher ratio of the membrane surface to the solution volume, the reproducibility of measurements performed for 25 mL solutions was slightly better.

The isotherm courses of BSA for the membranes with the exclusion cutoffs of 10 and 100 kDa are presented in Fig. 2A. In nonfiltration mode, i.e. at $\text{TMP} = 0$, the permeate flux was equal to zero, therefore, the contribution of CP to the surface deposition could be excluded. It can be observed that the isotherm course is the same for both membranes. This indicates that in both cases adsorption of BSA occurred at the membrane surface; the protein did not penetrate larger-sized pores with 100 kDa cutoff. This was additionally confirmed by the concentration analysis of filtrate.

A concave isotherm shape observed for BSA in Fig. 2A was characteristic for the presence of adsorbate–adsorbate interactions that are reported as type V isotherm [38]. We hypothesize that it arises from the aggregation of BSA, which is prone to unfold upon adsorption on hydrophobic surfaces [39–41]. Unfolded protein molecules can interact to form aggregates onto the membrane surface. The phenomena might be enhanced by the presence of dimers in the commercial BSA product. The dimer content, determined by size exclusion chromatography (Section 2.1), was 6% with respect to the total protein concentration. To reproduce the isotherm course we adopted the BET isotherm model (Eq. (15)). The isotherm parameters were determined by fitting the predictions with the isotherm model to the experimental equilibrium data using an optimization routine. The objective function, which was minimized by the routine, was the sum of the squares of the differences between the experimental data and simulations. The resulting parameters were almost the same for both membranes. The following values were estimated and used in further calculations: $H = K_S q^\infty = 0.1150 \pm 0.018 \text{ L m}^{-2}$, the equilibrium constants: $K_L = 0.0568 \pm 0.0065 \text{ L g}^{-1}$, $K_S \sim 0$. Since the inflection point of the isotherm was not localized within the investigated range of the protein concentration, the

Figure 2. BSA and LYZ deposition onto the membrane surface. (A) BSA adsorption versus the equilibrium concentration in the buffer tank, C_{eq} , at TMP = 0 (solid circles = 10 kDa, open circles = 100 kDa membrane) and TMP = 1 bar (triangles). Symbols = experimental data; lines = simulations: for TMP = 0 simulations with the BET isotherm (solid lines), for TMP = 1 bar with the steady state ultrafiltration model coupled with the BET isotherm (dashed lines); (B) LYZ adsorption; symbols and lines as in Fig. 2A, but simulations with the Langmuir isotherm; (C) steady state permeate flux, J_p , normalized with the reference to the protein-free solution $J_{p,0}$, versus the equilibrium protein concentration in the feed tank, C_{eq} , at TMP = 1 bar, the feed flowrate $Q_F = 40 \text{ mL min}^{-1}$.

value of the equilibrium constant for the first adsorption layer, K_S , tended to be zero in the optimization routine.

Since LYZ permeated large pores of 100 kDa cutoff membrane, the adsorption measurements for that protein were performed using solely the 10 kDa cutoff membrane. The isotherm course for LYZ (Fig. 2B) differed significantly from that for BSA. The structure of LYZ is stable under the operating conditions used in this study [42]; hence, it is much less prone to unfold and aggregate than BSA. Therefore, LYZ exhibited typical for solid–liquid adsorption equilibrium a concave shape of the isotherm course, which could be described by the Langmuir isotherm equation (Eq. (14)). The determined values of the isotherm coefficients were: $H = 0.849 \pm 0.036 \text{ L m}^{-2}$, $K_S = 0.230 \pm 0.021 \text{ L g}^{-1}$.

3.1.2 Determination of the interplay between adsorption and CP

In the next step, we analyzed the ultrafiltration process, where the solution passed through the membrane at various TMP values. Both fluxes of retentate and permeate were recycled into the feed tank. In such a case, the bulk volume in the feed tank remained constant. That procedure was meant to mimic the operation of solvent exchange at constant protein concentration, where the rate of fresh buffer delivery was the same as the filtration rate. The steady state permeate flowrate was measured, as well as the amount adsorbed onto the membrane for different initial protein concentrations in the feed tank. The relative drop in the permeate flux, with the reference to the solution free of the protein ($J_p/J_{p,0}$), depended on the protein concentration, which is illustrated in Fig. 2C. It can be observed that for both proteins, BSA and LYZ, the concentration dependence of the membrane permeability was noticeable only at low

protein concentration, and it diminished after reaching a certain concentration level. Because of low protein concentration, the influence of osmotic pressure on the filtration rate was negligible [15]. This may indicate that the entrances of smaller-sized pores are blocked by the adsorbed molecules of proteins already at a low adsorbed phase concentration. Further deposition of the protein at increased concentration does not substantially influence on the membrane permeability and filtration rate. The pore blockage is particularly significant for LYZ. The molecules of LYZ have better access to the membrane pores compared to BSA that is characterized by about four-fold larger size.

The protein concentration in the adsorbed phase in the filtration mode was determined according to the procedure described in Section 2.3.2. The adsorption data for BSA and LYZ obtained at TMP = 1 bar are depicted in Fig. 2A and B, respectively. The course of the adsorption curves for both proteins remained similar to that recorded at TMP = 0, i.e. concave for BSA and convex for LYZ, but with an increase in the amount of the adsorbed protein.

The adsorption enhancement can be attributed to the CP layer formation. As mentioned above, because of low protein concentration in the solution, the effect of gel formation could be excluded—the solubility of both BSA and LYZ in aqueous solution is very high, it exceeds 100 mg mL^{-1} [43, 44].

To evaluate the interplay between the protein adsorption and CP, the steady state model of ultrafiltration that consisted of Eqs. (6)–(12) was employed. The steady state longitudinal concentration profiles of the protein in the CP layer as well as in the bulk phase were calculated for each initial protein concentration in the feed tank. The permeate velocity, $w_p = Q_p/A$, was calculated using the values of the permeate flowrates measured at steady state (see the data reported in Fig. 2C).

Table 1. Adsorption behavior of BSA under various TMPs and feed flowrates at steady state; initial protein concentration in the feed tank, $C_{F,0} = 2 \text{ mg mL}^{-1}$

TMP (bar)	Q_F (mL min ⁻¹)	$Q_{p,eq}$ (mL min ⁻¹)	q^* (g m ⁻²)	δ (m) $\times 10^6$
BSA 10 kDa				
1	40	3	0.517	2.0
2	40	4.9	0.670	3.1
3	40	6.1	1.586	4.9
3	60	6.1	0.600	2.9
BSA 100 kDa				
1	20	6	1.600	4.2
1	40	8	1.220	2.1
1	80	8	0.186	~0
2	40	10	1.710	2.8
LYZ 10 kDa				
1	40	0.60	1.46	39
1	80	0.63	1.35	29
2	40	1.20	1.94	40

Since the value of the diffusion coefficient of both proteins in aqueous solution was nearly constant within the concentration range investigated [45–47], a constant value of $D_m = 5.7 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$ for BSA, and $1.1 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ for LYZ, was adopted for the mass transfer simulations. The thickness of the CP layer was calculated using Eqs. (12), and (14)–(16) for the adsorbed phase concentration measured at the highest protein concentration in the feed stream, for which the highest experiment reproducibility was achieved. The calculated δ values for both proteins are shown in Table 1. Possible deviations of the CP layer thickness due to experimental errors in determining the adsorbed phase concentration were for BSA about $2 \times 10^{-7} \text{ m}$, and for LYZ, $2 \times 10^{-6} \text{ m}$.

The δ values determined for both proteins are significantly different from each other. This is expected to arise from differences in the mechanism of interactions between the protein and membrane surface. As it is shown in the following Section 3.4.2, BSA is attracted to the surface by hydrophobic interactions, whereas for LYZ electrostatic interactions play a main role in the adsorption mechanism. Further analysis of the results obtained revealed that the value of the CP layer thickness was specific for the protein type, and that it was not equivalent to the laminar layer thickness. The latter was evaluated based on general correlations for the Sherwood number; for BSA it was found to range from about 2 to $3 \times 10^{-5} \text{ m}$, depending on the retentate flowrate, whereas the estimated CP layer thickness was of order 10^{-6} m . The possibility of the formation of the CP layer with the thickness of order 10^{-5} m was excluded on the basis of the comparison between the adsorption data acquired in the filtration mode and the model predictions. In case of LYZ, the values of δ and the laminar layer thickness were of the same order of magnitude.

Next, the δ value that was evaluated for the highest protein concentration was used to predict the amount of adsorbed proteins for other feed concentrations corresponding to the same hydrodynamic conditions. The predictions of the adsorbed phase concentrations using the determined δ value were superimposed on the experimental data. From the adsorption data of BSA

reported in Fig. 2A, it is evident that adsorption of the protein measured in the filtration mode was stronger for the 100 kDa membrane than for 10 kDa one. The reason for that was higher filtration rate for the larger-pore membrane, which intensified the protein concentration in the solution while it passed over the membrane. The concentration of the bulk solution induced the corresponding increase in the protein concentration in the CP layer, and in the adsorbed phase. Nevertheless, under similar hydrodynamic conditions in terms of TMP and the retentate flowrate, similar values of the CP layer thickness were obtained. It is evident that for a given hydrodynamic condition, one adsorption measurement can be sufficient to assess the protein deposition in the ultrafiltration process, provided that the protein solubility limit is not exceeded.

A change in hydrodynamic conditions altered the adsorbed phase concentration. An increase in TMP accelerated the filtration rate and the protein concentration, which resulted in the enhancement of the protein adsorption. It was particularly noticeable for BSA, for which increase in TMP caused growth of the CP layer (Table 1). In case of LYZ, the effect of TMP on the CP layer thickness was of minor importance. This may be attributed to a large thickness of the CP layer for that protein, which reached the value of the laminar layer thickness. The opposite trend was recorded for changes in the feed flowrate, whose increase intensified the retentate flux turbulence, and reduced the CP layer thickness. The trend was valid for both proteins. Typical adsorption data are presented in Table 1. The concentration at the membrane surface, $C_\delta(x)$, which was determined according to Eqs. (12) and (13), was averaged over the spatial coordinate, and overlaid in Fig. 2A B. It can be observed that the value of $C_\delta(x)$ is noticeably higher than the corresponding bulk phase concentration, $C_b(x)$, yet, the solubility limit was not exceeded and the possibility of gel formation at the membrane surface could be excluded. Nevertheless, the increased concentration at the membrane surface caused enhancement of adsorption of both proteins according to the isotherm course.

The CP layer thickness, δ , was estimated using the average adsorbed phase concentration. Therefore, the δ value should be considered as an average CP layer thickness over the longitudinal direction. That value can accommodate various possible distributions of the CP layer thickness along the axial coordinate, provided that their integral average is the same. Moreover, we found that δ was weakly dependent on the value of the apparent dispersion coefficient, $D_{L,a}$, chosen for the calculations. The relative difference between the average concentration of the protein at the membrane surface calculated for $D_{L,a} = 0$ with Eqs. (12) and (13), and for $D_{L,a} = 1 \times 10^{-11} - 1 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$ with Eqs. (6)–(12), was <5%. The maximum value of $D_{L,a} = 1 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$ used in the simulations corresponded to nearly uniform distribution of the protein concentration in the axial direction, with the SD about 3%. Therefore, Eq. (10) can be replaced with Eq. (13) that has a very simple formula.

The yield losses of the protein, caused by adsorption onto the membrane surface, can be calculated based on the average equilibrium concentration in the adsorbed phase: $m_{\text{loss}} = \bar{q}_{\text{tot}}^* A$. It depended on the protein concentration, TMP, hydrodynamic conditions, membrane pore sizes, and the solution volume. For instance, when a 0.5 L solution with the protein content of 5 mg mL^{-1} was filtrated using the 10 kDa membrane, at $\text{TMP} = 1$

Figure 3. Typical kinetic curves of adsorption for BSA and LYZ onto the 10 kDa membrane, at TMP = 0, the initial concentration in the feed tank: $C_0 = 5.58 \text{ mg mL}^{-1}$ for BSA, $C_0 = 6.06 \text{ mg mL}^{-1}$ for LYZ. Symbols = experiment, lines guide the eye.

bar, m_{loss} of the protein was 0.6% with the reference to the initial amount delivered into the unit, when 0.05 L of the same solution was processed, m_{loss} was one order of magnitude higher, i.e. m_{loss} was approximately 6%. The yield reduction was enhanced for higher filtration rates. In case of BSA for instance, the mass loss on the 100 kDa membrane was 1.5% for a 0.5 L solution, and 15% for a 0.05 L one. This indicates that operating conditions with high ratio of the membrane surface to the solution volume should be avoided. A similar calculation can be performed for LYZ, e.g. the yield reduction on the 10 kDa cutoff membrane was about 15% for a 0.05 L solution with the protein concentration of 5 mg mL^{-1} .

3.2 Adsorption kinetics

The adsorption kinetics was measured using the same dynamic method as that employed for the isotherm determination, i.e. in nonfiltration mode (TMP = 0) with full recycle of the retentate flux. The adsorption kinetics was monitored by analyzing the protein concentration in the feed tank over different time intervals. The rate of the adsorption process for both proteins was high; a rapid drop in the bulk protein concentration, which corresponded to the adequate concentration increase in the adsorbed phase concentration, happened in the first 5 min. In the next few minutes, the concentration changes diminished and the system approached the adsorption equilibrium. The volume of the solution in the feed tank was sufficiently small, i.e. 25 mL, to enable detection of the concentration changes over the whole operation period (see Section 2.3.2). Typical results of measurements are reported in Fig. 3.

3.3 Filtration at varying solution volume

Finally, the process was performed at varying volume of the bulk solution, i.e. without the permeate recycling. A BSA solution was subjected to ultrafiltration with the 10 kDa cutoff membrane.

The changes in the permeate flux and the concentration of BSA in the feed tank are depicted in Fig. 4. In the case illus-

Figure 4. Course of ultrafiltration with varying volume of the BSA solution (circles = experiment) and hypothetical protein concentration change in the feed tank, for which the adsorption term was neglected (stars), solid triangle = the protein concentration measured at the end of filtration; open triangle = evaluated by the model at 3 min delay in the degree of concentration. The initial protein concentration: $C_0 = 0.52 \text{ mg mL}^{-1}$; the final protein concentration: experimental $C_{\text{final}} = 8.21$, and evaluated $C_{\text{final}} = 8.05 \text{ mg mL}^{-1}$, C_R is the temporary protein concentration of retentate. Lines guide the eye.

trated there, 353 mL of 0.52 mg mL^{-1} protein solution was filtered out to obtain the volume of 19 mL, at TMP = 1 and Q_f 40 mL min⁻¹, for which CP layer thickness was evaluated to be $\delta = 2.0 \times 10^{-6} \text{ m}$, as reported in Table 1.

A hypothetical concentration change in the feed tank was calculated from the ratio of the initial and final solution volumes at neglecting the protein adsorption. To evaluate the mass loss of the protein, the concentration at the membrane surface and in the adsorbed phase was calculated based on the determined δ value. To account for the adsorption kinetics, a 3 min delay in the degree of the protein concentration in the bulk phase was assumed. The yield reduction of the protein due to its adsorption onto the membrane surface was represented by the difference between the final concentrations evaluated in both cases (see Fig. 4). Despite the rough estimation we made, the yield reduction was predicted with a reasonably good agreement with the experimental data. The mass deficiency was about 15% with respect to the initial amount of the protein introduced into the system.

It can be expected that for ultrafiltration processes performed at varying volume of the bulk solution, the yield reduction of the protein may be noticeable, particularly, when dilute solutions are filtrated out to obtain a small volume of concentrated retentate. In such a case, because of a significant concentration of the solution during filtration, the protein adsorption can be strongly enhanced.

3.4 Influence of the solution additives on the process performance

3.4.1 Membrane permeability

The permeability of the membrane in the crossflow ultrafiltration was measured for different solutions, which contained

Figure 5. Membrane permeability in crossflow ultrafiltration in the presence of (A) ammonium sulfate, sodium chloride, urea, and phosphate buffer; (B) PEG. Symbols = experimental data, lines guide the eye.

sodium chloride, ammonium sulfate, urea, or PEG, at various TMPs. The results of measurements are presented in Fig. 5, where the abscissa values correspond to the TMP normalized with respect to the solution viscosity (TMP/η). The viscosity data for the solutions were taken from literature [48–50].

As it can be observed in Fig. 5, the curves are almost linear, and the maximal permeability illustrated by the end point of each curve weakly depends on the additive concentration.

This indicates the validity of the Darcy's equation (Eq. (1)). The membrane permeability in the presence of sodium chloride and ammonium sulfate was almost the same as that recorded for phosphate buffer free of the inorganic salts. The marked drop in the permeability observed for the urea solutions might be attributed to a change in swelling properties of the membrane in the presence of urea. Moreover, the increase in solution viscosity caused the reduction of maximal permeability (compare the end points of the curves).

In case of PEG solutions, the dependence of the membrane permeability on the solution concentration was very strong. However, the slope of the curves Q_F vs. TMP/η was independent of the PEG concentration. Therefore, the permeability drop mainly stemmed from increasing viscosity of the solution, whereas the contribution of the PEG deposition onto the membrane surface reported in literature [19] was of minor importance.

3.4.2 Influence of additives on protein adsorption and filtration rate of protein solutions

The protein adsorption was also measured in the presence of the additives. The comparison of the adsorption data obtained for various solutions is presented in Table 2. It can be observed that for BSA the presence of both inorganic salts and PEG enhanced adsorption. This was caused by the intensification of hydrophobic interactions between the protein and the membrane surface, which was induced by the reduction in the protein solubility.

The enhancement of the protein adsorption reduced the permeate flux in the filtration mode ($\text{TMP} > 0$). Hence, the degree of the protein concentration in the bulk solution passing over membrane was lower in the presence of salts and PEG, than that in the presence of phosphate buffer free of additives.

The CP layer thickness was estimated in the same way, as described in Section 3.1. As it can be observed in Table 2, the presence of PEG caused significant increase in the CP layer

Table 2. Influence of solution additives on the protein adsorption onto the polyethersulfone membrane, $C_{F,0} \cong 4 \text{ mg mL}^{-1}$

Additive (% w/v)	$q^*/C_{F,eq}$ TMP = 0	Q_P/Q_{P0}	TMP = 1	$\delta \pm 0.2 \times 10^6$ (m) TMP = 1
BSA				
Buffer	0	0.15	0.67	2.0
NaCl	15	0.25	0.55	2.3
$(\text{NH}_4)_2\text{SO}_4$	7	0.22	0.42	2.9
PEG	3	0.28	0.70	7.9
Urea	3	0.088	0.61	~0
LYZ				
Buffer	0	0.46	0.10	39
NaCl	7	0.26	0.49	7.8
$(\text{NH}_4)_2\text{SO}_4$	7	0.35	0.24	15
PEG	3	0.89	0.20	—
Urea	3	0.44	0.10	—

thickness. It can be expected that PEG molecules contribute to the CP layer formation. Nevertheless, the effect of PEG on the CP layer formation requires a detailed investigation.

In case of LYZ, the presence of additives affected adsorption and ultrafiltration efficiency in a completely different manner. An addition of salts significantly reduced the amount of protein adsorbed on the membrane surface and accelerated the filtration rate. This suggests that electrostatic interactions contribute to the adsorption mechanism of LYZ, thereby, the salt ions can compete with the protein for adsorption sites on the membrane surface. Because LYZ is positively charged at pH 7, it can be expected that the membrane surface was to some extent negatively charged.

The presence of urea had no effect on adsorption of LYZ. Nevertheless, the membrane permeability in the presence of the protein and urea was so low that the process was inefficient. The same happened for solutions of LYZ in the presence of PEG. In this case, the protein solutions need to be markedly diluted before the use in ultrafiltration process.

4 Concluding remarks

A simplified procedure for assessing protein deposition onto the membrane surface and resulting yielded reduction in crossflow

ultrafiltration has been proposed. The procedure consists of the following stages: determining the isotherm equation, evaluating the CP layer thickness, and predicting the concentration dependency of the protein adsorption in the ultrafiltration process using the simplified mathematical model.

Aqueous solutions of two proteins, BSA and LYZ, were used as model systems. In case of BSA the isotherm course was concave, which is characteristic of the presence of adsorbate–adsorbate interaction. Such an adsorption behavior was attributed to aggregation of unfolded BSA molecules due to strong hydrophobic interactions with the membrane surface. The presence of inorganic salts, sodium chloride and ammonium sulfate, enhanced the interactions and thus protein adsorption, whereas an addition of urea to the protein solution diminished the adsorption.

The molecule of LYZ is less prone to unfold and aggregate, therefore, it exhibited different adsorption behavior–its isotherm was convex and followed typical Langmuirian shape. Moreover, in case of LYZ, a contribution of electrostatic interactions with the membrane surface to the adsorption mechanism was suggested. The presence of salts markedly reduced the adsorption strength for that protein. Such a phenomenon is typical for ion exchange, where salt ions compete with the solutes for adsorption sites. Since LYZ was positively charged in the solutions examined, it was concluded that the membrane surface was negatively charged, which intensified electrostatic interactions.

Adsorption of both proteins enhanced with increase of TMP and reduced with increase of the feed flowrate, which resulted form the changes in the CP layer thickness. The mathematical model was used to estimate the CP layer thickness under the specified hydrodynamic conditions. The values obtained were markedly different for both proteins, which resulted form the differences in the mechanism of their interactions with the membrane surface. This also indicated that the CP layer thickness is specific for the protein types. Nevertheless, because the CP layer thickness was found to be concentration independent, only one ultrafiltration experiment under given hydrodynamic conditions was sufficient to evaluate adsorption changes in the ultrafiltration process at varying protein concentration. The applicability of the approach proposed is limited to ultrafiltration of relatively dilute solutions, for which the solubility limit for the protein concentration on the membrane surface is not exceeded and gel formation does not occur.

Practical application

The simplified procedure suggested in this study allows predictions of the amount of the protein adsorbed onto the membrane surface in crossflow ultrafiltration based on a small number of experimental measurements. The procedure does not require sophisticated equipment and numerical methods, therefore, it can be efficiently used for the optimization of operating conditions of the ultrafiltration process.

Nomenclature

A	[m ²]	Surface area
C	[mg mL ⁻¹]	Concentration
$D_{t,a}$	[m ² s ⁻¹]	Apparent dispersion coefficient
H	[m]	Height of the membrane channel
J	[m s ⁻¹ or L m ⁻² h ⁻¹]	Permeate flux
J_0	[m s ⁻¹ or L m ⁻² h ⁻¹]	Initial permeate flux
k	[s ⁻¹]	Kinetic coefficient
K	[L g ⁻¹]	Equilibrium constant
l	[m]	Length of the membrane channel
p	[bar]	Pressure
q	[g m ⁻²]	Adsorbed phase concentration
Q	[m ³ s ⁻¹]	Flowrate
R	[m ⁻¹]	Membrane resistance
s	[m]	Width of the membrane channel
t	[s or min]	Time coordinate
V	[mL or m ³]	Volume
w	[m s ⁻¹]	Linear velocity
x	[m]	Space coordinate
y	[m]	Space coordinate

Greek symbols

δ	[m]	CP layer thickness
η	[Pa s]	Viscosity
ρ	[kg m ⁻³]	Density

Subscripts and superscripts

b	Denotes bulk
CP	Denotes concentration polarization layer
F	Denotes feed
l	Denotes length
P	Denotes permeate
tot	Denotes total amount
0	Denotes initial conditions
$*$	Denotes equilibrium conditions
$-$	Denotes average amount

The authors have declared no conflict of interest.

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