A combined fouling model to describe the influence of the electrostatic environment on the cross-flow microfiltration of BSA

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A R T I C L E   I N F O

Article history:
Received 29 October 2007
Received in revised form 12 February 2008
Accepted 22 February 2008
Available online 29 February 2008

Keywords:
Microfiltration
BSA
Transmission
Fouling
Model

A B S T R A C T

In this paper, the influence of pH in the 3–9 interval and NaCl concentration up to 25 mM on the cross-flow microfiltration of BSA was studied. A tubular ceramic membrane with a mean pore size of 0.14 μm was employed. The evolution of permeate flow and BSA transmission with time was determined at 30 °C, a cross-flow velocity of 3.28 m/s and a transmembrane pressure of 100 kPa. The flow data were discussed by means of combination of two fouling mechanisms: complete and standard blocking when transmission of proteins occurred and complete blocking and cake formations otherwise. The effective radius of the protein and the electrostatic interactions protein–membrane explained the transmission protein values.

1. Introduction

Proteins purification and separation have been widely studied because of their important properties and applications in biotechnology and food industries. Membrane technology is one of the most important processes implemented due to their economical advantages, compared to other separation techniques, and the ease of scale-up.

From the first experiments, many authors have focused on the influence of chemical environment in the separation process. Consequently, there are a significant number of works which have studied the variation of flux, adsorption, fouling or transmission of proteins as a function of pH or ionic strength.

Fane et al. [1] discussed the ultrafiltration of BSA solutions with polyethersulfone membranes (20 and 30 kDa) over a range of pH values (2–10) and salt concentrations (0–0.2 M NaCl). A minimum flux was obtained at pH 5 in the absence of salts, but it was lowest in the presence of salt at pH 2, increasing with pH. The final flux was correlated with the adsorbed protein. Maximum adsorption occurred at the isoelectric point (~pH 5) and was even greater with added salt.

Aimar et al. [2] studied the adsorption of BSA for pH values of 2.0, 4.7 and 7.2 and a concentration rate from 0.1 to 50 g/L with a 20 kDa polyacrylonitrile membrane. Adsorption isotherms correlated to Freundlich laws. The adsorbed protein increased when: (i) bulk protein increased, (ii) contact time increased and (iii) pH decreased, up to 10 g/L. For more concentrated solutions, the protein adsorbed was lowest at the isoelectric point. The presence of calcium ions increased the hydraulic resistance at pH values different from isoelectric point whereas an opposite effect was observed at iep.

Opong and Zydney [3] analyzed the filtration of BSA through ultrafiltration (30–1000 kDa) and microfiltration (0.16 μm) polyethersulfone membranes. The hydraulic permeability of protein deposit decreased with increasing filtration pressure, although variation with pressure was slow for time longer than 100 min. The permeability of the protein deposit was studied at different pH (2.0–7.4) and ionic strength (0–0.5 M NaCl) values, decreasing with increasing ionic strength and was maximum at the BSA isoelectric point.

Mochizuki and Zydney [4] employed a 0.16 μm polyethersulfone membrane to evaluate the variation of transmission of BSA with operating parameters. The flux decay was due to the formation of a protein deposit located on the surface of the membrane. The sieving coefficient also decreased along time, because of rejection of BSA by the protein deposit. The highest value was obtained at the isoelectric point of the protein. For values over and below this pH, the transmission obtained was lower and depended on the ionic strength and composition of the solution (Ca2+, Na+).

Palecek and Zydney [5] obtained data for hydraulic permeability of deposits formed during the microfiltration of BSA, lysozyme, ribonuclease A, hemoglobin, and immunoglobulins solutions (5 g/L and 69 kPa) through a 0.16 μm polyethersulfone membrane. The
steady-state permeability for all proteins was minimum at its isoelectric point, and decreased when increase salt concentration at pH values above and below the pI. An increase in ionic strength resulted in a rise in flux followed by a relatively slow flux decline.

Oppenheim et al. [6] analyzed the internal surface coverage of BSA in 100 kDa polysulfone membranes at different pH values (5 and 7) and salt concentrations (0.05 and 0.15 M NaCl) up to 4 h of ultrafiltration. The results showed an important protein accumulation in the membrane after a short time of exposure. At pH 7, the protein was likely to be lodged in the ultrathin skin area of the membrane. However, at pH 5 and low ionic strength, BSA was lodged and adsorbed throughout all the membrane structure.

Herrero et al. [7] studied the flux decline of BSA solutions with 0.1 μm polycarbonate membranes at several pH values (4, 5, 6.8 and 8) and two ionic strengths (0 and 0.15 M NaCl). In all the cases membrane fouled in two steps: a rapid initial internal blocking dependent on operation parameters, followed by an external blocking with lower sensitivity of flux on operation conditions. For those pH values far from the isoelectric point fouling was especially low when no additives were presents (neutral pH and absence of salt).

Jones and O’Melia [8] evaluated the adsorption of BSA and humic acid onto a 30 kDa regenerated cellulose ultrafiltration membrane. They calculated adsorption isotherms and rate of adsorption by measuring adsorbed mass as a function of time. Experiments were carried out at differing conditions of pH, ionic strength and bulk feed concentration. For both compounds, adsorption was higher at lower pH values and adsorption decreased as pH increased. The increase in salt concentration reduced electrostatic repulsion between like-charged material (increasing adsorption), and decreased electrostatic attraction between oppositely charged material (decreasing adsorption). They analyzed these interaction using two models, and concluded that an adequate control of electrostatic interactions could reduce adsorption onto the membrane, and consequently, long-term membrane fouling. The same authors [9] calculated reversible and irreversible fouling resistances, to study the effect of convective flow and electrostatic interactions on fouling behaviour. They filtered BSA and humic acid, varying conditions of pH and ionic strength, through 30–100 kDa cellulose membranes. Convective forces increased the amount of material accumulated near the membrane, but electrostatic forces were stronger, affecting reversible and irreversible resistances. These resistances were higher at the isoelectric point of the membrane, and decreased at higher pH values. On the other hand, humic acid adsorption decreased when pH was increased from 4.7 to 10.

Persson et al. [10] investigated the transmission of BSA through two MF membranes (nylon, 0.2 μm and polyethersulfone, 0.16 μm). The transmission was highest for the polyethersulfone membrane, and was affected by the pH. At pH 5 the transmission was 100% (almost constant during the entire experiment), and much lower at pH values of 3 and 7 (even 40%). However, an increase in the ionic strength resulted in an increase of the transmission for both MF membranes (at pH 3 and 7). This involved that protein–filter cake and protein–membrane electrostatic interactions affected the transmission. The increase in transmission near its isep (pH 5) was due to the lack of electrostatic repulsion. High ionic strength shields charged proteins from each others and from the membrane and the filter cake by the ions in the solution, acting as though they were uncharged, increasing the transmission of BSA.

Mehta and Zydney [11] observed the effect of membrane charge density on hydraulic permeability and protein transport during ultrafiltration. Using a series of charge–modified cellulose membranes, the membrane charge was evaluated from streaming potential measurements. Protein transmission decreased by a factor of 100 as the membrane potential increased from 0.3 to 6.6 mV. The protein sieving data were discussed according to a partitioning model, while the hydraulic permeability data were related to a model accounting for the effects of counter-electroosmosis.

More recently, Bowen and Williams [12] have performed a study on the quantitative predictive modelling of protein ultrafiltration processes. According to colloidal interactions and hydrodynamics, particle–particle interactions may be calculated using a cell-model description of electrostatic interactions coupled with quantification of London–van der Waals forces and the entropic pressure. The predictive calculations may be used in a number of different ways, depending on the level of knowledge of the colloidal properties available.

The aim of this work was to evaluate the electrostatic interactions in the microfiltration of a model protein through a ceramic membrane. To this aim, the effects of pH and salt concentration on the evolution of permeate flow and transmission on the cross-flow filtration of BSA were investigated. The results obtained were discussed according a combined fouling model and the effective protein size.

2. Experimental

2.1. Materials and experimental rig

BSA (molecular weight 69 kDa) was obtained from Sigma (USA). Ceramic membrane employed was Céram Inside 25 (pore radius 0.14 μm) from Tami Industries (France). This mineral membrane was tubular and consisted of a support made of a mixture of aluminium/titanium/zirconium oxides with an active layer of zirconium oxide. The membrane length was 25 cm and its hydraulic diameter 3.6 mm, giving a total surface area of 93.8 cm².

The experimental rig (Fig. 1) included the membrane housing, a variable frequency vane pump (Cole Parmer, USA), a flow meter (Badger Meter, Germany) and an analytical balance (Mettler Toledo, Switzerland), two pressure gauges located before and after the membrane and pH and temperature probes.

2.2. Determination of the pzc

The method described by Mullet et al. [13] was employed to determine the point of zero charge of the membrane, which corresponds to zero surface charge density, i.e., to equivalent amounts of negative and positive charges developed by proton equilibria.

The pzc of this membrane was calculated in a previous work [14] and was about 6.9. At initial pH values below the pzc, the hydroxyl groups at the surface of the membrane (MOH) become protonated and so positively charged (MOH⁺). On the other hand, at initial pH values above the pzc, the hydroxyl groups become deprotonated and therefore negatively charged (MO⁻).
2.3. BSA microfiltration

BSA (0.25 g/L) was dissolved in MilliQ water and adjusted to working pH (using HCl or NaOH) and salt concentration (using NaCl). All reagents were analytical grade products. In order to study the influence of pH, experiments were performed at pH values of 3, 4, 4.9, 6, 7 and 9 without addition of salt. The influence of salt concentration was tested at 0, 1, 3, 5, 10 and 25 mM in NaCl at a fixed pH of 4.9. Each experiment was carried out during 60 min under the following conditions: transmembrane pressure 100 kPa, cross-flow 400 L/h (velocity 3.28 m/s), temperature 30 °C. During cross-flow microfiltration, both retentate and permeate were recirculated to the feed tank. Permeate flow was measured by volume measurements in an analytical balance. Samples from filtrate and retentate were taken in order to determine their protein concentration by UV spectrophotometry with detection at 280 nm. The observed transmission was calculated as the ratio between the concentrations of BSA in the permeate and the retentate side.

In order to regenerate the membranes after operation, a cleaning procedure was performed consisting of an initial rinse with demineralized water, followed by recirculation of a 20 g/L NaOH + 2 g/L SDS solution at 50 °C for 30 min and a final rinse with demineralized water until neutrality. Complete recovery of the membrane was checked after each filtration by measuring the water membrane resistance.

3. Theory

3.1. Influence of electrostatic environment

The charge of a protein depends on the solution pH. The isoelectric point of a protein is the pH at which the protein has an equal number of positive and negative charges. Below the isoelectric point proteins carry a net positive charge and above it a net negative charge. For BSA its isoelectric point is around 4.9 [15,16], and the surface charge density is a function of the pH. According to Smith and Deen [17], the surface charge density for BSA, is given by the following expressions:

\[ \sigma_S = 0.0145(4.9 - \text{pH}) \]  \hspace{1cm} (1)

\[ \sigma_S = 0.0060(4.9 - \text{pH}) \]  \hspace{1cm} (2)

On the other hand, effective hydrodynamic volume of charged protein is increased by the presence of an electrical double layer, whose thickness is expressed by the Debye length:

\[ \kappa^{-1}(\text{nm}) = \sqrt{\frac{0.303}{C_{\text{salt}}}} \]  \hspace{1cm} (4)

Table 1

<table>
<thead>
<tr>
<th>pH</th>
<th>(\kappa^{-1}) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>9.6</td>
</tr>
<tr>
<td>4</td>
<td>30.4</td>
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<td>4.9</td>
<td>85.5</td>
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<tr>
<td>6</td>
<td>303.5</td>
</tr>
<tr>
<td>7</td>
<td>959.9</td>
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<tr>
<td>8</td>
<td>303.5</td>
</tr>
<tr>
<td>9</td>
<td>96.0</td>
</tr>
</tbody>
</table>

Pujar and Zydney [19] studied the protein separation in size-exclusion chromatography and membrane ultrafiltration as a combination of steric and long-range (electrostatic) interactions between the protein and the pore boundaries. In this way, effective solute radius is expressed by:

\[ r_{\text{eff}} = r_s + \frac{4r_s^2\sigma_S^2}{F \varepsilon_0 kT} \lambda^2 (1 - \lambda)^{-1} \]  \hspace{1cm} (5)

where \(r_s\) is the solute radius, \(k\) is the Boltzmann’s constant \((1.381 \times 10^{-23} \text{m}^2 \text{kg/s}^2 \text{K}), T\) is the absolute temperature and \(\lambda\) is the ratio between the solute and the pore radius of the membrane, \(r_{\text{pore}}/r_s\).

Considering \(r_s\) around 3.5 nm (Stokes radius for BSA [10]) and the expression for \(\sigma_S\) (1) and (2), Eq. (5) may be rewritten as a function of pH of the solution:

\[ \text{pH} \leq 4.9, \quad r_{\text{eff}}(\text{m}) = 3.5 \times 10^{-9} + 12.7(4.9 - \text{pH})^2 \lambda(1 - \lambda)^{-1} \]  \hspace{1cm} (6)

\[ \text{pH} \geq 4.9, \quad r_{\text{eff}}(\text{m}) = 3.5 \times 10^{-9} + 2.175(4.9 - \text{pH})^2 \lambda(1 - \lambda)^{-1} \]  \hspace{1cm} (7)

As pore diameter of the membrane is 0.14 μm, \(\lambda\) is 0.05 and therefore:

\[ \text{pH} \leq 4.9, \quad r_{\text{eff}}(\text{nm}) = 3.5 + 0.603(4.9 - \text{pH})^2 \lambda^{-1} \]  \hspace{1cm} (8)

\[ \text{pH} \geq 4.9, \quad r_{\text{eff}}(\text{nm}) = 3.5 + 0.103(4.9 - \text{pH})^2 \lambda^{-1} \]  \hspace{1cm} (9)

Assuming that partition coefficient (\(\phi\)) for an uncharged solute (due to steric effects only) can be calculated from the following expression:

\[ \phi = \frac{\pi(r_p - r_{\text{eff}})^2}{\pi r_p^2} = (1 - r_{\text{eff}}/r_p)^2 = (1 - \lambda_{\text{eff}})^2 \]  \hspace{1cm} (10)

the variation of \(r_{\text{eff}}\) and \(\phi\) with pH of the solution can be obtained (Table 2).

As we can observe, in those experiments carried out without addition of salt, there will be no transmission of BSA at pH 7, 8 and 9, because of the big effective size of the protein. At pH of the isoelectric point, the effective radius is the lowest and the highest amount of protein will pass through the membrane. Above and below this point, less amount of BSA will pass, being lower for basic than for acidic values of pH.

Table 2

<table>
<thead>
<tr>
<th>pH</th>
<th>(r_{\text{eff}}) (nm)</th>
<th>(\phi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>24.4</td>
<td>0.424</td>
</tr>
<tr>
<td>4</td>
<td>18.3</td>
<td>0.545</td>
</tr>
<tr>
<td>4.9</td>
<td>3.5</td>
<td>0.903</td>
</tr>
<tr>
<td>6</td>
<td>41.5</td>
<td>0.166</td>
</tr>
<tr>
<td>7</td>
<td>440.9</td>
<td>0.000</td>
</tr>
<tr>
<td>8</td>
<td>304.9</td>
<td>0.000</td>
</tr>
<tr>
<td>9</td>
<td>170.2</td>
<td>0.000</td>
</tr>
</tbody>
</table>
All the experiments carried out at the isoelectric point will have the same effective radius. In these cases, where the protein has the same number of positive and negative charges, addition of salt will result in an increase of the size of BSA, due to the ion shielding, reducing the aggregate formation. This will enhance the transmission of BSA through the membrane.

3.2. Flow decline models

Among the theoretical models for the flow decline, the ones based on the blocking mechanisms are well known [20]. To sum up the assumptions included in these models [21], there are four possibilities of blocking the membrane:

(i) Complete blocking: where it is assumed that each particle arriving to the membrane blocks some pore or pores, without superposition of particles.

(ii) Standard blocking: if it is considered that each particle arriving to the membrane deposits onto the internal pore walls, decreasing the pore volume.

(iii) Intermediate blocking: in this case each particle can either settle on other particle, already blocking some pore, or block directly some membrane area.

(iv) Cake filtration: finally, if it supposed that particles locates on others, previously arrived and blocking some pores, with no more space to directly obstruct more membrane area.

Usually, no individual models are susceptible of explaining the flow evolution with time, being necessary to appeal to a combination of them. Complete blocking is normally present in all the experiments [22]; in this model, flow evolution is given by:

\[ Q_f = Q_{f0} e^{-\frac{B t}{(1 + B t)}} \]  \hspace{1cm} (11)

where the flow filtration, \( Q_f \), is a function of the initial flow filtration, \( Q_{f0} \), and \( B \), constant of the complete blocking laws, related to the membrane surface blocked per unit of total volume permeated through the membrane. As pore size of the membrane is not uniform, only a fraction \( 1 - \alpha \) is susceptible of being completely blocked. The rest of pores \( (1 - \alpha) \) will be fouled by other mechanism.

The first combination is assumed in those experiments with transmission of protein through the membrane. In this case, part of the proteins passes through the membrane and deposits on the pore walls, being described by standard blocking:

\[ Q_f = \frac{Q_{f0}}{(1 + B t)^2} \]  \hspace{1cm} (12)

where \( B \), constant of the standard blocking law, is a function of the decrease in the cross-section area of the pores per unit of total permeate volume.

Finally, the second combination is assumed in those experiments with no transmission of solute, with a cake formation over the pores no susceptible of complete blocking:

\[ Q_f = \frac{Q_{f0}}{\sqrt{1 + C t}} \]  \hspace{1cm} (13)

with \( C \), constant of the cake filtration law, related to the total permeate volume per unit of membrane area and the hydraulic resistance of the cake.

In accordance to those possibilities, the flow evolution of permeate will be given by one of these two equations:

\[ Q_f = \alpha Q_{f0} e^{-\frac{Q_{f0}}{(1 + B t)^2}} + (1 - \alpha) \frac{Q_{f0}}{\sqrt{1 + C t}} \]  \hspace{1cm} (14)

or

\[ Q_f = \alpha Q_{f0} e^{-\frac{Q_{f0}}{(1 + B t)^2}} + (1 - \alpha) \frac{Q_{f0}}{\sqrt{1 + C t}} \]  \hspace{1cm} (15)

4. Results and discussion

4.1. Influence of solution pH

4.1.1. Transmission

The evolution of transmission as a function of pH is showed in Fig. 2. The highest values of transmission were obtained at the acid range of pH, from pH 3 to the isoelectric point of BSA (pH 4.9).

At pH 4.9 the transmission was the highest (65% at 5 min), remaining a considerable transmission up to 15 min of operation. At other acid values of pH, the amount of protein passing the membrane was significant, higher at pH 3 (51% at 1 min) than at pH 4 (14% at 2 min), decreasing with time and reaching a final value of transmission similar to the one at the isoelectric point (≈6%).

On the contrary, at those values of pH over the isoelectric point, the transmission was nearly null along all the experiments, with a very small pass of protein in the first minutes at pH 7 and pH 8.

The isoelectric point (\( \text{iep} \)) of BSA and the pzc of the membrane determine different pH ranges with respect to the electrostatic interactions protein–membrane. At \( \text{pH} < \text{iep} \), both protein and membrane are positively charged. If \( \text{pH} = \text{iep} \), BSA is uncharged while the membrane remains positively charged. While \( \text{iep} < \text{pH} < \text{pzc} \), protein and membrane are negatively and positively charged, respectively. When \( \text{pH} = \text{pzc} \), the membrane has no net charge and BSA is negatively charged. Finally, at \( \text{pH} > \text{pzc} \) both membrane and BSA are negatively charged. These differences in the relative signs of the charges of the membrane and BSA are crucial for the discussion of the results obtained in the filtration experiments.

The values of transmission obtained at pH 7, 8 and 9 were nearly null because of the effective pore radius of the BSA at those pHs (Table 2). In these experiments, the effective radius was greater than the pore radius of the membrane and therefore, no transmission of the protein was expected.

At pH 6 the protein (41.5 nm) was able to pass through the pore of the membrane, though the partition coefficient, only due to the BSA itself, was quite low (16.6%). Besides this, both membrane and protein were oppositely charged, enhancing BSA adsorption...
Fig. 3. Time evolution of $Q_t/Q_{t0}$ at pH 3, 4 and 4.9. Solid lines correspond to fitting of experimental data to Eq. (14).

Fig. 4. Time evolution of $Q_t/Q_{t0}$ at pH 6, 7, 8 and 9. Solid lines correspond to fitting of experimental data to Eq. (15).

Table 3

<table>
<thead>
<tr>
<th>pH</th>
<th>$\alpha$</th>
<th>$B$ (min$^{-1}$)</th>
<th>$C$ (min$^{-1}$)</th>
</tr>
</thead>
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<tr>
<td>3</td>
<td>0.534</td>
<td>0.000689</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.719</td>
<td>0.002685</td>
<td></td>
</tr>
<tr>
<td>4.9</td>
<td>0.892</td>
<td>0.003673</td>
<td></td>
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<tr>
<td>6</td>
<td>0.424</td>
<td></td>
<td>0.015044</td>
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<td>7</td>
<td>0.526</td>
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<td>8</td>
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</tr>
<tr>
<td>9</td>
<td>0.492</td>
<td>0.003396</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 5. Influence of pH over the fraction of pores susceptible of being completely blocked, no salt addition.

4.1.2. Permeate flow

The time evolution of the permeate flow at the pHs assayed, without salt addition, is shown in Figs. 3 and 4. In all cases, the main flow drop took place in the first 30 min of filtration (except at pH 6), while steady flows were practically achieved in the last 20 min. The lowest permeate flow was obtained at the isoelectric point of BSA (pH 4.9). On the contrary, the highest permeate flows were attained at pH 3, 6, 7, 8 and 9. An intermediate flow was obtained at pH 4.

In the previous figures, experimental data are shown together with the flow decline model which better fits to the results. Eq. (14) is used for experiments at pH 3, 4 and 4.9. At these pH values the effective radius of the protein (calculated with Eqs. (8) and (9)) is lower than the membrane pore radius. Then, one could expect significant protein transmission and consider that standard blocking predominates on cake filtration. The opposite applies for pH 7, 8 and 9. Here, Eq. (15) is employed since the effective radius is higher than the pore radius, transmission is very low or null and cake filtration predominates. A special case is pH 6. Although the effective radius is lower than the pore radius, Eq. (15) was used because null transmission was observed as a consequence of the attractive forces between protein (positively charged) and membrane (negative).

The values obtained for the parameters $\alpha$, $B$ and $C$ are given in Table 3. The value of $A$ fluctuated around 1.5 min$^{-1}$ for all the experiments. This involved the blockage of 95% of susceptible pores after only 2 min of filtration: $\exp (-1.5 \times 2) = 0.05$, that is, only 5% of pores remain unblocked.

Fig. 5 shows the influence of pH over the fraction of flow which pass through the fraction of pores susceptible of being completely blocked ($\alpha$). The highest value of $\alpha$ was obtained at the isoelectric point of BSA (89%); in the experiments at pH > 4.9 this fraction was...
the lowest, around 50%, while at values of pH < 4.9 the value of $\alpha$ was intermediate (50–70%).

The smaller the effective radius of BSA was, the higher value of $\alpha$ was obtained. As the particle was smaller, it might block a greater number of pores. As a consequence of this the value of $\alpha$ at pH 4.9 was the higher, with a protein size corresponding to the Stokes radius. As well as this, there was no net charge of BSA at this experiment, which eased the formation of aggregates, together with the irregularity and tortuosity of pores [10].

At values of pH above and over the isoelectric point, $\alpha$ decreased with the pH, as a result of an increase of the effective radius of BSA, reaching a residual value of 50% at pH 7, 8 and 9, where the size of the proteins was bigger than the pore radius of the membrane.

On the other hand, the variation of the parameters $B$ and $C$ with pH is shown in Fig. 6. Firstly, in those experiments with no transmission of protein through the membrane, the maximum value of parameter $C$, constant of the cake filtration law, happened at pH 7, decreasing at both sides of this point. At that value of pH, the point of zero charge of the membrane, the membrane had no net charge, allowing the formation of a more compact cake. For values of pH > 7 both, protein and membrane were negative charged, being more difficult to create the cake. For these values, a decreasing linear correlation exists between the parameter $C$ and pH. At pH 6, they had opposite charge, forming a cake over the membrane and narrowing their pores; due to this the parameter $C$ was lower than the obtained at pH 7.

The value of the parameter $B$, constant of the standard blocking law, increased linearly with the value of pH, being maximum at the isoelectric point of the membrane, where it was easier to adsorp onto the pores of the membrane and the protein layers previously deposited. The less the pH of the experiment, the less value of $B$ was obtained, due to the higher repulsion between protein and membrane, both negatively charged.

4.2. Influence of salt concentration

4.2.1. Transmission

The effect of salt concentration in transmission is represented in Fig. 7. A working pH of 4.9 was selected for these experiments in order to study the influence of ionic strength at a value of protein charge equal to zero (i.e. constant effective radius [24]).

Under these conditions, the presence of positive and negative ions, protein aggregation was hindered since both $\text{Na}^+$ and $\text{Cl}^-$ ions may compete with the BSA molecules for the local charges in protein. According to this, transmission increased from 65% at 5 min without salt addition to about 100% (after 5 min with NaCl addition).

However, there was not significant improvement in further addition of NaCl up to 25 mM in protein transmission compared to the results obtained for 3 mM. The final value of transmission in the rest of experiments was similar (≈50%). This suggests that the local charges in the BSA molecules and the positive positions of the membrane may become saturated in the 3–25 mM range.

4.2.2. Permeate flow

The evolution permeate flow at different salt concentrations of the solution is represented in Fig. 8. In the previous figures, experimental data are shown together with the flow decline model. Eq. (14) has been used at all the experiments. The values obtained for the parameters $\alpha$ and $B$ are given in Table 4. As before, the value of $A$ fluctuated around 1.5 min$^{-1}$ for all the experiments.

Fig. 9 shows the influence of ionic strength over the fraction of flow which pass through the fraction of pores susceptible of being completely blocked ($\alpha$). An increase in the ionic strength, due to salt addition, caused a decrease in the value of $\alpha$, from 0.892 (without
Table 4
Parameters of flow decline models

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>( \alpha )</th>
<th>( B ) (min(^{-1}))</th>
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<tr>
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<td>0.003673</td>
</tr>
<tr>
<td>1</td>
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<td>25</td>
<td>0.824</td>
<td>0.011367</td>
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</table>

BSA filtration with salt addition.

5. Conclusions

The effect of pH and salt concentration on permeate flow and transmission evolution of BSA with time was studied. All the experiments were explained in terms of electrostatic processes. The curves of permeate flow were explained according to blocking models. In the experiments in which protein transmission had taken place the flow decline had been explained by combination of complete and standard blocking mechanisms. In the experiments in which there had been no transmission, the combination of complete blocking and cake formation fitted the obtained flow data. The protein transmission had been discussed based on the effective radius of the protein and the repulsive or attractive forces protein–membrane. The variation of pH determined three different regions, depending on the electric charge of the membrane and the protein surfaces. The point of zero charge of the membrane (6.9) and the isoelectric point (4.9) of BSA were the limits of these regions. As well as this, effective radius of charged proteins was a function of pH, due to surface charge density of the solute and the ionic strength of the solution.

The higher transmission along time and the minimum permeate flow were obtained in the isoelectric point. An increase of salt concentration at this point, hindered the protein aggregation, increasing the transmission of BSA and reducing the pores susceptible of being completely blocked.

Acknowledgements

This research was supported by the Spanish Plan Nacional I+D+I, under the Projects PPQ-2002-02235 and CTQ-2005-02653/PPQ.

Nomenclature

- \( A \): constant of the complete blocking law (s\(^{-1}\))
- \( B \): constant of the standard blocking law (s\(^{-1}\))
- \( C \): constant of the cake filtration law (s\(^{-1}\))
- \( C_i \): concentration of the electrolyte ions (mol m\(^{-3}\))
- \( C_{salt} \): concentration of acid or alkali needed to adjust the pH (mol m\(^{-3}\))
- \( F \): Faraday’s constant (=96485 A s mol\(^{-1}\))
- \( k \): Boltzmann’s constant (=1.381 \times 10^{-23} m^2 kg/s^2 K)
- \( Q_F \): permeate flow (m\(^3\) s\(^{-1}\))
- \( Q_{F0} \): initial permeate flow (m\(^3\) s\(^{-1}\))
- \( r_{eff} \): effective solute radius (m)
- \( r_p \): pore radius (m)
- \( r_s \): solute radius (m)
- \( R \): universal gas constant (=8.314 kg m\(^2\) mol\(^{-1}\) s\(^{-2}\) K\(^{-1}\))
- \( t \): time (s\(^{-1}\))
- \( T \): temperature (K)
- \( z_i \): valence of the electrolyte ions

Greek symbols

- \( \alpha \): fraction of pores susceptible of being completely blocked
- \( \varepsilon_r \): relative permittivity of the bulk solution
- \( \varepsilon_0 \): permittivity of free space (A\(^2\) s\(^{-4}\) m\(^{-3}\) kg\(^{-1}\))
- \( \kappa \): Debye length (m)
- \( \lambda_{eff} \): ratio solute effective radius/pore radius of the membrane
- \( \sigma_s \): surface charge density (A s/m\(^2\))
- \( \phi \): partition coefficient
References


