Reducing the Protein Fouling of Polysulfone Surfaces and Polysulfone Ultrafiltration Membranes: Optimization of the Type of Presorbed Layer

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SUMMARY

The mechanisms of whey-protein fouling of polysulfone ultrafiltration (UF) membranes were studied. The results of whey-protein adsorption at a nonporous polysulfone model surface were compared with permeate flux measurements of polysulfone UF membranes fouled by filtration of a whey-protein solution. The effect of presorbing the model surface and the membranes with anionic, non-ionic and cationic polymers and surfactants on the adsorption of whey proteins and the membrane flux decline was established. Protein adsorption was found to be a major cause of the observed decline in membrane flux. Especially non-ionic, hydrophilic polymers were found to minimize protein adsorption as well as to decrease the membrane resistance during UF, while the application of surfactants and ionic polymers was generally less successful. Some of the observed effects of surface presorption can be explained by considering physico-chemical interactions between the protein and the modified surface.

INTRODUCTION

A common problem of many membrane separation processes is the decline of permeate flux during filtration. It is becoming increasingly apparent that, besides the well-known effects of concentration polarization, fouling phenomena due to solute/membrane-interactions can also result in severe flux loss [1–11]. While the effects of concentration polarization, often reversible in nature, can be diminished by altering the operating conditions or by improving the design of the membrane module [12], the most appropriate approach to reduce the
influence of solute/membrane-interactions seems to be modification of the membrane surface [10,13].

Two methods of modification of polymeric membranes can be distinguished regarding the stability of the applied modification. A permanent alteration of the surface properties is obtained by surface treatment with a reactant to modify the chemical structure of the polymer molecules at the surface. Sulfonation of polysulfone and poly(phenylene oxide) membranes [14–17] or the grafting of hydrophilics to a hydrophobic membrane [18,19] are typical examples. Permanent modification can also be achieved by physical adsorption of e.g. a polymer followed by chemical cross-linking of the adsorbates [20]. Adjustment of the surface properties by physical adsorption alone can be considered as a different class of modification techniques. In this case the stability of the presorbed layer (henceforth indicated as 'coating') is dependent on the (ir)reversibility of the adsorption process and on the adsorption affinity of the coating for the surface in relation to the affinities of the foulants. Though the inherently lower stability of the physically adsorbed coating can be regarded as a disadvantage, this type of membrane modification offers certain distinct advantages. It is relatively simple to apply and can be utilized in existing membrane installations. The structure of the membrane is not likely to be affected by the adsorbed molecules. Finally, assuming that the coating can be desorbed completely from the surface, a multi-purpose membrane is obtained by applying the appropriate coating for a given separation.

Recently several examples of the beneficial effects of applying the non-permanent coating technique during filtration of protein-containing solutions were given [21–26]. In view of the complex nature of protein adsorption at solid-liquid interfaces (e.g. [27]), it is, however, not surprising that knowledge concerning the mechanisms of the non-fouling action of a coating is still insufficient. Moreover, it is often not clear why one coating is superior to the other in reducing protein fouling. Therefore, in this work a study is presented concerning some of the fundamental aspects of non-permanent membrane modification techniques. We are investigating as a model the ultrafiltration (UF) of cheese whey with polysulfone as the membrane material. Whey UF is an commercially important unit-operation in the dairy industry [10], and it is well-known that protein fouling is severely restricting the whey-permeate flux (e.g. [28]). Furthermore, polysulfone, one of the most widely used polymers in membrane technology, is known to adsorb (whey) proteins in considerable quantities [5,6]. The effects of a large number of physically adsorbed coatings, with strongly differing chemical structures, on the whey-protein adsorption at a non-porous coated polysulfone model surface were determined. Also, the influence of these coatings on the reduction in permeate flux of a coated polysulfone membrane during UF of a whey-protein solution was established.
Comparison of these two types of experimental data could then provide insight into the relation between type of coating and the extent of membrane fouling due to UF of a protein solution.

Various surface-active agents (ionic and non-ionic surfactants and polymers) were found not only to change the adsorption levels of the whey proteins markedly but also to alter the flux decline rate during UF of a whey-protein solution. Especially non-ionic, hydrophilic polymers were successfully applied to reduce the amount of absorbed protein as well as to reduce membrane fouling. Some of the observed effects can be explained by considering the physico-chemical interactions between the coating material and the protein.

EXPERIMENTAL

Chemicals

Bovine serum albumin (Cat. No. A 0281) was obtained from Sigma, St. Louis, MO, USA. α-Lactalbumin (α-LA) and β-lactoglobulin (β-LG) were both isolated from desalted, clarified casein whey by ion-exchange chromatography (Pharmacia Stack KS 370/15, DEAE Sepharose Fast Flow Anion Exchanger). The protein content of the samples was determined from the Kjeldahl Nitrogen content (Kjeldahl factor 6.38), while the purity of the protein fraction was analyzed by reversed-phase HPLC. The purity of the isolated proteins was found to be relatively high (>95%). Though bovine serum albumin (BSA) was claimed to be essentially globulin free, it appeared that this protein contained considerable amounts (15 to 20%) of immunoglobulins. The McIlvaine buffer (0.01 M citric acid / 0.02 M phosphate) was used to dissolve the proteins and as the mobile phase in the column experiments (see below). In case of the chromatographic analysis of protein adsorption the buffer solutions were degassed prior to use. All aqueous solutions were prepared using demineralized water, which was prefiltered before use by reverse osmosis (Osmonics PA 99 RO-module). The polymers and surfactants used as coating materials are presented in Table I. These coatings are all cold or hot water-soluble. Trifluoroacetic acid (TFA) (HPLC/Spectro grade) and acetonitrile (chromatography grade) were purchased from Pierce, Rockford, IL, USA and Baker Chemicals, Deventer, The Netherlands, respectively. Before use the membranes were cleaned at 50°C for 30 minutes with a 0.5 vol.% alkaline cleaning and disinfectant solution commonly used in the dairy industry (Reca VL, obtained from Centrale Aankoop FNZ, Arnhem, The Netherlands; contains no surfactants).
### TABLE I

Polymers and surfactants used as coating agents

<table>
<thead>
<tr>
<th>Coating</th>
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<td><strong>Polymers</strong></td>
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<td>Poly(ethyleneimine)</td>
<td>PEI</td>
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<tr>
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<td>Poly(acrylic acid)</td>
<td>PAA</td>
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<td>Poly(sodium 4-styrene sulfonate)</td>
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<tr>
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<td>PVP</td>
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<td>DAC</td>
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<tr>
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<td>SDS</td>
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<tr>
<td><em>Non-ionic</em></td>
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<tr>
<td>Octylphenol-polyethylene-glycol ether (Triton X-100)</td>
<td>TRIT</td>
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*Sulfate content < 0.10%

### Preparation of polysulfone model surface

Udel polysulfone granulate (Union Carbide, product No. CU 4750) was ground in an ultracentrifugal mill (Retsch, type ZN 1) equipped with a 200 μm ring sieve. During the grinding process sufficient liquid nitrogen was added to prevent scorching of the polysulfone particles. The average surface volume
diameter, \( d_{3.2} \), of the irregularly shaped particles, as determined by light scattering (Malvern Particle Size Analyzer), was about 100 (±5) \( \mu \text{m} \). The specific surface area of the particles as determined by BET nitrogen adsorption (Micromeritics) was 0.27 (± 0.06) \( \text{m}^2/\text{g} \). By combining the determined size and the specific surface area of the particles (density UDEL polysulfone 1.24 \( \text{g/cm}^3 \)) it can be concluded that the adsorbent is non-porous. This eliminates the possibility that size-exclusion effects play a role in the protein adsorption measurements.

**Protein adsorption measurements**

**Polysulfone model surface**

The theory and practice of the chromatographic analysis of protein adsorption has been reviewed recently by Koltisko and Walton [29]. Protein adsorption measurements, coating and column regeneration were performed at room temperature. The unmodified polysulfone particles (9.4 g) were packed in a glass chromatography column (Bio-rad Laboratories, 1.0 × 30 cm) and cleaned by pumping a trifluoroacetic acid (TFA)/acetonitrile/water (0.1:40:60, vol. %) mixture through the column for about 30 minutes. After displacement of the cleaning solution with the McIlvaine buffer of the appropriate pH, at the same time equilibrating the column material, the buffer feed solution was replaced by a protein solution in the buffer (about 0.7 g/l) with a pH equal to that of the equilibrating buffer. The protein concentration of the column effluent was monitored by a UV photometer (LKB Uvicord S), of which the signal (280 nm) was recorded. The protein solution was applied to the column until the effluent concentration reached a plateau, which corresponded with the protein concentration of the feed solution. From the obtained effluent concentration profile, the flow rate (usually about 1.5 ml/min) and the void volume of the column system (typically 8 to 14 ml), the adsorbed amount of protein (mg) and the surface concentration (mg/m\(^2\)) can be deduced by using the frontal analysis technique [29]. Dispersion effects in the column do not influence the accuracy of this technique. The void volume was estimated from the effluent concentration profile measured during the subsequent replacement of the protein solution by the buffer, assuming that desorption of protein from the hydrophobic polysulfone as a result of mere dilution can be neglected (see e.g. [30] and also results below). The thus obtained void volumes correlated linearly with the measured values of the column height, confirming the correctness of the above assumption.

The polysulfone particles were coated by pumping a 1% (w/v) aqueous solution of the coating through the column for approximately 30 minutes. This should provide sufficient time to complete the adsorption as adsorption of
polymers [31] and surfactants is usually fairly rapid on nonporous adsorbents. Subsequently, the coating solution was replaced by the buffer solution and the protein adsorption capacity of the coated particles was then established in the same way as described above.

Desorption of protein from the column material was achieved by contacting the packed particles with the TFA/acetonitrile/water mixture. Desorption of proteins or peptides from hydrophobic surfaces using this solution is a standard procedure in reversed-phase HPLC analysis (e.g. [32]). The column regeneration made it possible to use the same polysulfone packing several times without loss of reproducibility. In case of regeneration of coated particles, the polysulfone packing was coated again with the same polymer or surfactant to assure complete adsorption of the pertinent coating. However, a fresh column packing was prepared for each series of measurements with a specific coating.

**Polysulfone membranes**

To compare with the results of adsorption at unmodified polysulfone model surface, protein adsorption measurements were also carried out with (unmodified) polysulfone UF membranes (DDS GR 61 PP and GR 51 PP) as the adsorbent. From infrared spectrometry measurements (IR and ATR-IR) it was concluded that the membranes were of the Udel polysulfone type (Union Carbide), i.e. the same type of polysulfone as used for the preparation of the polysulfone model surface. The membranes were cleaned in Reca VL. By clasping the membrane between a glass container and its cover, only the active side of the membrane (disk with external surface area of 13.2 \(10^{-4}\) m\(^2\)) was exposed for 70 hours at 5°C (to prevent bacterial growth) to a buffered whey protein solution (about 20 ml) of known concentration (between 0.05 and 1 g/l). The effect of pH on protein adsorption at the membranes was measured using a 0.7 g/l protein solution. The adsorbed amount of protein could be calculated using the depletion technique. The applied adsorption time (70 h), necessary to reach maximal adsorption, was established from separate kinetic measurements. The adsorbed amount of protein could also be deducted from the quantity of protein desorbed from the membrane under influence of exposure to the TFA/acetonitrile/water mixture. As the result of these two methods were found to be in agreement, only the depletion method was applied.

**Flux decline measurements**

The polysulfone membrane type used for the protein adsorption measurements (DDS GR 61 PP, Udel) was also employed for establishing the influence of the coatings on the degree of membrane flux decline due to UF of a \(\beta\)-LG solution. All experiments were carried out at room temperature and
performed twice in view of the limited reproducibility (see results below). The membrane disks (effective surface area 25.5 $10^{-4}$ m$^2$) were cleaned as above in Reca VL, after which the membranes were purged for 15 minutes in a stirred dead-end filtration cell (Amicon model 202) with demineralized and filtered water to displace the cleaning solution. Subsequently, the permeate flux was determined by weight measurements of the permeate ($\Delta P$ 1.5 $10^5$ Pa, measurement time 15 min) to determine the membrane resistance of the clean, unmodified membrane. In case of experiments with modified membranes, the coating was applied by passive adsorption from a 1% (w/v) coating solution in demineralized and filtered water for thirty minutes. After extensive cleaning of the UF cell to remove excess coating solution, the permeate flux was measured as described above. A buffered solution of $\alpha$-LG (180 ml, about 1 g/l) was then filtered in the UF cell ($\Delta P$ 1.5 $10^5$ Pa) for two hours during which period the permeate flux was measured. As the protein concentration in the permeate was found to be negligible, the protein content of the retentate could be calculated from a single mass balance. Finally, the UF cell was rinsed thoroughly and the resistance of the fouled membrane determined by water flux measurements as described before.

RESULTS AND DISCUSSION

_protein adsorption measurements with polysulfone model surface_

Flushing the unmodified or modified column packing with the TFA/acetonitrile/water mixture after a protein-adsorption experiment resulted in a broad desorption peak as measured by the UV photometer at a wavelength of 280 nm. By reversed-phase HPLC analysis of eluate fractions it was confirmed that this phenomenon was caused by desorption of the whey protein used in the adsorption experiment. The desorbed amount of protein, calculated from the area under the desorption peak and using the measured product of the optical path length and the extinction coefficient of the pertinent, native protein, agreed with the adsorbed amount determined by frontal analysis. This underlines the reliability of the latter technique. Furthermore, repetition of the adsorption experiment (unmodified or modified polysulphone) after flushing with TFA/acetonitrile/water gave reproducible results, suggesting that the desorption was complete. Also, experiment repetition without regeneration yielded negligible adsorption values ($0 \pm 0.1$ mg/m$^2$), showing that no additional protein could be adsorbed at a polysulfone surface previously saturated with protein. It also shows that desorption did not occur as a consequence of dilution with buffer.
The influence of some experimental variables, flow rate, buffer type and ionic strength, on the adsorption measurements with the polysulfone model surface was examined. Variation of the flow rate (0.5 to 2.5 ml/min) did not alter the adsorption levels significantly. The use of an acetic acid/acetate buffer (0.02 M, pH about 5.2) instead of the McIlvaine buffer (citric acid/phosphate) was also found not to change the amount of BSA adsorbed to the polysulfone surface. Furthermore, adsorption levels were not affected drastically by increasing the strength of the McIlvaine buffer (0.1 M citric acid/0.2 M phosphate). The latter is in accordance with results of Suzawa et al. [33,34], who studied the effect of ionic strength on adsorption of BSA onto lattices of various polymers. A tenfold increase of the ionic strength resulted in relatively minor changes of the dependence of BSA adsorption levels on the pH (3 < pH < 7). All the experiments with the (coated) polysulfone model surface described hereafter were performed at the standard conditions given in the experimental section.

The isotherms of adsorption of α-LA, β-LB and BSA at the ground polysulfone granulate at pH 5.2 are given in Fig. 1. The adsorption levels (0.5 to 1.6 mg/m²) indicate monolayer adsorption [35]. In the case of β-LB a plateau in the adsorbed amount of protein seems not to be established in the concentration range shown. In case of α-LA and BSA clear plateau values can be recognized. It is, however, possible that these plateau would prove to be semi-plateaux. For example, Matthiasson [5] found that, in case of BSA adsorption on polysulfone UF membranes, an increase of the bulk concentration

![Fig. 1. The isotherms of adsorption of α-LA (+), β-LG (o) and BSA (x) at the polysulfone model surface at pH 5.2.](image-url)
from about 1 g/l to about 10 g/l showed a second plateau value in the higher concentration range. For practical reasons all adsorption experiments with the modified model surface were performed at a bulk concentration of about 0.7 g/l.

In case of adsorption experiments with coated polysulfone particles the possibility exists that a coating (polymer or surfactant), with insufficient affinity for the polysulfone surface, is displaced by the whey protein. The desorbed coating could then in theory interfere with the spectroscopic measurement of the protein concentration if the polymer or surfactant shows pronounced UV absorbance at 280 nm. This would result in erroneously low values for the calculated amount of adsorbed protein. However, the extinction coefficients of most coatings were found to be much lower than the corresponding values of the whey proteins, thereby minimizing the experimental error in case of displacement of the coating. Only the extinction coefficients of Triton X-100 and poly(sodium 4-styrenesulfonate) (PSSS) were in the same order of or even greater than those of the proteins. As Triton X-100 seems to reduce the level of protein adsorption somewhat (see below), while PSSS does the opposite, it was checked whether this observed reduction in adsorption level was not caused by displacement of the coating. This was performed by reversed-phase HPLC analysis of eluate fractions to determine independently the protein content of the column outlet. Only minor differences in concentration levels were observed, while the calculated adsorption levels using the two break-through curves were found to be in agreement.

Protein adsorption on polysulfone model surface versus adsorption on polysulfone surfaces prepared by phase inversion

Polysulfone UF membrane are usually fabricated by the phase-inversion method. In this process phase-separation phenomena in ternary solutions of polysulfone in mixtures of a solvent and a non-solvent give rise to the asymmetric structure of the membrane [36]. In previous work the authors [37] showed that the adsorption characteristics of ground polysulfone granulate, the model surface used in this study, and of a polysulfone latex prepared by phase inversion do not seem to differ greatly. This would indicate that there exists only a minor influence of the phase-inversion process on the protein adsorption capacity of the polysulfone surface. It also implies that the protein adsorption results obtained with the polysulfone model surface can be used to explain the results concerning the fouling of polysulfone membranes.

The levels of adsorption at the (Udel) polysulfone model surface can also be compared directly to those measured at (Udel) polysulfone membranes prepared by the phase-inversion method. However, our experimental attempts to determine the effective surfaces of the applied polysulfone membranes using
Fig. 2. The pH dependence of the levels of adsorption of \(\alpha\)-LA (a), \(\beta\)-LG (b) and BSA (c) from solution (about 0.7 g/l) at the polysulfone model surface \((\Gamma_{m})\) and at polysulfone membranes \((\Gamma_{m}, \bigcirc: \text{GR 61 membrane, } \square: \text{GR 51 membrane});\) adsorption levels of membranes are expressed as mg/m\(^2\) external surface; standard deviation of the model surface measurements indicated by vertical lines; shown values of the membrane experiments were obtained by averaging two measurements.
adsorption of nitrogen or methylene blue were not successful. Hence, the observed levels of protein adsorption on the membranes are expressed as mg/m² external surface of the membrane. The isotherms of adsorption on polysulfone membranes resemble the corresponding isotherms on the model surface (Fig. 1), i.e. a clear plateau can be recognized in case of α-LA adsorption while the adsorbed amount of β-LG increases to some degree with higher concentrations (0.25 to 1.0 g/l). In Fig. 2 the amounts of α-LA, β-LG and BSA adsorbed at the membranes are given as function of the pH and are compared with the pH profiles measured using the polysulfone model surface. The adsorption levels found at membranes are comparable to previous results of adsorption of β-LG to polysulfone membranes [38]. From Fig. 2 it can be concluded that in case of membranes as well as the model surface adsorption maxima occur at the same pH (α-LA: pH about 5, β-LG and BSA: pH about 4.5). This also underlines the above mentioned assumption concerning the minor effects of the phase-inversion process on the protein adsorption characteristics of polysulfone. More strikingly is the apparently large difference in protein adsorption capacity of the two surfaces (in case of α-LA and β-LG three orders of magnitude). However, the latter phenomenon can be explained by adsorption of the proteins in the pores of the membrane [11].

Interestingly, the amount of BSA adsorbed to the standard GR-61 membrane (cut-off $M_r=20\,000$) is found to be very low, while the adsorbed amount to a comparable polysulfone membrane with larger pores (GR-51, cut-off $M_r=50\,000$) is clearly much higher (Fig. 2c). This points out that the larger BSA molecules ($M_r=66\,267$) are unable to penetrate the interior of the GR-61 membrane, and it confirms the above stated hypothesis concerning internal adsorption. Furthermore, these results are also in agreement with results of Amano et al. [39], who found that adsorption of BSA to porous silicas decreased markedly when adsorbents were used with pore diameters below 50 nm. The average pore size of the GR-61 membrane is also believed to be considerably lower than 50 nm (GR-61: 17–18 nm, [38]). The very low amount of α-LA adsorbed on the membrane at low pH values (Fig. 2a) could be explained by assuming that at these pH values swelling of the net positively charged protein molecule prevents penetration of the polysulfone membrane.

**Coating optimization**

**Effect of coating on protein adsorption at polysulfone model surface**

**Protein adsorption experiments.** The polymers and surfactants listed in Table I were coated on the ground polysulfone granulate, after which the amount of β-LG and BSA that can be adsorbed at the modified model surface was established. In Figs. 3 to 5 the adsorption levels thus obtained are compared
with the adsorption results determined with the unmodified polysulfone model surface (from Fig. 2b and 2c). It was not checked whether or not exposure of the polysulfone particles to the coating solution resulted in an adsorbed layer of the polymer or surfactant, which is moreover not removed by flushing the column with buffer after the coating procedure. However, in case of an effect of the applied coating on the level of protein adsorption it is most probable that the polysulfone surface is indeed coated with the polymer or surfactant, and that this coating caused the increase or decrease of the adsorbed amount of protein.

Fig. 3. The effects of coating the polysulfone model surface with ionic polymers on the adsorption levels ($r$) of $\beta$-LG (a) and BSA (b); (O) unmodified model surface; coatings: (+) PEI, (x) CMC, (□) PAA, (●) PSSS.
In those (incidental) cases where little or no effect on the adsorption was found it is unknown whether the coating procedure did not result in a protective, adsorbed layer or the protein was able to displace the (weakly) adsorbed polymer or surfactant. Both these situations, however, indicate that the adsorption affinity of the coating for polysulfone is insufficient. The latter can be illustrated by the effects of coating the polysulfone particles with the polysaccharides agarose (sulfate content < 0.10% to minimize ionic phenomena) and dextran (Fig. 4). Especially in case of adsorption of β-LG these two polymers hardly seem to influence the adsorption capacity for protein of the

Fig. 4. The effects of coating the polysulfone model surface with non-ionic polymers on the adsorption levels ($\Gamma$) of β-LG (a) and BSA (b); (○) unmodified model surface; coatings: (△) AGAR, ( △) DEX, (+) MC, ( * ) PEG 6000, (□) PAAm, ( x ) PVA, ( ● ) PVME, (■) PVP.
polysulfone model surface. Though it cannot be concluded unambiguously from the experiments, it is, however, likely that in this case the affinity of the very hydrophilic polysaccharides for the relatively hydrophobic polysulfone is too low to result in a stable coating layer.

A notable agreement can be seen in Figs. 3 to 5 between the effects of coatings on β-LG adsorption and those on BSA adsorption. The only clear dissimilarity is observed with a coating of PEI at pH 6.7, which seems not to influence the BSA adsorption level, while the adsorbed amount of β-LG at PEI-coated polysulfone is remarkably high (about 8.6 mg/m²). The latter seems to

**Fig. 5.** The effects of coating the polysulfone model surface with surfactants on the adsorption levels (Γ) of β-LG (a) and BSA (b); (©) unmodified model surface; coatings: (Δ) ABDAC, (□) DAC, (+) SDS, (●) TRIT.
indicate coagulation of β-LG (negatively charged at this pH) at the positively charged, adsorbed PEI resulting in multi-layer adsorption [35]. The otherwise profound similarity between the coating effects on the β-LG and BSA adsorption levels underlines the soundness of the applied experimental procedure and, furthermore, suggests that the guidelines discussed below might also be valid for other proteins.

**Ionic versus non-ionic coatings.** By comparing the effects of ionic polymers (Fig. 3) and those of non-ionic polymers (Fig. 4) it can be noticed that the adsorbed amount of β-LG or BSA is nearly always reduced in case of non-ionic coatings, while increased as well as decreased adsorption levels are found under influence or ionic coatings. This increase or decrease is dependent on the pH of the buffer medium, which can be observed from the fact that the adsorption pH-profiles are often no longer symmetrical relative to the iso-electric points of β-LG and BSA (pH about 5). The decrease, if any, in adsorbed amount of protein in case of coating with non-ionic polymers is rather independent of pH, which gives rise to more or less symmetrical profiles. The effects of coating with surfactants (Fig. 5) affirm the results obtained with polymers, i.e. the non-ionic surfactant (TRIT) reduces the adsorption levels, while ionic surfactants (ABDAC, DAC and SDS) can increase or decrease the adsorbed amounts.

An important conclusion that can be drawn from the results of the coating experiments is the substantial role that electrostatic interactions can play in determining the extent of protein adsorption. This can be deduced from the pronounced effects of ionic coatings (Fig. 3). In many cases these effects can be understood in terms of electrostatic attraction or repulsion between the protein and the adsorbed coating. Especially at pH values below the iso-electric points of β-LG and BSA (pH < about 5), where the proteins have a net positive charge, a clear picture emerges. The cationic polymer (PEI) diminishes protein adsorption, while the still more or less negatively charged polymers (CMC, PAA, PSSS) give rise to an increased adsorption. At pH 6.7 the situation is less clear. As could be expected, the effect of the cationic PEI at higher pH is to increase the adsorption of the negatively charged proteins, especially of β-LB. The amount of adsorbed protein at polysulfone coated with the anionic polymers is usually less than the observed adsorption at lower pH. However, despite the equal signs of the charges of protein and adsorbed anionic, the adsorbed amount of protein is not reduced significantly, and in one case (PSSS, BSA) it is even higher than the adsorbed quantity at unmodified polysulfone. A similar phenomenon was reported by Norde and Lyklema [40], who found that negatively charged proteins adsorbed spontaneously and exothermally at a negative polystyrene surface. It demonstrates the well-known phenomenon that
besides electrostatic interactions, though important, other interaction types (e.g. hydrophobic interaction, hydrogen bonding [27,30,41]) can play a decisive role in determining the extent of adsorption of proteins at surfaces.

**Non-ionic coatings.** A further conclusion that can be derived from Figs. 3 to 5 is the capability of non-ionic coatings to counteract the adsorption of proteins. The chance that an arbitrary non-ionic polymer reduces the adsorption level is fairly high, though large differences are found between the effects of individual non-ions. This is also in agreement with the above stated conclusion concerning the importance of electrostatic interactions. Coating the polysulfone surface with a non-ionic polymer on surfactant eliminates the possibility of electrostatic (attractive) forces between the protein and the surface, which is apparently adequate (Fig. 4) in many cases to reduce protein adsorption levels drastically.

As noted above, at least two other interaction types, hydrophobic interactions and hydrogen bonding, could lead to strong protein/surface interactions and/or free enthalpy loss. However, the first interaction type is not likely to play a significant role in the experiments presented here as all the non-ionic (and ionic) coatings tested so far are water soluble, and thus more or less hydrophilic. Therefore, an adsorbed coating would hydrophilize the polysulfone surface, thereby diminishing the water ordering and reducing the possibilities for entropy gain (free enthalpy loss, hydrophobic bonding) upon protein adsorption [27].

The second interaction type, hydrogen bonding, may also cause proteins to adsorb at surfaces (e.g. see [42]). The two non-ionic polymers, which were found to be most effective to minimize protein adsorption, are MC and PVME (Fig. 4). It is remarkable that both these substances contain methoxylated groups (-O-CH3), of which it is known that the oxygen atom contained in it is weak proton acceptor [43]. Thus, the effectiveness of MC and PVME could be explained by assuming that these polymers prevent electrostatic interactions (due to their non-ionic nature), hydrophobic interactions (due to their hydrophilicity) as well as hydrogen-bond formation (due to their lack of groups capable of forming strong hydrogen bonds). The D-glucose unit of methylcellulose also contains, however, free hydroxyl groups [44], which could, in theory, form hydrogen bonds with proteins. The latter apparently does not occur. Most of the other non-ionic polymers (AGAR, DEX, PAAm, PVA, PVP) all contain polar groups (-OH, -CO-N), which could give rise to hydrogen-bond formation between proteins and adsorbed polymers (and possibly other polar charge-transfer interactions). This seems to be in agreement with the experimental phenomenon that the protein-adsorption reducing capacity of these non-ions is clearly not as effective as MC and PVME. The results of PEG 6000 are
inconclusive. The reduction in adsorption level in case of β-LG is comparable to those of MC and PVME, probably due to the lack of strong polar groups in this polymer, but the results for BSA do not confirm this hypothesis.

**Effect of coating on flux decline of polysulfone membranes**

Membrane fouling experiments. Polysulfone UF membranes (DDS GR 61 PP) were coated with the above-mentioned hydrophilics and the effect of the coating on the rate of ultrafiltration of a solution at β-LG was established. The relative increase of the total hydraulic resistance of the modified membranes ($R_f/R_m$) was calculated from the permeate fluxes ($J_v$) measured during ultrafiltration:

$$R_f/R_m = J_{v,0}/J_v$$  \hspace{1cm} (1)

with $R_m$ the hydraulic resistance of the unmodified membrane and $J_{v,0}$ the permeate flux of the unmodified membrane, which was measured for each membrane disk used previous to the coating procedure. The membranes were contacted with the coating solution for thirty minutes. This contact time is probably sufficient to allow an adsorbed layer of polymer or surfactant to form on the external surface of the membrane. In case of coating with methylcellulose it was observed that a contact time of only 2 minutes did not diminish the advantageous effect of this coating (see below). Also a possible effect of the cleaning procedure with Reca VL was tested experimentally. Cleaning of the membranes with demineralized and prefilted water only ($50^\circ\text{C}, 30$ minutes) did not produce results that were significantly different from the corresponding results with membranes cleaned with Reca VL. Finally, it was observed that coating by merely contacting the membranes with a solution of the coating (passive adsorption) seems to yield more favourable results than coating by ultrafiltration of the coating solution. A similar phenomenon was found by Kim et al. [24].

The increase in hydraulic resistance of unmodified polysulfone membranes during ultrafiltration of a β-LG solution at pH 3.2, 4.7 and 6.7 is presented in Fig. 6 as a function of the concentration factor $K$ (calculated protein concentration in the cell during ultrafiltration divided by the initial protein concentration). It emerges that at the start of the ultrafiltration the membrane resistance increases very rapidly, while a much slower increase occurs during the remainder of the filtration process. It can also clearly be seen in Fig. 6 that the highest flux decline occurs at pH 4.7, which is near the iso-electric point of β-LG. At this pH the protein adsorption at the polysulfone model surface and membranes is also maximal (Fig. 2b), indicating that protein adsorption could be
Fig. 6. The relative hydraulic resistance ($R_t/R_m$) of unmodified polysulfone membranes as a function of concentration factor ($K$) during UF of a $\beta$-LG solution at pH 3.2 (+), 4.7 (□) and 6.7 (x).

...a major cause for the observed decline of membrane flux. The foregoing is in accordance with previous work [11,38,45]. Furthermore, though not shown in Fig. 6, the membrane resistance levels determined from the measurements of the water flux after ultrafiltration and after rinsing the cell with demineralized and filtered water revealed that the fouling phenomenon is largely irreversible as these resistances were always (unmodified and modified membranes) only about 10 percent lower than the highest resistance measured during the ultrafiltration. This suggests that, under these conditions, the fouling is mainly due to irreversible adsorption of protein to the polysulfone membrane and only to a minor extent due to (reversible) concentration polarization effects. The experimental observation that variation of the stirrer speed as well as of the dimension of the dead-end filtration cell (internal diameter 3.8 and 5.7 cm), both determining mass transfer coefficients, did not have a significant effect on the permeate fluxes obtained confirms the above stated hypothesis. As compared with ultrafiltration of a $\beta$-LG solution, UF of a BSA solution at pH 4.7 has less deteriorating effects on membrane fluxes ($R_t/R_m$ : 2 to 3). Again, this is in agreement with the low amount of BSA adsorbed at the GR 61 PP-membrane (Fig. 2c), and it shows that a close relation exists between protein adsorption and flux decline.

Coating effects. Coating polysulfone membranes can result in an increase as well as in a decrease of the membrane resistance as compared to that of an
unmodified membrane. In Fig. 7 three typical examples are given. The total membrane resistances given in this study include the increase in resistance due to coating with polymer (in case of surfactants see below). This increase (usually $R_d/R_m = 1$ to $2$) is always much smaller than the increase due to ultrafiltration of a β-LG solution. A coating consisting of polyacrylamide does not seem to have a clear effect on the membrane resistance level. This is especially true when one considers that the reproducibility of the measurements with modified membranes appears to be somewhat lower than those with unmodified membranes (Figs. 6 and 7). A clear increase of the membrane resistance results when the membranes are coated with PEI, while the opposite is the case with a PVME coating. The effects of the applied coatings listed in Table I on the membrane resistance at a concentration factor $1.2$ are compared in Fig. 8. It can be seen that, as in the case of adsorption to the polysulfone model surface, coating with non-ionic polymers is often advantageous to reduce flux decline. A lowering of the resistance of the modified membrane during ultrafiltration can be noticed in case of coating with AGAR, DEX, MC, PEG 20000 and, especially, PVME. Three other non-ions (PEG 6000, PAAm and PVA) seem to have little influence on the flux behaviour of the membrane, while only application of PVP worsens the situation somewhat. The observed differences between the two types of PEG tested could indicate that for the formation of a stable, adsorbed polymer layer the molecular weight of the polymer should not be too small. Kim et al. [24] also found that coating polysulfone membranes with larger molecular weight

Fig. 7. The relative hydraulic resistance ($R_d/R_m$) of an unmodified polysulfone membranes (□) and of membranes coated with PEI (●), PAAm (x) or PVME (+) as a function of concentration factor during UF of a β-LG solution at pH 4.7.
polymers resulted in a greater flux increase during ultrafiltration of a BSA solution as compared with lower molecular weight polymers. It is remarkable that one of the two non-ionic coatings that were most effective in preventing the proteins β-LG and BSA to adsorb at polysulfone surfaces (PVME) is also the coating that reduces the flux decline of polysulfone membranes to the greatest extent (about 50%). The situation is quite different with ionic coatings. Two polymers result in a very large increase of the resistance ($R_d/R_m > 100$ for PAA), little effect is noticed from using PSSS, while only a CMC coating reduces the flux decline. The dramatic effects of ionic coatings show some resemblance with the effects of these coatings on protein adsorption on polysulfone (Fig. 3).

Interestingly, the initial effect of treatment of the membranes with each of the surfactants tested was an increase of about 20 to 40% in water permeability as compared with that of the untreated membrane. A similar phenomenon was noted by Swaminathan et al. [46], who ascribed this flux rise to swelling of the membrane. The decrease in flux during the subsequent ultrafiltration step was,

![Graph showing the relative hydraulic resistance ($R_d/R_m$) of coated membranes at a concentration factor 1.2 during UF of a β-LG solution at pH 4.7; shown bars represent duplicate measurements; solid line indicates average resistance of unmodified membranes with standard deviation indicated by broken lines.]

Fig. 8. The relative hydraulic resistance ($R_d/R_m$) of coated membranes at a concentration factor 1.2 during UF of a β-LG solution at pH 4.7; shown bars represent duplicate measurements; solid line indicates average resistance of unmodified membranes with standard deviation indicated by broken lines.
however, comparable to that of unmodified membranes, if the ultrafiltration fluxes were compared with the enhanced fluxes after surfactant treatment (as is the case in Fig. 8). Again, this is in agreement with the work of Swaminathan et al. [46], who found only little improvement (about 6%) in flux behaviour of a Teepol treated UF membrane during BSA ultrafiltration. Thus, apart from the above mentioned effect of enhancement of the initial flux, the use of surfactants seems not to be helpful to reduce fouling. A similar conclusion can also be drawn from the effects of surfactants on protein adsorption (Fig. 5).

The foregoing results seem once more to confirm the above stated hypothesis concerning the relation between membrane flux decline and protein adsorption. The prevention of proteins to adsorb at and/or in the membrane seems to reduce flux decline during UF of protein solutions. Coatings that were found to prevent effectively adsorption of the proteins also tend to decrease the disadvantageous effects of protein fouling of membranes.

There are, however, some distinct discrepancies between the coating effects on protein adsorption and those on flux decline. Agarose and dextran both were found to have little influence on adsorption levels, especially in case of β-LG (Fig. 4). Nevertheless, these coatings decrease the membrane resistance levels during UF. Coating polysulfone with carboxymethyl cellulose resulted in increased amounts of adsorption at pH 4.7 (especially in case of β-LG, Fig. 3), though the membrane resistance did not increase but even decreased. These results suggest that protein adsorption is not the sole mechanism determining the extent of flux decline. The effects of other mechanisms, like pore blockage (at the pore opening and/or in the pores), and their mutual interactions could prevent simple explanations to be valid. The complex nature of protein adsorption at interfaces also complicates the analysis of fouling phenomena. For example, adsorbed CMC molecules, which were found to promote protein adsorption (Fig. 3) and could be expected to increase membrane fouling, could give rise to characteristically adsorbed protein layer(s) that are well permeable for water and that (partly) prevent the more severe fouling due to direct protein adsorption at unmodified polysulfone.

The effectiveness of PVME was also tested at pH values below and above the iso-electric point of β-LG. During ultrafiltration at low pH (3.2) the decrease of the membrane resistance as a result of coating with PVME was again about 50% (Fig. 9). This shows that the non-fouling properties of this non-ionic coating seem not to be dependent on pH, which once more resembles the pH-independent effect of non-ionic polymers on protein adsorption (Fig. 4). At pH higher than the iso-electric point (pH > about 5) flux decline is less severe than is the case at lower pH (Fig. 6). Coating with PVME in this case is not beneficial as the increase in resistance due to PVME coating is in the same order of magnitude as the increase of an unmodified membrane due to protein fouling.
Part of the results presented here are in agreement with recently published work. Michaels et al. [26] showed that in case of lipophilic UF membranes pretreatment with non-ionic polymers was more favourable with respect to minimizing flux decline than the use of anionic polymers, which corresponds with the general picture of Fig. 8. However, the results of Fig. 8 also disclose that exceptions to this rule are possible (e.g. CMC versus PVP). Furthermore, as depicted in Fig. 3, the effect of ionic coatings is also likely to be very dependent on the pH of the medium, and thus on the charges of coating and proteins. Kim et al. [24] found that MC was to be preferred as coating above PVA and PVP with respect to fouling of a polysulfone membrane due to ultrafiltration of a BSA solution. The same conclusion can be obtained from Fig. 8. The latter authors also reported an increase of the resistance of the polysulfone membrane due to coating with nonylphenol-polyethylene glycol ethers and positive effects of membrane treatment with these surfactants during ultrafiltration of a BSA solution [23,24]. Both these phenomena are in contradiction with the initial flux-enhancing effects of detergents and with the neutral effects on permeate fluxes during UF that were observed in the present work.
CONCLUSIONS

More insight into membrane fouling due to solute-membrane interactions during UF of protein containing liquids can be obtained by comparing protein adsorption measurements at non-porous (modified) membrane materials with measurements of permeate fluxes of (modified) membranes. From several types of experimental observations made in this work it was concluded that a close relationship exists between protein adsorption and membrane flux decline. Modification of the polysulfone model surface or of polysulfone membranes by physical adsorption of polymers or surfactants can have important effects on protein adsorption and on membrane fluxes during UF, respectively. A large number of these surface-active compounds was screened for their fouling-preventing capabilities. Especially coating with hydrophilic, non-ionic polymers was found to diminish adsorption of proteins as well as to reduce flux decline. This can be explained by assuming that adsorbed polymer molecules prohibit electrostatic interactions between the protein and the surface, due to their non-ionic properties, as well as minimize the possibility of hydrophobic bonding due to their hydrophilicity. Furthermore, the most successful non-ionic polymers also seem to prevent the formation of strong hydrogen bonds between protein molecules and the surface.

The strong effects of anionic and cationic polymers on protein adsorption and membrane fouling show that electrostatic interactions can dominate over other interaction types. This may make it feasible to reduce adsorption and fouling by selecting polymers with charges equal of sign to those of the foulants. Possible fouling due to charge-induced protein aggregation or precipitation must, however, be taken into account.

From certain pronounced discrepancies between the protein adsorption results and the flux decline results it was also concluded that adsorption of proteins at and in the membrane is not the sole mechanism determining the membrane fouling properties. Present research is concerned with further optimization of various coating techniques as well as to clarify other possible fouling mechanisms involved.

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REFERENCES