Electrostatic repulsion as a mechanism in fouling of ultrafiltration membranes
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ABSTRACT

Studies of electrostatic repulsion in ultrafiltration membranes are limited to applications of different organic compounds carrying a set of unique characteristics, or to changes of general water parameters such as ionic strength and pH. The proposed method of deliberate alteration of surface charge of organic molecule by succinylation or by guanidination provides an opportunity to selectively investigate the electrostatic mechanism without changing size or hydrophobic properties of investigated molecule. The approach was successfully implemented on BSA protein, and new inside into the mechanism of electrostatic mechanism was obtained. The electrostatic repulsion becomes important when zeta potential of the protein exceeded 20 mV, when before the threshold the interactions were mainly governed by size exclusion.

Key words | BSA, MWCO, PES, protein

INTRODUCTION

Ultrafiltration (UF) membranes are implemented in various water treatment processes, such as in pre-treatment to reverse osmosis membranes, in tertiary treatment of wastewater streams, as stand-alone filtration process for surface water streams, etc. Further expansion of UF membrane technologies is currently slowed down by fouling caused by organic matter typically present in the water. The fouling causes reduction of flux and therefore requires special treatment by means of either pre-treatment of raw water, or periodic backwash of the fouled membranes. Continuous research of fouling phenomenon detected three main fouling mechanisms of size exclusion, hydrophobic interaction and electrostatic repulsion.

Size exclusion is usually considered in a context of retention of particles bigger than average pore size in the membrane. To be implemented for retention of organic matter, molecular weight of a molecule is converted into hydrodynamic radii by comparing the diffusivities of the spheres calculated with Stokes-Einstein equation, with diffusivities of organic molecules (Arkhangelsky & Gitis 2008). That approach might be problematic as organic molecules are likely to have a shape that deviates from a sphere. The shape might change as a result of changes in solution chemistry and after interaction with other molecules, or to stretch out under high pressure (Arkhangelsky et al. 2008). As a result, macromolecules with an equivalent diameter much higher than average membrane pore size might penetrate the UF membranes through, as it is depicted on Figure 1 for proteins. Hydrophobic interactions are viewed as adsorption or capture of organic matter by membrane material until equilibrium or saturation is reached. The effect depends on chemical affinity of the solute to polymer material. The adsorption mechanism is hence very specific to membrane and solute and also influenced by solution chemistry.

Charge repulsion and electroneutrality are usually described as solutes having the same charge as the membrane are being retained more, while solutes with the opposite charge are attracted by the membrane and subsequently permeate more easily. For UF membranes that phenomenon is also attributed to the size ratio between organic molecule and pore—if the pore is large enough in

comparison to molecule size, than having similar charges on both will increase repulsion from pore walls and result in smaller retention. Opposite charges when sizes of pore and organic molecule are of the same order are likely to result in ionic bonding and increased retention. The brief description of the three main mechanisms is leaving outside other fouling mechanisms that might be important, such as hydrophobic interactions, hydrogen bonding, bringing mechanisms, as well as specific and charge interactions.

The experimental approach for studies of fouling phenomenon is based on filtration experiments performed with either natural or artificial solutions having organic content. There are three groups of parameters—feed water characteristics, membrane characteristics, and operational conditions—that might influence degree of fouling. The brief description of the three main mechanisms is leaving outside other fouling mechanisms that might be important, such as hydrophobic interactions, hydrogen bonding, bringing mechanisms, as well as specific and charge interactions.

However changing characteristics of the organic material is not a trivial task. The usual approach is to perform separate UF membrane tests with different organic compounds, sometimes taken from a similar functional group (Mehta & Zydney 2006). The approach is as simple and useful as problematic if we take into account that each organic molecule carries a full unique range of parameters. Simplifying the organic molecule down to its functional group and macroscopic characteristics, we must agree that each molecule will have its own molecular weight, hydrophobic behavior and surface charge. The conclusions usually drown on the retention mechanisms will be of general character since the retention itself is governed by all the properties. The other way to assess the importance of electrostatic repulsions is to amend the characteristics of feed water such as pH, ionic strength and concentration of organic matter (Matsumoto et al. 2003). That approach is also less than optimal since the change will affect the characteristics of water, the organic molecule and membrane surface. That might influence the secondary structure of organic material changing its shape from globular to linear form as it is shown on Figure 1. Hence application of the currently available tools for studies of fouling phenomenon is likely to not be able to differentiate between the main fouling mechanisms, and even if a researcher intends to study the electrostatic phenomenon alone more likely that all the fouling mechanisms will be involved.

Here we suggest a novel way to assess the importance of electrostatic interactions in fouling of UF membranes by a deliberate alteration of surface charge on the organic molecules. Selective alteration can be performed by either succinilation or by guanidination resulting in formation of the same compounds with extensive negative or positive charge, as illustrated on Figure 2 for proteins. The obtained molecule has no influence on the solution chemistry and its introduction guards the system status-quo except for the degree of electrostatic interactions.

**MATERIALS AND METHODS**

Commercially available 20-kDa MWCO UF membranes (Sterlitech Corporation, Kent, WA, USA) made of polyethersulfone (PES-20) were used. The membranes were
supplied as flat sheets and were stored dry under ambient conditions. Nominal MWCO, contact angle, and permeability of the membranes, have been reported previously (Arkhangelsky et al. 2007), as was a detailed description of the lab-scale 150ml stirred cell used for the experiments. In brief, pieces of membrane taken from a flat sheet were cut into round shapes with cross sectional areas of 0.0017 m². The membrane pieces were thoroughly rinsed with deionized water at 30°C by shaking at 100 rpm on a mechanical shaker for 1h before an experiment, after which the membrane sheets were placed in the cell. Membrane compaction experiments were performed by filtration of deionized water for 30 min at 2 bars N₂ (99.99% purity) pressure. In these experiments, membrane flux was calculated gravimetrically as:

\[
J = \frac{\Delta m}{\rho S \Delta t P}
\]

where \(\Delta m\) is the permeate weight difference (kg), \(\Delta t\) is the frequency interval (h), \(S\) is the active membrane surface area (m²), \(P\) is TMP (bar), and \(\rho\) is the permeate density (kg/m³).

Measured membrane permeability values were 80 ± 2 L/m²-h-bar. Membrane samples that showed significant deviations from the measured average permeability values were discarded.

The streaming potential was measured in the stirred cell by pumping a KCl solution through the membrane pores in a transmembrane mode (Arkhangelsky & Gitis 2008). The membrane sample was placed above a PTFE spacer (0.2 mm in thickness) with the membrane skin-layer facing the flow channel (200 × 74 mm). The electrolyte solution was forced through the membrane by static pressure given by nitrogen. The membrane was equilibrated by soaking it in a 0.001 M KCl solution overnight. Measurements were performed for 10 mM KCl at pressures of 0.5 and 1 bar in the pH range 3–10.5 (at pH values higher than 10.5, the electrode is unstable; at pH measurements lower than 3, solution conductivity will be too large and will affect the measurement). Measurements were conducted with Ag/AgCl reference electrodes (model 725/735, Metrohm Ltd., Switzerland) and a high impedance digital multimeter (EDM 2347, Escort, Taiwan). The asymmetry potential of the electrode pair was less than 1 mV.

Well known protein bovine serum albumin (BSA) was modified following the procedure previously described by Habeeb et al. (1958). That is however can serve as a premise for developing a full range of modified compounds. Briefly, for the current study 12 moles of succinic anhydride (Sigma-Aldrich Israel Ltd., Rehovot, Israel) were gradually added to 15 ml of a 5% solution of the cold alcohol precipitated BSA (Sigma-Aldrich) in deionized (DI) water (MilliQ quality). The pH was adjusted to pH 8 and maintained by 1 N NaOH, if needed. The reaction was performed under continuous magnetic stirring keeping the temperature at 4°C for 30 min. After that the solution was dialyzed against 0.1 M phosphate buffer at pH 7.5 for 48 hours. The resulted solution was lyophilized. As result of this procedure amino groups of lysine were replaced with a \(\text{NHCO} \left(\text{CH}_2\right)_2\text{COO}^-\).

Guanidination of BSA was performed with 1-guanyl-3,5-dimethylpyrazole Nitrate (GDMP) prepared from amidoguanidine nitrate (Sigma-Aldrich) and 2,4-pentanedione (Habeeb 1972). Five percent (by weight) BSA were reacted in 0.5 M GDMP at pH 9.5 and 0°C for 7 days. After reaction

![Diagram of Electrostatic repulsion of native (a), succinylated (b) and guanidinated (c) BSA.](https://iwaponline.com/wst/article-pdf/58/10/1955/435878/1955.pdf)
the solution was brought to pH 7.5 and dialyzed against phosphate buffer, ionic strength 0.1 M. The reaction replaces the positively charged NH$_3^+$ group of lysine residues with $-$NH–C(–NH$_2$)–NH$_2$ groups thus increasing positive charge of the protein.

Concentration of BSA in feed and permeate was detected using Bradford assay (Bradford 1976) which involves binding of Coomassie Brilliant Blue G-250 (Sigma-Aldrich) to the protein. The binding causes a shift in the absorption maximum of the dye from 465 to 595 nm; it is the increase in absorption at 595 nm that was monitored with Synergy HT Multi-Mode Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA). Zeta potential of native and modified BSA was measured with ZetaPlus (Brookhaven Instruments Corporation, Holtsville, NY, USA). Transition electron microscopy (TEM) was performed at 120 kV with a JEM-1230 equipped with a TemCam-F214 (TVIPS Company, Germany) camera.

The filtration experiments were performed at 20 ± 1°C and pH range between 5 and 10 with 30 mg of BSA diluted in 100 ml of buffered sterile deionized water. The solution was pressed through the membrane by nitrogen at 1 bar TMP for time periods of 30 min per run.

RESULTS AND DISCUSSION

Figure 3 presents zeta potential–pH curves for native and modified BSA (Figure 3a) and PES membrane (Figure 3b). BSA is a protein with a molecular weight of 67 kDa having a point of zero charge at pH 4.2, in accordance with earlier reported data (Matsumoto et al. 2003). The protein is constructed from a library of just twenty aminoacids, which share a common “backbone” but are distinguished by their chemically diverse “sidechains”. Each sidechain contains amino and carboxyl end groups that will attribute to the protein a positive charge at low pH, and negative charge at high pH. Than looking on the zeta potential–pH plot of native BSA the zeta potential values of 30 mV for pH of 3 and −30 mV for pH of 8 and higher were detected. The succinylated BSA is more negatively charged in comparison with BSA that did not undergo succinylation. BSA and succinylated BSA had no pronounced charge differences at pH range from 3 to 5. However from pH 5 to 10.5 these two proteins have a significant difference in zeta potential values. For this pH range zeta potentials of native and modified BSA are changing from −9 mV to −30 mV and from −22 mV to −40 mV respectively. Guanidination of the protein resulted in a shift in pzc from 4.2 to pH 5.8 and more positive charge in the area of low pH values. For example, at pH 4 zeta potential of native BSA was 3 mV, of succinylated BSA 0 mV and of guanidinated BSA 25 mV. Same difference was depicted at alkaline pH values. At pH 8 zeta potential of native BSA was −27 mV, of succinylated BSA −40 mV and of guanidinated BSA −15 mV. The membrane was negatively charged for the entire pH range (Figure 3b), and as the pH became more alkaline, the absolute values of the streaming potential measured in 10 mM KCl increased from −7 mV at pH 5 to −12 mV at pH 10. Since PES has no dissociated functional groups, specific ion adsorption is the only possible process for the formation of surface charge, i.e., the initial charge can be attributed to the adsorption of hydroxyl ions on the PES membrane surface.

Figure 3 | Zeta potential–pH curves for native and modified BSA (a) and PES membrane (b).
Influence of charge modification on the BSA size is depicted in Figure 4.

The same 50,000 magnification allows comparing the approximate size and shape of native and modified BSA. The native BSA is of a round shape with an average size of 27–33 nm. Both succinylation and guanidination resulted in a change of BSA shape to a prolonged one, and had some visible effect on the BSA size. The observed diameter of succinilated BSA was in a range of 23–27 nm, and the diameter of guanidinated BSA was in the range of 18–23 nm. The obtained results were approved by dynamic light scattering measurements (data not shown) at 90° angle. The observed decrease in the average diameter of modified BSA can single out the electrostatic repulsion since the retention of BSA due to size exclusion should be bigger for native protein than for a modified one. The results of retention of native and modified BSA as a function of pH are depicted on Figure 5.

Degree of retention of BSA depends on its surface charge. For low pH values of 5 and 6 the guanidinated BSA has a positive charge when both native and succinylated BSA are negatively charged. In comparison with negatively charged membrane the native and succinylated BSA that are retained due to electrostatic repulsion and size exclusion, the guanidinated BSA can be retained also due to charge interactions. That results in higher degree of removal along with more severe fouling. At pH 7 and 8 degree of retention of succinylated BSA has dropped from 92 to 87%, probably due to increase in absolute zeta potential values. Degree of retention of native and guanidinated BSA remained the same as it was for lower pH values. At pH of 9 and 10 degree of retention has dropped from 95–97% removal to 87–88% removal, probably due to increased electrostatic repulsion. At that pH range lower degree of fouling is expected as well. The obtained tendencies point on the presence of a threshold in electrostatic repulsion. When the absolute value of zeta potential of the macromolecule is below 20 mV, the repulsion might take place but it is less dominant. That has a direct influence on the degree of fouling as depicted on Figure 6 for pH values of 5, 7, and 9. Degree of fouling as a function of pH for native BSA had been reported in our previous communication (Kuzmenko et al. 2005). Here we report flux results for succinylated BSA (Figure 6a) and comparison of fluxes for native, succinylated and guanidinated BSA at pH 5, 7 and 9 (Figure 6b–d). Fluxes of succinylated BSA has shown that the higher absolute value of zeta potential, smaller degree of fouling and less significant flux drop. One common tendency that was depicted throughout the experiments is that flux drop occurs almost immediately, and that is strictly opposite to previously described (Iritani et al. 2007). The difference can be attributed to concentrated feeds and performing experiments in a dead-end mode, so the observed fluxes are introduced primarily for a relative comparison. Along with that we are confident that they are adequately representing the fouling tendencies on any scale. Flux drop at pH 5.2 where zeta potential of succinylated BSA were closer to electroneutrality than at any other pH was significant and for the first minute the flux reduction from 80 to 301/m²-h was observed. That was followed by a plateau for the rest of the experiment. For other pH values the flux drop was less

![Figure 4](https://iwaponline.com/wst/article-pdf/58/10/1955/435878/1955.pdf) | Electron micrographs of native and guanidinated BSA.
drastic and resulted in 50, 40 and 25% reduction for pH values of 7.1, 8.2 and 10 respectively.

Similar tendencies were observed for flux measurements performed for native, succinylated and guanidinated BSA at pH 5.2 (Figure 6b). As can be seen during 30 minutes filter run with native BSA the flux decreased from 80 to 20 l/m²-h, in comparison with 30 l/m²-h for positively charged and 50 l/m²-h for negatively charged BSA. Comparing the flux data with retention and zeta potential measurements it can be concluded that at that pH value the native BSA was close to electroneutrality, and therefore its interactions with membrane surface could result in selective adsorption and severe flux drop. Somewhat different tendency was observed at pH 7.1 (Figure 6c) where the guanidinated BSA was closer to electroneutrality and therefore the flux drop for experiment performed with positively charged protein resulted in more significant flux drop of 75%. At the same time charged native and succinylated BSA flux drop was in 40% range. As to the experiment performed at pH 9 (Figure 6d) the zeta potential values of all three proteins were almost identical so the degree of fouling was similar although the absolute flux values were surprisingly low.

CONCLUSIONS

Electrostatic interactions play an important role in membrane fouling by a BSA protein. At low pH values the BSA becomes either electroneutral or even positively charged. The low surface charge does not provide a strong electro-negative repulsion and results in fouling, the existence of which was proved by reduction of protein concentrations over time.

Although the general trend of reduced fouling with increased electrostatic repulsion was obvious, the approach
of selective alteration of surface charge of organic molecules was particularly useful for delineation of retention mechanisms. Applying the succinylation we were able to verify the previous assumptions without the need for further simplifications. The electrostatic repulsion becomes important when zeta potential of the protein exceeded 20 mV, when before the threshold the interactions were mainly governed by size exclusion.

REFERENCES


