Carbohydrate Polymers 116 (2015) 243–248

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Influence of ionic strength on membrane selectivity during the ultrafiltration of sulfated pentasaccharides

Camille Delcroix[a], Jean-Philippe Bonnet[a], Martial Etienne[b], Philippe Moulin[a,*]

[a] Aix Marseille Université, CNRS, M2P2, UMR 7340, Equipe Procédés Membranaires (EPM), Europôle de l'Arbois, pavillon Laennec, hall C, BP 80, 13545 Aix en Provence cedex, France
[b] Sanofi Chimie, Route d'Avignon, 30390 Aramon, France

ARTICLE INFO

Article history:
Received 20 December 2013
Received in revised form 24 June 2014
Accepted 3 July 2014
Available online 16 July 2014

Keywords:
Ultrafiltration
Sulfated polysaccharides
Charge effects
Salinity

ABSTRACT

Due to their numerous biological properties, natural sulfated polysaccharides have attracted the interest of the food and pharmaceutical industries. Membrane processes were thought to be especially suitable for their production at industrial scale. The aim of this study was to evaluate the effect of sodium chloride, often used as a preservative and a precipitation adjuvant, on the ultrafiltration of sulfated pentasaccharides. In pure water, results showed a complete retention of the polymers on membranes with molecular weight cut-off up to eight times the molecular weight of the studied pentasaccharides. When NaCl was added to a concentration of 0.5 mol L^{-1}, retention rates decreased significantly (≈−50%). As no relevant modification of the molecules size was observed through hydrodynamic radius measurements, these variations of selectivity were fully attributed to the screening of membrane surface charges by the electrolyte. Therefore, optimising the ultrafiltration of charged molecules need absolutely exammining electrostatic interactions.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

In recent years, a wide range of polysaccharides has emerged as an important class of bioactive natural products. Sulfated polysaccharides have particularly aroused the interest of many researchers because of their various applications in food and pharmaceutical industries. Indeed, some of them, such as pectin or carrageenans, have become valuable food additives due to their rheological properties. They are used as gelling agents, thickeners, stabilisers, emulsifiers or fat replacers (Hatziantoniou & Howell, 2002; Hodur, Kertesz, Beszedes, Laszlo, & Szabo, 2009; Moresi & Sebastiani, 2008). In addition, many of these biopolymers exhibit a wide range of biological activities that may find relevance in functional food and pharmaceutical applications. For example, glycosaminoglycans from the extracellular matrix of the vertebrate organisation (Barbucci, Magnani, Lamponi, & Albanese, 1996; Ben Mansour et al., 2009; Chavaroche, Van den Broek, & Eggink, 2013; Cui, Li, & Yuan, 2012; Yang, Chang, Weyers, Sterner, & Linhardt, 2012; Wu & Chen, 2006) or oligosaccharides derived from polysaccharides of seaweed, bacteria, fungi or plant origin (Mellal et al., 2008; Jiao, Yu, Zhang, & Ewart, 2011; Wijesekara, Pangestuti, & Kim, 2011; Cardozo et al., 2011; Chen et al., 2011) possess antitumor, antiviral, immunomodulant, anticoagulant and antioxidant properties. Therefore, many studies have been launched on polysaccharides isolation to improve both selectivity and yield of their production processes.

Sulfated polysaccharides are traditionally extracted by enzymatic hydrolysis and precipitated with ethanol. The purification step is mainly realised by gel filtration or anion exchange chromatography and a high grade of purification is required since only polysaccharides of specific degree of polymerisation show biological activities. Thus, when conventional laboratory techniques are employed, the purification process can become very expensive, which limits the production at industrial scale.

Due to a wide size range, membrane processes have seemed to be especially suitable for initial purification of bioactive and heat-sensitive materials at the industrial scale as they are operated under mild conditions, permit to treat large volumes and can be easily automatised. Furthermore, membrane processes offer a large number of benefits for industries: high product selectivity with consistent quality of permeate and retentate, low energy requirement, reduced operating costs, easy implementation, long operating life and low pollution. The clarification and concentration of sulfated polysaccharide solutions can be achieved by

* Corresponding author. Tel.: +33 4 42 90 85 01; fax: +33 4 42 90 85 15.
E-mail addresses: camille.delcroix@etu.univ-amu.fr (C. Delcroix), jean-philippe.bonnet@univ-amu.fr (J.-P. Bonnet), martial.etienne@sanofi.com (M. Etienne), philippe.moulin@univ-amu.fr (P. Moulin).

http://dx.doi.org/10.1016/j.carbpol.2014.07.013
0144-8617/© 2014 Elsevier Ltd. All rights reserved.

microfiltration or ultrafiltration membranes. The following steps of purification are thus facilitated, for example by reducing the volumes to be treated or the amount of alcohol needed for precipitation, and the overall quality of the final product is improved (Hodur et al., 2009). However, the filtrating conditions have to be optimised to control the decline in permeate flux and the variations in selectivity which occur during the filtration of complex natural products. Due to molecules/molecules and molecules/membrane interactions, the determination of the optimised operating conditions is not easy and requires a careful investigation at the laboratory scale (Hatziantoniou & Howell, 2002; El Rayess et al., 2012; Mellal et al., 2008).

Interactions between molecules and membrane during the filtration process are not limited to steric effects. Particularly, in the case of charged molecules, electrostatic phenomena can appear and strongly modify the mass transfer. Most membranes present an electrically charged active surface and these interactions become more and more important with decreasing pore size and thus have to be considered in ultrafiltration (Moritz, Benfer, Arki, & Tomandl, 2001). Regarding ceramic membranes, which are the most frequently employed in industrial filtration plants for this application, the metal oxides composing the active layer become hydroxylated in aqueous solution, leading to the development of an electrical charge at the membrane surface (Zhang, Jing, Fan, & Xu, 2008). Consequently, the pH and ionic strength of the solution may affect significantly the behaviour of sulfated polysaccharides in ultrafiltration (Broeckmann, Wintgens, & Schäfer, 2005; Lo, Yang, & Min, 1996; Pinelo, Moller, Prado-Rubio, Jonsson, & Meyer, 2013). In fact, during the process of polysaccharides isolation, sodium chloride is often added to the solution to prevent microbial development and to strongly reduce the alcohol quantity needed for precipitation (Garcia-Ochoa, Santos, Casas, & Gomez, 2000). The variations of the ionic strength by adding salt can modify the molecular conformation of charged molecules and their interactions with the membrane material. It is then expected that the presence of sodium chloride has an impact on molecules retention.

The present study was undertaken to determine the effect of sodium chloride addition on the membrane efficiency in terms of permeate flux and retention during the ultrafiltration of sulfated pentasaccharides, in order to identify the mechanism(s) controlling the purification and, thus, improve the industrial membrane processing of such molecules.

2. Materials and methods

2.1. Test molecules

Two synthetic polysaccharides provided by Sanofi Chimie (Aramon, France) were selected for ultrafiltration trials because of their structural similitude with natural anticoagulant polysaccharides (Fig. 1). There were a Linear Sulfated Pentasaccharide (LSP) with a molecular weight (M_W) of 1726 g mol^{-1} and the associated Biotinylated Sulfated Pentasaccharide (BSP) with a molecular weight of 2046 g mol^{-1} to determine the impact of their molecular configuration on their behaviour during filtration experiments. The graft of biotin, responsible for a gap of 16% in molecular weight, allows the inhibition of the pentasaccharide activity by adding avidin. These molecules present many sulfated and carboxyl groups, making them highly ionised in aqueous solution.

2.2. Membranes

The ultrafiltration membranes employed in this study were tubular ceramic membranes with molecular weight cut-off (MWCO) varying from 1 to 150 kDa (Inside Ceram™ and Filtanium™ series, Tami Industries, Nyons, France). Each monochanneled porous membrane presented a membrane area of 0.011 m^2, made either of titanium dioxide (TiO_2) or zirconiuim dioxide (ZrO_2). According to measurements of electrophoretic mobility (Verhnet & Grangeon, 2003), which consist in recording the time needed for a suspended compound subjected to an electric field to cross the measuring cell, TiO_2 powder showed isoelectric points (IEP) at pH 3.2 ± 0.5 which is consistent with literature data (Zhang et al., 2008) and ZrO_2 powder had an IEP at pH 3 ± 0.1 which is quite below the values given in literature (6–7). This can be explained by the origin and the treatment of the metal oxides solutions which surely differ from one manufacturer to another. In the study of Verhnet and Grangeon (2003), the oxide powders have been thermally treated in the same way as for the membranes elaboration, before being crushed and sieved. Thus, theses values reflect the surface charge of the membranes with higher accuracy.

2.3. Experimental setup

The ultrafiltration experiments were performed on a filtration module composed of four carters in parallel configuration,

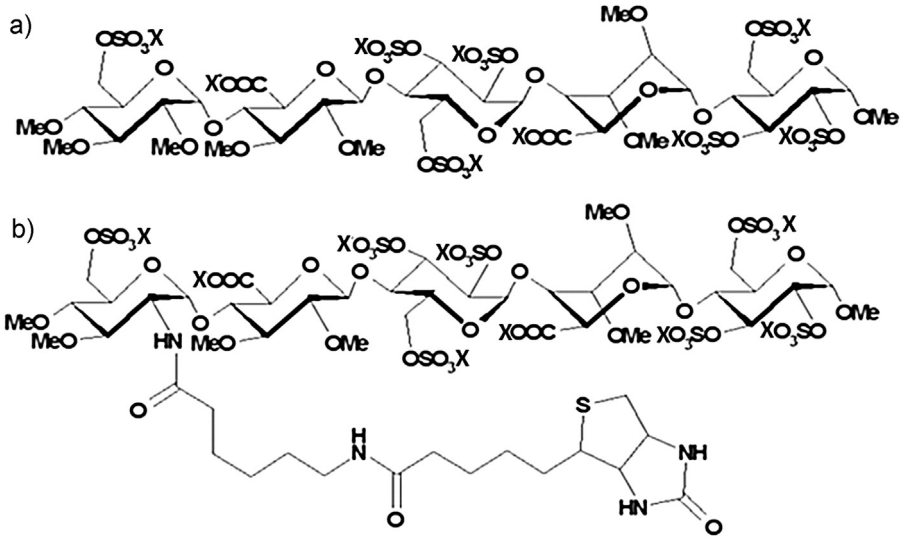

Fig. 1. Chemical structures of the test pentasaccharides (a) linear sulfated pentasaccharide: 1726 g mol^{-1}; (b) biotinylated sulfated pentasaccharide: 2046 g mol^{-1}; X = Na).

Table 1
Water permeabilities at 20 °C and membrane resistances.

MWCO (kDa)	R_m (10^{12} m^{-1})	Lp_0 (L h^{-1} m^{-2} bar^{-1})
1	7.1–8.9	40–51
5	5.4–7.8	46–66
10	6.0–7.1	50–60
15	6.6–7.6	47–55
100	1.8–2.1	168–204
150	1.8–2.4	148–197

adapted for comparative filtration tests (Helicopter, Tami Industries, Nyons, France). It allows the ultrafiltration of a solution on four different membranes simultaneously, insuring identical experimental conditions in terms of TMP, velocity and feed composition. Each experiment was conducted under a transmembrane pressure (TMP) of 1.50 ± 0.05 bar, a turbulent crossflow velocity of 4.9 ± 0.5 m s^{-1} (Re ≈ 30,000) and a temperature of 30 ± 2 °C. The volume of the feed solution was 5 L and the concentration of pentasaccharides in distilled water was fixed at 4 g L^{-1} to limit intermolecular interactions. Retentates and permeates were continually recycled to the feed tank to keep the feed concentration constant. After having reached the steady state, the permeate flux to the solution was measured and the feed and permeates samples were collected, according to the French Standard for the determination of retention rate of ultrafiltration membranes (French Standard NF X 45-103, 1997). To evaluate the effect of sodium chloride concentration, experiments were performed without NaCl addition and at two different salt concentrations, one low and one high (0.01 and 0.50 mol L^{-1}). The minimum concentration allows the significant decrease of intramolecular electrostatic repulsion providing flexibility to the molecules (Duclos, 1984), and the maximum concentration was set to meet the use limit of the chromatographic device employed for samples analysis.

2.4. Membrane cleaning and water permeability

Membranes were cleaned before the first use to remove the material left during the membrane preparation, as recommended by the manufacturer and highlighted in the literature (Nyström & Zhu, 1997; Zhu & Nyström, 1998), and after each filtration experiment following this protocol: a solution of sodium hydroxide at a concentration of 20 g L^{-1} was filtered during 30 min at a temperature of 80 °C and a TMP of 1.50 bar. After rinsing to neutrality with distilled water, a solution of nitric acid at a concentration of 5 mL L^{-1} was filtered during 15 min at a temperature of 50 °C and a TMP of 1.50 bar. Finally, the membranes were rinsed to neutrality with distilled water and the initial water permeabilities were evaluated. After filtration experiments if the loss of water permeability was higher than 10%, the cleaning sequence was repeated. The ranges of water permeabilities (Lp_0) and the corresponding membrane resistances (R_m) are presented in Table 1. They are in accordance with the permeability ranges given by TAMI industries.

2.5. Determination of fouling resistance

The permeate flux during the filtration of a polysaccharides solution (J_p) can be expressed through the equation of the resistance-in-series model (Rai, Rai, Majumdar, DasGupta, & De, 2006):

$$J_p = \frac{TMP}{\eta \times (R_m + R_r + R_i)} \quad \text{with} \quad R_r + R_i = R_f \tag{1}$$

where η is the dynamic viscosity (Pa s) and R_m, R_r, R_i (m^{-1}) are, respectively, the resistances of the membrane, the reversible fouling and the irreversible fouling. For each experiment, the membrane resistances were determined considering the water permeabilities and the total fouling resistances ($R_f = R_r + R_i$) with the permeabilities to the solution. At the end of the filtration, the membranes were rinsed with distilled water during 10 min at a TMP of 1.50 bar. The variation of the permeability associated to this step is defined as reversible fouling and the remaining fouling after rinsing corresponds to the irreversible fouling resistance (R_i). By way of example, for the filtration of the LSP in pure water on the 1 kDa membrane, the values of membrane permeability and corresponding resistances for each of the conducted measurements are illustrated on Fig. 2.

Table 2
Dynamic viscosity measurements at 25 °C.

[NaCl] (mol L^{-1})	η (mPa s)		
	Water	LSP (4 g L^{-1})	BSP (4 g L^{-1})
0	0.89	0.90	0.89
0.01	0.89	0.90	0.90
0.50	0.94	0.94	0.94

2.6. Viscosity measurements

For the calculation of resistances, the dynamic viscosity of the different feed solutions was measured at 25 °C (Table 2). Measurements were conducted on a rheometer with double Couette geometry (AR 550, TA Instruments, Guyancourt, France). The values of viscosity obtained for pure water and water with NaCl are in accordance with literature data (Copin-Montégut, 2002). The addition of LSP or BSP at the operating concentration of 4 g L^{-1} did not affect the viscosity values which were increased by 1%, at most.

2.7. Determination of retention rates

The concentration of pentasaccharides in the collected samples was determined through density measurements. The densimeter (DMA 5000 M, Anton Paar, Courtaboeuf, France) offers a fast and reliable measure with an accuracy of 5×10^{-6} g cm^{-3} and a repeatability of 1.10^{-6} g cm^{-3}. For each NaCl concentration and each molecule, a calibration curve was established, and the concentrations of permeates were then obtained from the density values. Thus, the retention rate values (RR) expressed in percentage of retained molecules, are accurate to 1%. These measurements were performed in duplicate and the average values are presented.

2.8. Hydrodynamic radius measurements

For the different conditions of salinity, the hydrodynamic radius (R_h) of the test molecules in solution was determined using size exclusion chromatography with triple detection (Viscotek TDA max, Malvern Instruments, Orsay, France). This device provides R_h values through the coupling of a refractometer, a right angle light scattering detector and a viscosimeter as shown by Eqs. (2)–(5). The measurements are realised in triplicate.

Equation of the refractometer:

$$RI = K_{RI} \times \frac{dn}{dC} \times C \tag{2}$$

with RI the refractometer signal (mV), K_{RI} the refractometer constant, n the refractive index of the sample and C the concentration of the sample (g L^{-1}).

Equation of the right angle light scattering detector:

$$RALS = K_{RALS} \times M \times \left(\frac{dn}{dC}\right)^2 \times C \tag{3}$$

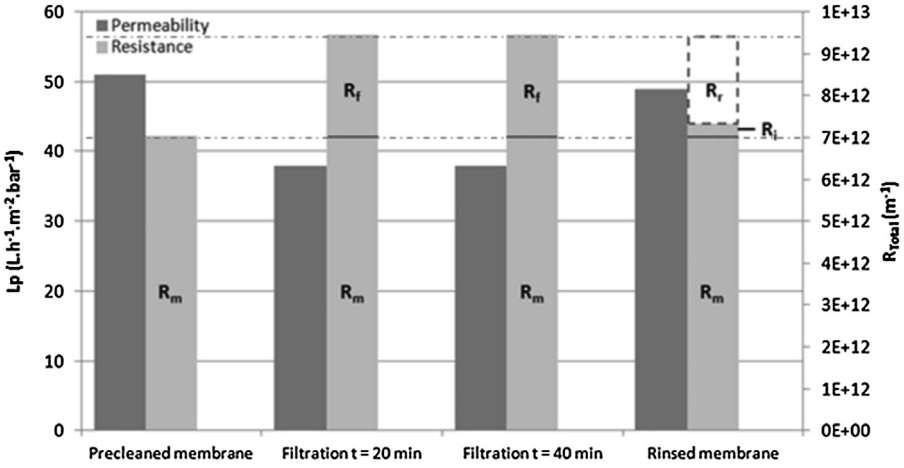

Fig. 2. Permeabilities and associated resistances obtained with the 1 kDa membrane for the filtration of LSP in pure water.

where *RALS* and K_{RALS} are the signal and the constant of the light scattering detector and M the molecular weight of the compound ($g\,mol^{-1}$).

Equation of the viscosimeter:

$$IV = K_{IV} \times [\eta] \times C \quad (4)$$

with IV the viscosimeter signal, K_{IV} its constant and $[\eta]$ the intrinsic viscosity of the compound ($L\,g^{-1}$).

The R_h is then calculated using the Einstein's viscosity equation:

$$[\eta] \times M = 2,5 \times \left(\frac{4}{3} \times \pi \times N_A \times {R_h}^3\right) \quad (5)$$

where N_A is the Avogadro number.

3. Results and discussion

3.1. Influence of salinity on selectivity

The obtained values of retention rate for the lower molecular weight cut-offs (1–15 kDa) (Figs. 3 and 4) showed that the two molecules were unexpectedly totally retained ($RR > 95\%$) in pure water and at low NaCl concentration. The pentasaccharides transfer was observed only from the 100 kDa membrane although their molecular weights are 50 times lower than the membrane MWCO, and even then the retention rates were still high (45–85%). Thus, the impact of the phenomenon responsible for the high retention of the polymers decreases with increasing membrane pore size. On the contrary, the addition of sodium chloride at a higher concentration ($0.50\,mol\,L^{-1}$) has strongly modified the filtration behaviour. Indeed, for both compounds and all MWCO, the retention values were lower than 50%, even for the 1 kDa membrane which cut-off is half the molecular weight of the polymers. A decrease of the RR was also noticed on the membranes with high MWCO, which already allowed the molecules to pass through at low salt concentration. Therefore, the membrane selectivity was clearly not only controlled by the steric effects. The presence of NaCl at high concentration has notably influenced the interaction between the sulfated pentasaccharides and the different membranes.

3.2. Influence of salinity on membrane fouling

The fouling resistances are presented in Table 3. The values have been gathered according to the similarity of selectivity results, as the mean values obtained for the low MWCO and the high MWCO. The addition of sodium chloride at $0.50\,mol\,L^{-1}$ has led to a decrease of the fouling resistance values for LSP and to an increase of these values for BSP. For low MWCO membranes, when no transfer of molecules was observed (i.e. in pure water and at low NaCl concentration), the fouling appeared to be mainly composed of reversible surface fouling, in particular for the LSP. This trend was still noticed at high concentration of salt for the linear polysaccharide whereas the proportions of R_r and R_i were reversed for the branched molecule. For high MWCO membranes, the behaviour of the two compounds was similar in any filtration conditions. The fouling was mainly reversible by rinsing with water and the part of irreversible fouling has increased with NaCl concentration. Thus, the LSP appeared to predominantly cause surface fouling, whereas the BSP seemed to be more prone to adsorption or to internal fouling. This difference in fouling mechanism is probably related to the structure and the conformation of the molecules.

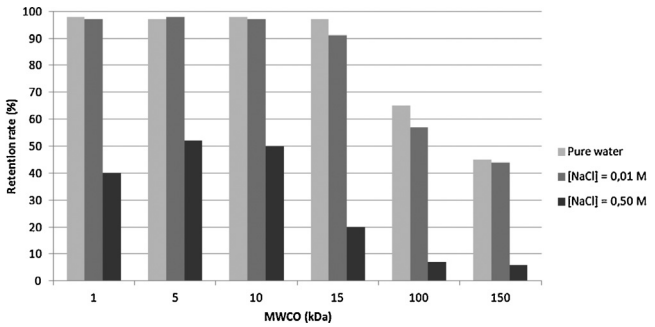

Fig. 3. Variation of the retention rate of the LSP as a function of MWCO for differents ionic strengths [$4\,g\,L^{-1}$, 1.50 bar, 30 °C, $4.9\,m\,s^{-1}$].

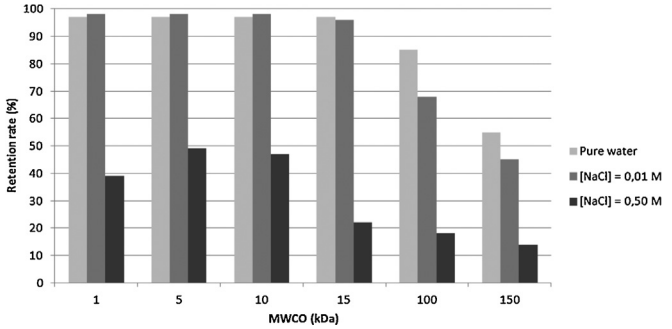

Fig. 4. Variation of the retention rate of the BSP as a function of MWCO for differents ionic strengths [$4\,g\,L^{-1}$, 1.50 bar, 30 °C, $4.9\,m\,s^{-1}$].

Table 3
Fouling resistances for the different operating conditions.

	LSP			BSP		
	R_f[a] (10^{12} m^{-1})	R_r[b]/R_f (%)	R_i[c]/R_f (%)	R_f (10^{12} m^{-1})	R_r/R_f (%)	R_i/R_f (%)
Pure water						
1–15 kDa	2.2	88	12	0.8	63	37
100–150 kDa	2.4	85	15	1.9	88	12
[NaCl] = 0.01 mol L^{-1}						
1–15 kDa	2.1	87	13	2.7	70	30
100–150 kDa	3.3	80	20	1.5	80	20
[NaCl] = 0.50 mol L^{-1}						
1–15 kDa	1.6	84	16	3.1	29	71
100–150 kDa	0.8	60	40	3.1	73	27

[a] Fouling resistance ($R_i + R_r$).
[b] Reversible fouling resistance.
[c] Irreversible fouling resistance.

3.3. Influence of salinity on molecules size

In order to determine the impact of ionic strength on the apparent size of the molecules, their hydrodynamic radius (R_h) was measured at the two concentrations of sodium chloride (Table 4). Although only few data on the hydrodynamic radius of low molecular weight polysaccharides are available in the literature, the values given by Morris, Adams, and Harding (2014) are in agreement with our results. The R_h of heparin molecules of 3.9 kg mol^{-1} is estimated to 1 nm in aqueous solution with 0.2 mol L^{-1} of NaCl and the one of a 6 kg mol^{-1} pullulan to 4 nm in aqueous solution with 3.10^{-3} mol L^{-1} of NaN_3. Moreover, the values of hydrodynamic radius obtained by Armstrong, Wenby, Meiselman, and Fisher (2004) for some others polymers with molecular weights similar to those of the LSP and the BSP, in aqueous solution with 0.01 mol L^{-1} of Na_2HPO_4, confirm our results. Indeed, PEGs of 1.45 and 2.0 kg mol^{-1} are characterised by a R_h of, respectively, 1.13 and 1.36 nm. For 0.01 M of NaCl, our results showed that the LSP radius was around 16% lower than the BSP radius, which is consistent with their difference in structure and configuration. Regarding the impact of ionic strength, the addition of NaCl at 0.50 mol L^{-1} did not affect the apparent size of the linear polymer and reduced the hydrodynamic radius of the branched molecule by only 11%. Consequently, the observed variations in selectivity could not be explained by size effects.

3.4. Influence of salinity on membrane surface charge

In order to clearly determine the influence of sodium chloride addition in the same conditions as during industrial processing, the pH of the different feed solutions was not modified. The values were of 6.4 ± 0.2 for BSP and of 7.6 ± 0.2 for LSP. According to the data of isoelectric points (pH ≈ 3), the membrane surfaces became negatively charged for these pH values. The electrostatic repulsion between the sulfated polymers and the active layers may explain the high retention rates observed in pure water. Measurements of electrophoretic mobility (μ_{ep}, m^2 V^{-1} s^{-1}) of metal oxides in aqueous solution of KNO_3, conducted by Verhnet and Grangeon (2003), showed the influence of electrolyte concentration on this parameter in the pH range of the feed solutions (Table 5). A shift in ionic strength from 10^{-3} to 10^{-2} mol L^{-1} have led to a decrease by 9% of the absolute values of μ_{ep}, which corresponds to an equivalent decrease in the apparent electric charge of metal oxides as these parameters are related according to the following equation:

$$\mu_{ep} = \frac{q}{6 \times \pi \times \eta \times R_h} \quad (6)$$

where q is the electric charge of the molecule. This low reduction was consistent with the absence of notable changes in terms of selectivity. In the case of a concentration shift from 10^{-3} to 10^{-1} mol L^{-1}, a strongly decrease by around 40% was noticed. As the experimental ionic strength was five times higher, this strong reduction of the apparent surface charge, by screening, was quite in agreement with the changes observed in retention rates. Therefore, the electrostatic interactions between molecules and membrane surface seemed to be fully responsible for the modifications in ultrafiltration performances. This phenomenon of charge screening by the presence of an electrolyte was also observed by Ricq, Pierre, Reggiani, Pagetti, and Foissy (1998) on ZrO_2 and by Velikovska and Mikulasek (2007) on TiO_2. As mentioned by Alkan, Demirbas, and Dogan (2005), in the case of monovalent electrolytes, there is no specific adsorption of ions on the oxides. Owing to the Coulomb's law, they accumulate as counterions in the electrical double layer and compress it, leading to a decrease of the apparent electric charge of the metal oxides.

Table 4
Mean values of hydrodynamic radius.

[NaCl] (mol L^{-1})	R_h (nm)	
	LSP	BSP
0.01	0.88 ± 0.02	1.05 ± 0.02
0.50	0.87 ± 0.02	0.93 ± 0.02

Table 5
Variations of the electrophoretic mobility of metal oxides with salt addition (Verhnet & Grangeon, 2003).

	TiO_2	ZrO_2
[KNO_3] (mol L^{-1})	$\Delta\lvert\mu_{ep}\rvert$(%)	$\Delta\lvert\mu_{ep}\rvert$(%)
0.001 → 0.01	−9	−9
0.001 → 0.1	−43	−36

4. Conclusion

The ultrafiltration of a linear and a biotinylated sulfated pentasaccharides in the presence of sodium chloride was studied. The retention values have shown an important modification of retention rates with NaCl concentrations ranging from 0 to 0.50 mol L^{-1}. At low salt concentration, both polymers were totally retained on membranes with molecular weight cut-offs up to 15 kDa, corresponding to nearly eight times the molecular weight of the pentasaccharides. When the amount of sodium chloride was increased to 0.50 mol L^{-1}, the retention rates decreased for all membranes below 50%. Regarding the measures of hydrodynamic radius, the salt addition did not modify the size of the molecules. The charge effects were found to be responsible for the observed variations in membrane selectivity. The presence of NaCl at high

concentration caused a charge screening effect and consequently a decrease of the apparent surface charge of the filtrating material. This phenomenon allowed the sulfated polysaccharides to pass through the membrane structure. The only difference between the two compounds in their filtration behaviour relied on membrane fouling. The linear pentasaccharide was more prone to cause surface fouling whereas the branched molecule has responsible for a higher irreversible fouling. Finally, during the ultrafiltration of charged molecules, the electrostatic interactions completely control the membrane performances, contrary to size parameters that usually define the membrane processes efficiency.

References

Alkan, M., Demirbas, O., & Dogan, M. (2005). Zeta potential of unexpanded and expanded perlite samples in various electrolyte media. *Microporous and Mesoporous Materials*, *84*, 192–200.

Armstrong, J. K., Wenby, R. B., Meiselman, H. J., & Fisher, T. C. (2004). The hydrodynamic radii of macromolecules and their effect on red blood cell aggregation. *Biophysical Journal*, *87*, 4259–4270.

Barbucci, R., Magnani, A., Lamponi, S., & Albanese, A. (1996). Chemistry and biology of glycosaminoglycans in blood coagulation. *Polymers for Advanced Technologies*, *7*, 675–685.

Ben Mansour, M., Majdoub, H., Bataille, I., Roudesli, M. S., Hassine, M., Ajzenberg, N., Chaubet, F., & Maaroufi, R. M. (2009). Polysaccharides from the skin of the ray Raja radula, partial characterization and anticoagulant activity. *Thrombosis Research*, *123*, 671–678.

Broeckmann, A., Wintgens, T., & Schäfer, A. I. (2005). Removal and fouling mechanisms in nanofiltration of polysaccharide solutions. *Desalination*, *178*, 149–159.

Cardozo, F. T. G. S., Camelini, C. M., Mascarello, A., Rossi, M. J., Nunes, R. J., Barardi, C. R. M., Mendonça, M. M., & Simoes, C. M. O. (2011). Antiherpetic activity of a sulfated polysaccharide from Agaricus brasiliensis mycelia. *Antiviral Research*, *92*, 108–114.

Chavaroche, A. A. E., Van den Broek, L. A. M., & Eggink, G. (2013). Production methods for heparosan, a precursor of heparin and heparan sulfate. *Carbohydrate Polymers*, *93*, 38–47.

Chen, T., Li, B., Li, Y., Zhao, C., Shen, J., & Zhang, H. (2011). Catalytic synthesis and antitumor activities of sulfated polysaccharide from Gynostemma pentaphyllum Makino. *Carbohydrate Polymers*, *83*, 554–560.

Copin-Montégut, G. (2002). Propriétés physiques de l'eau de mer. *Techniques de l'ingénieur*, K170.

Cui, Q., Li, G., & Yuan, C. (2012). Optimization of glycosaminoglycan extraction on *Patinopecten yessoensis* waste. *Procedia Environmental Sciences*, *16*, 131–137.

Duclos, J. P. (1984). *L'héparine, fabrication, structure, propriétés analyses*. Paris, Ed. Masson, p 399.

El Rayess, Y., Albasi, C., Bacchin, P., Taillandier, P., Mietton-Peuchot, M., & Devatine, A. (2012). Analysis of membrane fouling during cross-flow microfiltration of wine. *Innovative Food Science and Emerging Technologies*, *16*, 398–408.

French Standard NF X 45-103. (1997). *AFNOR Association Francaise de Normalisation*. French Standard NF X 45-103.

Garcia-Ochoa, F., Santos, V. E., Casas, J. A., & Gomez, E. (2000). Xanthan gum: Production, recovery and properties. *Biotechnology Advances*, *18*, 549–579.

Hatziantoniou, D., & Howell, J. A. (2002). Influence of the properties and characteristics of sugar-beet pulp extract on its fouling and rejection behaviour during membrane filtration. *Desalination*, *148*, 67–72.

Hodur, C., Kertesz, Sz, Beszedes, S., Laszlo, Zs, & Szabo, G. (2009). Concentration of marc extracts by membrane techniques. *Desalination*, *241*, 265–271.

Jiao, G., Yu, G., Zhang, J., & Ewart, H. S. (2011). Chemical structures and bioactivities of sulfated polysaccharides from marine algae. *Marine Drugs*, *9*, 196–223.

Lo, Y.-M., Yang, S.-T., & Min, D. B. (1996). Kinetic and feasibility studies of ultrafiltration of viscous xanthan gum fermentation broth. *Journal of Membrane Science*, *117*, 237–249.

Mellal, M., Jaffrin, M. Y., Ding, L. H., Delattre, C., Michaud, P., & Courtois, J. (2008). Separation of oligoglucuronans of low degrees of polymerization by using a high shear rotating disk filtration module. *Separation and Purification Technology*, *60*, 22–29.

Moresi, M., & Sebastiani, I. (2008). Pectin recovery from model solutions using a laboratory-scale ceramic tubular UF membrane module. *Journal of Membrane Science*, *322*, 349–359.

Moritz, T., Benfer, S., Arki, P., & Tomandl, G. (2001). Influence of the surface charge on the permeate flux in the dead-end filtration with ceramic membranes. *Separation and Purification Technology*, *25*, 501–508.

Morris, G. A., Adams, G. G., & Harding, S. E. (2014). On hydrodynamic methods for the analysis of the sizes and shapes of polysaccharides in dilute solution: A short review. *Food Hydrocolloids*.

Nyström, M., & Zhu, H. (1997). Characterization of cleaning results using combined flux and streaming potential methods. *Journal of Membrane Science*, *131*, 195–205.

Pinelo, M., Moller, V., Prado-Rubio, O. A., Jonsson, G., & Meyer, A. S. (2013). Mechanisms controlling retention during ultrafiltration of charged saccharides: Molecular conformation and electrostatic forces. *Separation and Purification Technology*, *118*, 704–709.

Rai, P., Rai, C., Majumdar, G. C., DasGupta, S., & De, S. (2006). Resistance in series model for ultrafiltration of mosambi (*Citrus sinensis* (L.) Osbeck) juice in a stirred continuous mode. *Journal of Membrane Science*, *283*, 116–122.

Ricq, L., Pierre, A., Reggiani, J. C., Pagetti, J., & Foissy, A. (1998). Use of electrophoretic mobility and streaming potential measurements to characterize electrokinetic properties of ultrafiltration and microfiltration membranes. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *138*, 301–308.

Verhnet, A., & Grangeon, A. (2003). Innovations dans la microfiltration des vins: Membranes et procédés. *Récents Progrès en Génie des Procédés*, *89*, 91–99.

Velikovska, P., & Mikulasek, P. (2007). The influence of Cl^-, SO_4^{2-} and PO_4^{3-} ions on the ζ-potential and microfiltration of titanium dioxide dispersions. *Separation and Purification Technology*, *58*, 295–298.

Wijesekara, I., Pangestuti, R., & Kim, S.-K. (2011). Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. *Carbohydrate Polymers*, *84*, 14–21.

Wu, X. Z., & Chen, D. (2006). Effects of sulfated polysaccharides on tumour biology. *West Indian Medical Journal*, *55*, 270–273.

Yang, B., Chang, Y., Weyers, A. M., Sterner, E., & Linhardt, R. J. (2012). Disaccharide analysis of glycosaminoglycan mixtures by ultra-high-performance liquid chromatography–mass spectrometry. *Journal of Chromatography A*, *1225*, 91–98.

Zhang, Q., Jing, W., Fan, Y., & Xu, N. (2008). An improved Parks equation for prediction of surface charge properties of composite ceramic membranes. *Journal of Membrane Science*, *318*, 100–106.

Zhu, H., & Nyström, M. (1998). Cleaning results characterized by flux, streaming potential and FTIR measurements. *Colloids and Surfaces A*, *138*, 309–321.