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Role of electrophoretic mobility of protein on its retention by an ultrafiltration membrane
Comparison to chromatography mechanisms

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Abstract

Lysozyme and lactoferrin, two globular proteins, were first studied separately in order to elaborate a strategy for the improvement of their separation by ultrafiltration (UF) with zirconia-based membranes of different charge sign and pore radius. The electrophoretic mobility (μ) at fixed pH and variable ionic strength was used for the characterisation of both proteins and zirconia particles, similar to the active layer of the membrane during the UF run. Specific adsorption of phosphate ions was shown for both proteins resulting in new isoelectric points. The occurrence of electrostatic exclusion mechanism in addition to the molecular sieving in UF of charged solutes was shown for:

- Low molecular weight solute: multivalent citrate at pH 6 was specifically adsorbed on zirconia and its transmission through the membrane (defined as the ratio of the concentration in the permeate to that of the feed solution) was reduced in the range 0.001–0.01 mol l^{-1} of citrate concentration
- Proteins: their transmissions increase when the ionic strength increases (ion-exchange is not the relevant mechanism because transmission is irrespective of the initial charge of the membrane compared with the protein charge).

A model based on convection, diffusion and electrophoretic migration mechanisms (CDE model) was proposed to take into account this behaviour. The CDE model predicts the possible existence of a depleted sub-layer of the charged protein in the concentration polarisation layer, located in the close vicinity of the membrane surface. A strategy for the separation of two proteins in mixed solution was proposed by varying both the physico–chemical environment in the feed solution (pH, ionic strength, chemical nature of the electrolyte) and the membrane pore radius. Maximum selectivity was obtained when the target protein (to be transmitted in the permeate side) is close to being uncharged due to specific adsorption of electrolyte ions. Ultrafiltration selectivity is enhanced with membrane of large pore radius, which provides high transmission of the target protein and efficient electrostatic exclusion of the solute to be retained in the retentate side. This UF approach corresponds roughly to the separation of one uncharged and one charged protein from a mixed solution by size exclusion chromatography of the uncharged protein combined with electrostatic exclusion of the charged protein due to packing of similar charge.

Keywords: Electrophoretic mobility; Ultrafiltration membrane; Proteins

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1. Introduction

Ultrafiltration and liquid chromatography are both processes used in order to concentrate, to extract and to purify proteins. Although these techniques correspond to quite different targets, a comparison can be made about the separation mechanisms:

(i) Molecular sieving or size exclusion is the sole mechanism of ultrafiltration (UF) and size exclusion chromatography (SEC) of neutral solutes. Partition or retention is mainly based on the dimensionless ratio λ of solute radius (R) to pore radius (r_p.)

(ii) Electrostatic interactions are more or less involved in exclusion or retention of charged solutes. Ion exchange chromatography (IEC) is commonly used for the separation of solutes (proteins) of opposite charge to that of the exchanger-group. Regnier and co-workers [1,2] have proposed a stoichiometric displacement model allowing the number of contacts (z) between the solute and the chromatographic support to be determined. Displacement of the adsorbed protein occurs more efficiently by the use of eluent that containing multivalent co-ion of the protein to be desorbed [3]. The electrophoretic mobility (roughly the ratio of the charge to the radius) has been proposed as a useful tool for understanding the elution order of protein in ion-exchange chromatography [4].

For solutes similarly-charged as the stationary phase (either chromatographic column or membrane) repulsive electrostatic interactions occur. Non ideal size exclusion chromatography of protein is shown by an abnormal low retention time of negative protein on negatively charged silica-based supports at ionic strength lower than 0.010–0.1 mol l^{-1} [5]. Many papers deal with the abnormal calibration curve obtained with polyelectrolyte similarly charged as the support: either negatively charged as silica and polystyrene sulfonate [6,7] or conversely positively charged as amine grafted silica and chitosan [8]. Consequently, the hydrodynamic volume of the charged solute is overestimated until pore volume accessible to solute does not increase as well with the increase of the ionic strength of the eluent. Briefly in those cases the solute is a co-ion of the charged support.

In UF the retention of a charged solute (namely tetracycline, antibiotics) is unexpectedly high at low ionic strength compared to the retention of a neutral solute (sucrose) of close molecular weight. When varying the ionic strength, the retention of the charged solute decreases linearly with the reciprocal of the square root of ionic strength ($I^{-1/2}$) [9,10] whereas the retention of the neutral solute remains constant over the ionic strength range [9]. For protein, a high ionic strength (I=0.5–1 mol l^{-1}) is needed for the cancellation of the electrostatic interactions either for proteins of opposite sign to that of the support or for proteins similarly charged as the support [11,12].

Retention mechanisms involve size exclusion, electrostatic repulsion in any case and hydrophobic interactions with supports bearing both charged and hydrophobic moieties. A semi-empirical model called ‘Ionic Strength Control of Retention model’ (ISCR) has been proposed in UF that relates the retention of a charged solute and the ionic strength through $I^{-1/2}$ [9,12].

An in-depth characterisation of a model protein, namely lysozyme a protein from the white egg was performed. It shows that the electrophoretic mobility of the protein (roughly the ratio of its charge to its radius) is dependent on pH and ionic strength. Moreover, it also depends on the chemical nature of the electrolyte solution when specific adsorption occurs on protein [13].

However, it is shown that the zirconia-based membrane, whatever its original charge, becomes similarly-charged as the protein due to the occurrence of fouling provoked by the convection and adsorption mechanisms. Direct membrane characterisation can be achieved by experimental measurements of the streaming potential (dynamic measurement of the electrical potential allowing the determination of the zeta potential). Both the organic membranes [14] and the inorganic membranes [15] acquire the potential of the free protein by adsorption of the protein during UF. Consequently the ‘active’ membrane surface becomes similar to that of adsorbed protein. Briefly, the fouled membrane becomes self-rejecting towards the free protein in the feed solution.

Separation of proteins in mixed solution is performed in several ways using very different membranes. Among these studies, it is pointed out that fractionation is better when the pH is set at the

isoelectric point of the target protein to be transmitted in the permeate side regardless to the protein sizes [16,17].

In this paper, the occurrence of the electrostatic exclusion was shown in UF either for small organic ion (citrate) which can not be size excluded, or for proteins (lysozyme, lactoferrin) by variation of the ionic strength of the feed solution.

For UF of a solution of a single protein, namely lysozyme, a description of the solute concentration profile in the close vicinity of the membrane wall based on the CDE model was proposed. The electrostatic exclusion mechanism was taken into account by the use of the experimental electrophoretic mobility of the solute and the zeta potential of the fouled membrane material.

From the deduced trends, UF separation of a mixture of two proteins, namely lysozyme and lactoferrin, was achieved by variation of the physico–chemical environment in the feed solution: ionic strength, pH and chemical nature of the electrolyte.

2. Theory

2.1. Partition coefficient

Partition of a solute at a cylindrical pore entrance depends on the ratio (λ) of the R solute radius to the r_p pore radius:

$$\lambda = R/r_p \tag{1}$$

The partition coefficient (K) depends on the cross section of pore accessible to the solute [7,18] according to:

$$K = (1-\lambda)^2 \tag{2}$$

2.2. Size exclusion chromatography

In size exclusion chromatography (SEC) the solute is eluted according to its hydrodynamic volume [19]. The K_D capacity factor or partition coefficient (dimensionless) is defined according to:

$$K_D = (V_e - V_o)/(V_t - V_o) = (1-\lambda)^2 \tag{3}$$

with: V_t the total volume or mobile phase volume of the column; V_o the void volume of the column; and V_e the elution volume of the solute.

Eq. (3) gives the fraction of the porous volume accessible to the solute. K_D ranges from 0 (large solute, not retained) to 1 (small solute, total retention).

2.3. Electrophoretic mobility and zeta potential of surface

Electrophoretic mobility (μ) occurs for charged species when placed in an electric field (E). According to the Debye–Huckel–Henry's equation, which accounts for the effect of the ionic strength (I) for a protein, μ is expressed as [20]:

$$\mu = [Ze/6\pi\eta R]\cdot[X(\kappa R)/(1+\kappa R)] = \mu_0\,[X(\kappa R)/(1+\kappa R)] \tag{4}$$

with: Ze the net charge of the solute (C); η the electrolyte viscosity (Pa s); R the solute radius (m); and κ the reciprocal Debye length (m^{-1}).

For water at 25°C and with the ionic strength (I) expressed in mol l^{-1}:

$$\kappa = 3.28\times10^9\times I^{1/2} \tag{5}$$

The right hand side of Eq. (4) shows that μ_0, given by the Nernst–Einstein equation, is the mobility in an insulating medium ($I=0$). As the correcting term of Henry $[X(\kappa R)/(1+\kappa R)]$ varies from 1 to 0 when the ionic strength (I) increases from 0 to infinity, then μ_0 is a maximum value. Eq. (4) takes into account the ionic strength (via κ) besides the well-known ratio of the net charge to the protein radius (R) without attention paid to the electrolyte chemical nature. In fact two types of electrolytes have to be considered, according to the type of interactions occurring with the protein:

(i) For indifferent electrolyte media, interactions are only attractive electrostatic ones. Then, the experimental electrophoretic mobility of the protein agrees well with the mobility expected on the basis

of amino-acid content and Eq. (4). NaCl is relevant of this type of electrolyte towards lysozyme.

(ii) For specifically adsorbed electrolyte, non-electrostatic interactions (Van der Waals) occurred also. Then, experimental electrophoretic mobilities are lower than predicted from Eq. (4) and sometimes the protein sign is reversed. Phosphate is relevant of this type of electrolyte towards both lysozyme and lactoferrin. The most important consequence of the specific ion adsorption is the change of the isoelectric point of a protein: p*I* of lysozyme (10.7) shifts to 9 in phosphate for a particular $I = 0.010$ mol l^{-1} [13].

From the electrophoretic mobility measurement of particles, one can characterise the electric potential of a surface, called its zeta potential. Difference between the zeta potential of a clean surface and the zeta potential of the same surface fouled with protein, highlights the adsorption of the protein on the surface. For a particle of large size regard to Debye length, the electrophoretic mobility (μ) is proportional to its zeta potential (ψ) [20]. In water at 25°C:

$$\psi\,(\mathrm{mV}) = 12.85\mu(10^{-8}\ \mathrm{m^2\ V^{-1}\ s^{-1}}) \quad (6)$$

2.4. Ultrafiltration

UF performances are generally expressed in terms of permeate flux J and of solute retention (Ret) or Transmission (Tr) by the membrane:

$$\mathrm{Tr} = 1 - \mathrm{Ret} = C_p/C_r \quad (7)$$

with: C_p the concentration of the solute in the permeate (passing through the membrane); and C_r the concentration of the solute in the retentate (remaining over the membrane). Observed transmission of the solute by the membrane is obtained when using concentration of solute in the bulk solution (C_b) instead of concentration at the membrane wall (real retention).

2.5. Molecular sieving

Molecular sieving is the basic principle in ultrafiltration (UF) of neutral solutes. In 1936, Ferry has proposed a relationship between the retention and the λ ratio of the solute radius to the radius of a cylindrical pore in the membrane [21]

$$\mathrm{Ret} = 1 - \mathrm{Tr} = [\lambda(2-\lambda)]^2 \quad (8)$$

2.6. Model of convection-diffusion (CD model)

Applied pressure is the driving force for convection of solutes towards the membrane wall: concentration polarisation occurs during the UF process due to the accumulation of solute before the membrane wall (thickness: d) The most commonly used transport mechanism is based on convection and diffusion [22].

The concentration of a neutral solute in the polarisation layer can be described by:

$$JC_i - D\frac{dC_i}{dx} = JC_p \quad (9)$$

with: C_i the concentration of solute within the polarisation layer (boundaries: C_b, away from the membrane and C_w at the membrane wall); D the diffusion coefficient of the solute ($m^2\ s^{-1}$); and x the distance from the bulk to the membrane.

2.7. Model of convection-diffusion-electrophoretic migration (CDE model)

For charged solutes in nanofiltration (NF), the extended Nernst–Planck equation is applied [23,24]: a 'diffusion' mechanism due to the electric field inside the charged membrane pore is added to convection and diffusion mechanisms.

Recently, the CDE model taking into account convection, diffusion and electrophoretic migration has been proposed for UF of charged proteins [25,26]. The main difference arises from the localisation of electrostatic interactions. In the CDE model they are considered mainly before the membrane pore entrance. As the membrane material is carrying the same charge as the solute, due to fouling occurring during UF, a repulsive electric force ought to be taken into account:

$$JC_i - D\frac{dC_i}{dX} - \mu_i C_i \kappa \psi_z \exp(-\kappa(d-x)) = JC_p \quad (10)$$

with: ψ the electric potential (zeta) of the charged

surface, induced by the charged solute (zeta potential of the membrane); μ_i the electrophoretic mobility of the charged solute; X the distance from the membrane wall ($X=d-x$); d the thickness of the polarisation layer (m); $C_{w,CDE}$ is the concentration at the membrane wall; and $C_{max,CDE}$ is the maximum concentration in the polarisation layer, located at the X_{max} distance from the membrane wall (see Discussion).

Eq. (9) of the CD model is in fact a reduced form of Eq. (10). The main feature of the CDE model is to predict a depleted sub-layer of highly charged solute in the close vicinity of a highly charged membrane surface, due to an electrostatic exclusion mechanism.

3. Experimental

3.1. Samples and reagents

All reagents were of an analytical grade. Citric acid, potassium dihydrogenophosphate, NaOH and HCl were from Merck (Darmstadt, Germany).

Acetonitrile (Carlo Erba) and trifluoroacetic acid (TFA, Pierce Chemicals) of spectroscopic grade were used for HPLC experiments.

Lysozyme, a protein from the white egg, in hydrochloride form was from Ovonor (Tregueux, France), and lactoferrin, a metallo-protein (binding iron) from cows milk, was kindly provided by Armor Proteines (Saint-Brice en Coglès, France). Table 1 sums up their characteristics.

3.2. Liquid chromatography (RP-HPLC)

The HPLC system included a pump unit (Beckman 126), an automatic sample injector (Gilson 231-401) equipped with a valve (Rheodyne 7125) and a diode-array UV detector (Beckman 168) at 280 and 220 nm. The data were processed with Gold 8 software (Beckman). The stationary phase was made of polystyrene divinylbenzene, 300 A, 8 μm, 150× 4.6 mm, PLRP-S from Polymer Laboratories. The flow-rate was 1 ml min^{-1} and 50 μl samples were injected. The eluents were 0.1% (v/v) trifluoro acetic acid (TFA) in water (eluent A) and 0.1% (v/v) TFA in water–acetonitrile (20:80, v/v) (eluent B). Elution pH was roughly 2. A gradient elution mode was used to determine the protein concentration in retentate and permeate (Table 2). The accuracy on concentration was 5% and consequently 10% on UF transmission.

Table 2
Elution gradient for the RP-HPLC[a]

Ve (ml)	% B
0	44
1	44
4	100
5	100
5.5	44
12	44

[a] Eluent A: 0.1% (v/v) trifluoro acetic acid (TFA) in water. Eluent B: 0.1% (v/v) TFA in water–acetonitrile (20:80, v/v). flow-rate 1 ml min^{-1}.

Reversed phase HPLC in isocratic elution mode (32% acetonitrile + 0.1% TFA) was performed for the characterisation of the hydrophobicity of lysozyme.

3.3. Electrophoretic mobility

The electrophoretic mobilities were measured with a Delsa 440 (Coultronics). The electrophoretic mobility of free proteins was measured at various ionic strengths and fixed pH: NaCl at pH 7 and potassium phosphate at pH 9 [13].

Unmodified and modified zirconia particles (same

Table 1
Molecular mass, Stokes radii and isoelectric points of lysozyme and lactoferrin from literature data

Protein	Molecular mass (g mol^{-1})	Stokes radius (R_s: nm)	Isoelectric point (pI)
Lysozyme (LYS)	14 400	1.8 [31]	11 [34]
Lactoferrin (LF)	80 000	2.2[a] [30]	8–9 [32,33]
	320 000[a] [30]	4.4[a] [30]	

[a] In NaCl 200 mmol l^{-1} and 1000 mmol l^{-1}, pH 7: tetramer form [30].

history as the membrane) were let to withstand in solutions of single or mixed proteins in different electrolytes, in order to simulate the adsorption on the membrane surface during ultrafiltration. Such particles were assumed to have the same zeta potential as the fouled-membranes.

3.4. Ultrafiltration

3.4.1. UF runs

In UF the velocity of the feed solution is tangential to the membrane wall. During all runs, the retentate and the permeate were sampled and remixed in the feed tank in order to keep a constant concentration of the feed solution (volume reduction ratio VRR = 1). Other conditions are given on figures.

The ionic strength variation during UF runs was obtained by step by step by addition of a concentrated NaCl (pH 7) or potassium phosphate (pH 9) stock solution in the feed tank.

3.5. Zirconia based membranes and powders

Multichannel inorganic membranes (Kerasep 300 kDa, Orelis, France) – 7 channels, 2.0 cm outer diameter and 0.45 cm channel diameter, 40 cm length, 0.0396 m^2 filtering area) with 300 kg mol^{-1} molecular mass cut-off and pore radius of about 14 nm were used (manufacturer data). Membrane matrix is based on alumina.

Monotubular inorganic membranes (Carbosep M1, Orelis, France) – 1.0 cm outer diameter and 0.6 cm channel diameter, 60 cm length, 0.0113 m^2 filtering area) with 150 kg mol^{-1} molecular mass cut-off and a pore radius of about 10 nm were used (manufacturer data). Membrane matrix is based on carbon.

For both membranes, the separation layer was mainly composed of zirconium oxide (zirconia, ZrO_2) sintered on the internal wall of the channels.

Zirconia powder (P316, Orelis, surface area was about 33 $m^2\ g^{-1}$) with the same history process as zirconia on membrane was kindly given by Orelis.

Chemical irreversible modifications were performed on zirconia membrane and zirconia particles by:

- polyethyleneimine adsorption and cross-linked with diglycidylether of bisphenol A, (further called PEI membrane) [12]
- grafting with organo titanate coupling agent bearing either ethylenediamine groups (cationic group, further called EDA membrane) or pyrophosphate groups (anionic group, further called PP membrane).

4. Results and discussion

4.1. UF of citrate by zirconia membrane

Citrate (MW = 192 g mol^{-1}, hydrated radius R = 0.24 nm [27], pK_a's: 3.1, 4.8, 6.4, respectively) was ultrafiltered with a zirconia membrane (Kerasep 300 kDa, pore radius r_p = 14 nm). From the ratio of the solute radius to the membrane pore radius close to 0.02 no retention due to molecular sieving is expected. According to pK_a's, a pH close to 6 was selected in order to get a highly charged solute (citrate charge number is about -2.3) of low molecular weight.

Fig. 1a shows the transmission of citrate at steady state versus its concentration in the feed. Observed transmissions varied from 0.4 to 1.0 when the citrate concentration increased from 0.001 to 0.100 mol l^{-1}, respectively.

Fig. 1b shows the electrophoretic mobility of zirconia particles versus the pH. The zirconia was positively charged below pH 7, which is the isoelectric point (iep) of zirconia in 10^{-2} mol l^{-1} KCl acting as an indifferent electrolyte. In 10^{-3} mol l^{-1} citrate solution, zirconia particles became negatively charged as pH was roughly greater than 3. The difference of isoelectric point of zirconia particles highlights the specific adsorption of citrate on zirconia, as citrate becomes increasingly negatively charged over pH 2 according to its pK_a's. Fourier Transform Infrared study (FTIR) has shown that an acid–base Lewis bond between zirconium of zirconia and nitrogen of amine group (in PEI) is involved [28]. It can be assumed a similar bond between zirconium and oxyanions

Accordingly, both the 'active' membrane, i.e. the 'citrate fouled membrane', and the solute are negatively charged at pH 6: electrostatic exclusion occurs

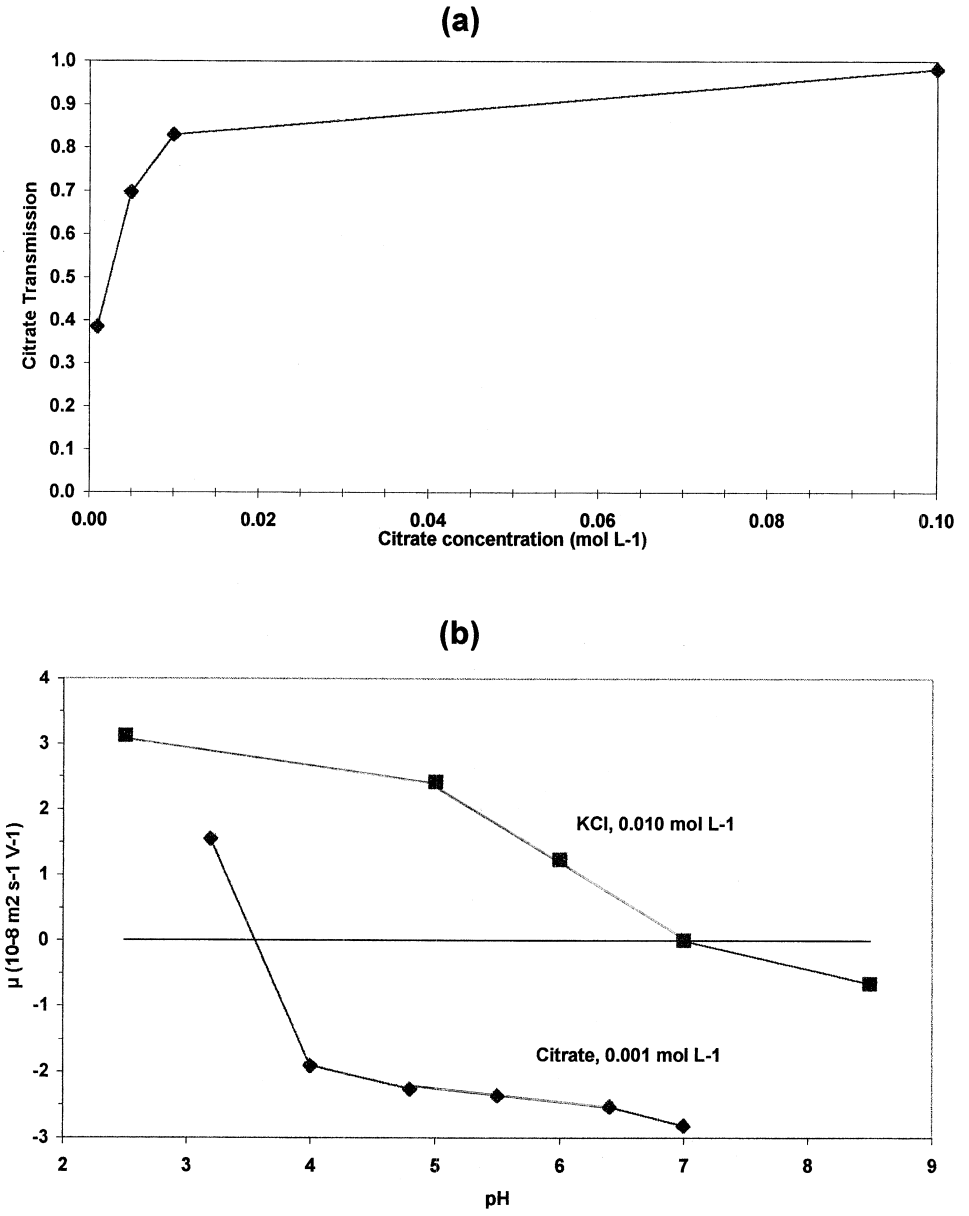

Fig. 1. (a) Transmission of citrate with a zirconia membrane (Kerasep 300 kDa) versus the citrate concentration at pH 6. (UF conditions: 1.2 m s^{-1}, 0.4 bar, 30°C, VRR = 1). (b) Electrophoretic mobility (μ) of zirconia particles versus pH, in 0.010 mol l^{-1} KCl (■■) and in 0.001 mol l^{-1} citrate (◆◆).

from the citrate-fouled-membrane and the free multivalent citrate. Only electrostatic exclusion mechanism is involved in the unexpected low transmissions of citrate at concentration up to 0.01 mol l^{-1}. For citrate concentration close to 0.1 mol l^{-1} (ionic strength about 0.4) the electrostatic repulsive interactions are screened and the transmission (1.0) is as expected from molecular sieving.

4.2. UF of protein by modified zirconia membranes

4.2.1. Role of the electrostatic interactions on the protein transmission

Lysozyme (pI=10.7) was ultrafiltered at pH 7 with modified membranes bearing either cationic groups (EDA) or anionic groups (PP).

From its electrophoretic mobility (μ, Fig. 2) in KCl at pH 7, lysozyme is positively charged within the ionic strength range of I=0 to 1 mol l^{-1}, in good agreement with its pI. μ decreases with the ionic strength as expected from the double electric layer compression in good accordance with literature [13,29].

Fig. 3a and b shows the transmission of protein in single solution, lysozyme and lactoferrin respectively, versus the increase of ionic strength by KCl addition (indifferent electrolyte). Whatever the initial charge of the membrane (cationic EDA or anionic PP groups), transmission of lysozyme increased with the increasing ionic strength, whereas its electrophoretic mobility decreased (Fig. 2). As this phenomenon occurred regardless the initial charge of the membrane (EDA or PP), it is evidenced that ion-exchange mechanism is not relevant for the protein transmission at steady state. However, it could be involved in the first adsorption step allowing membrane fouling (comparisons on UF transmissions can be made because of quite equivalent hydrodynamic conditions in each figure).

This behaviour is a general trend, not dependent on the initial chemical nature and initial sign of the membrane. Adsorption of protein on the membrane is clearly evidenced here, and confirmed by electrophoretic measurement of zirconia particles fouled by protein (not shown here). A mechanism based on electrostatic exclusion is clearly involved at low ionic strength, where transmissions of both proteins were low.

4.2.2. In-depth characterisation of the hydrophobicity of protein during UF

Fig. 4 shows chromatograms (RP-HPLC) running in isocratic elution mode of both retentate and permeate of lysozyme (dissolved in water) during UF by a PP membrane (Kerasep 300 kDa-PP membrane). Both in the feed solution and in the final retentate, the native protein was eluted as a main

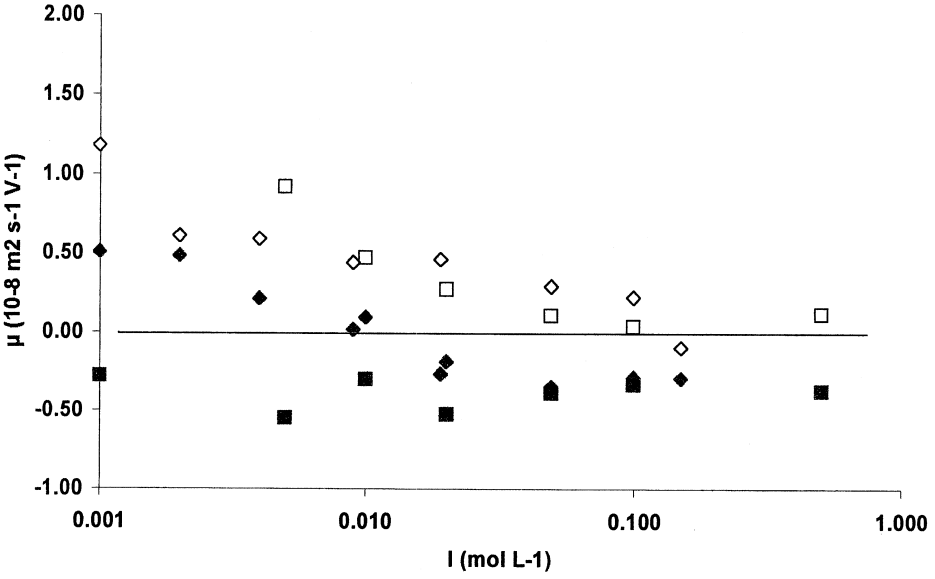

Fig. 2. Electrophoretic mobility of lysozyme (◆, ◇◇) and lactoferrin (■■, □□) versus the ionic strength of KCl (or NaCl) at pH 7 (open symbols) and phosphate at pH 9 (closed symbols); accuracy: 0.2×10^{-8} for mobility close to zero.

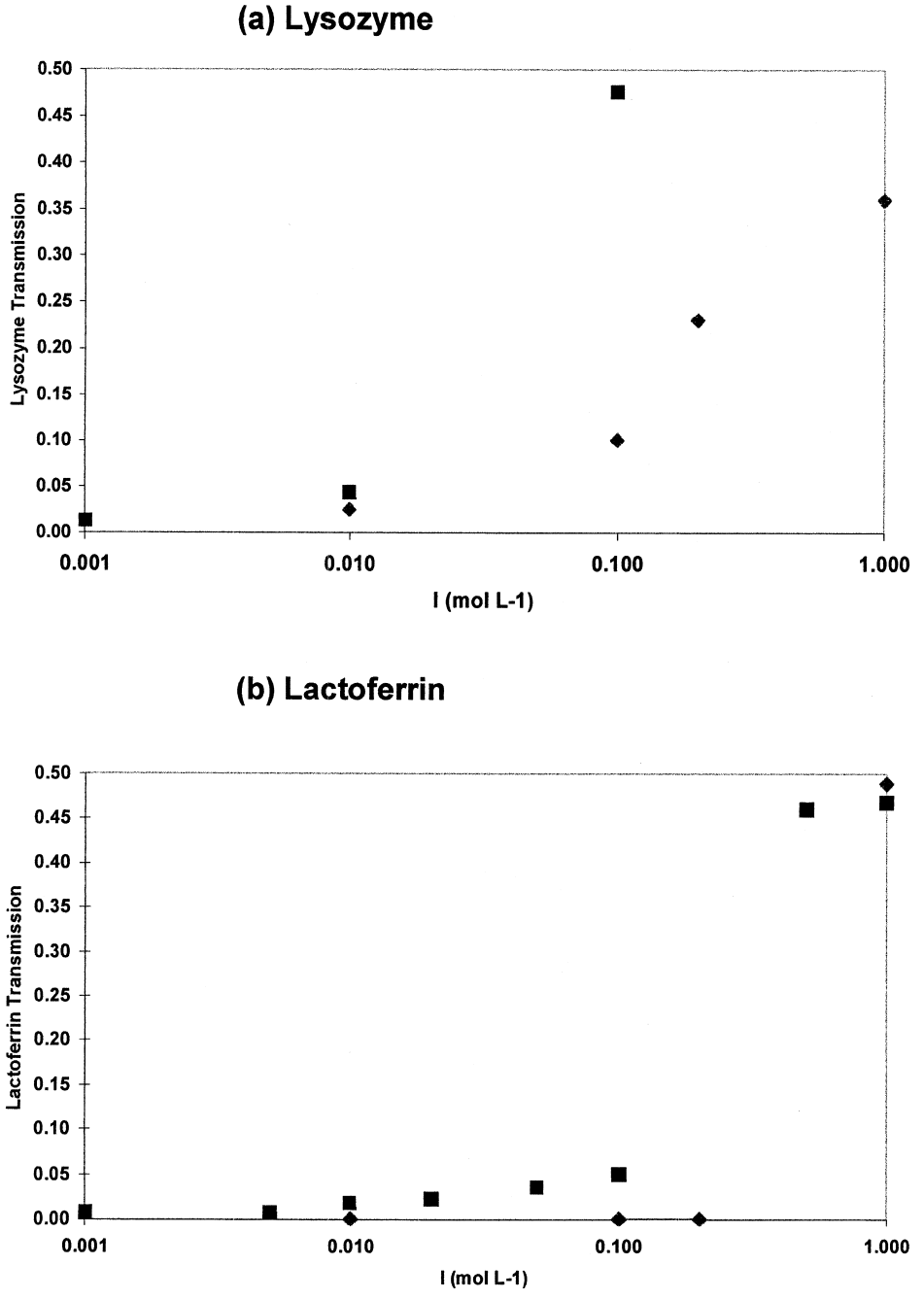

Fig. 3. Transmission of lysozyme (a) and lactoferrin (b) in single solution with the cationic EDA membrane (M1-EDA, ◆◆) and the anionic PP membrane (Kerasep 300 kDa-PP, ■■) versus the ionic strength of KCl at pH 7. UF conditions: 1.5 bar, 12°C, VRR = 1, tangential velocity: 1 m s^{-1} (lactoferrin) and 4 m s^{-1} (lysozyme).

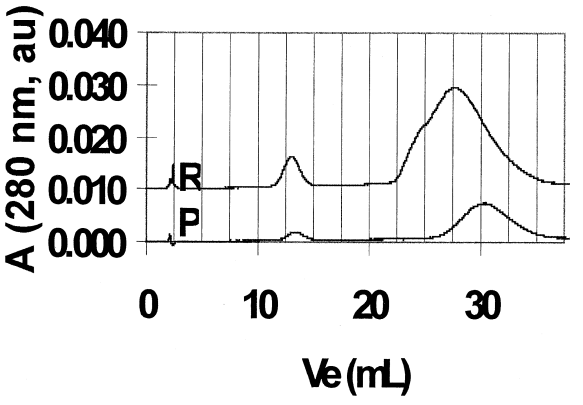

Fig. 4. RP–HPLC chromatograms of retentate (R) and permeate (P) during the UF of lysozyme in water at pH 7 with Kerasep 300 kDa-PP membrane. isocratic elution mode: 0.1% (v/v) TFA in water–acetonitrile (68:32 v/v), flow-rate 1 ml min^{-1}.

peak at V_e close to 27.5 ml (the minor peak at V_e close to 13 ml was not identified, and only the main contribution is discussed in the following). In the retentate, the lysozyme peak was a double contribution with more or less hydrophobic species evidenced by an asymmetric peak with a shoulder. Only the more hydrophobic part was detected in the permeate. It must be noticed that capillary electrophoresis (CE) shows only one symmetric gaussian peak, whereas CE is well suitable to resolve the two genetic variants of β-lactoglobulin, which differ only by one charge [29]. Consequently, the two species of lysozyme observed in RP-HPLC can not be differently charged but differ only by their hydrophobic characters.

Although the transmission of lysozyme was calculated from the whole asymmetric peak, this new and unexpected result shows that UF membrane can be able to separate components of an apparently 'pure' protein by a mechanism (not stated) different of electrostatic exclusion and molecular sieving, and probably involving hydrophobic interactions.

4.3. Incidence of electrostatic exclusion mechanism in the close vicinity of the membrane: CDE model

In order to show the role of electrostatic exclusion on the charged solute transport through a membrane, some experiments were performed with another membrane modified with positively charged polyethyleneimine (PEI). The main advantage of this membrane is to have a quite constant permeate flux regardless the ionic strength of the solution, due to an important 'pre-fouling' of the membrane by the adsorbed polymer.

UF of lysozyme was performed in various ionic strengths obtained by step by step addition of KCl concentrated solution at pH 7. Table 3 shows the increase of the transmission from 0.13 to 0.73 according to the ionic strength increase from 10^{-3} mol l^{-1} to 0.100 mol l^{-1}, respectively [26].

From these results, the concentration profile of lysozyme in the polarisation layer was calculated according to the CDE model by numerical resolution of Eq. (10). Fig. 5 shows the lysozyme concentration within the polarisation layer of thickness $d = 13$ μm according to the CDE model. The concentration (C_x) increases from the bulk (C_{bulk} 1 g l^{-1}) to the membrane until a maximum concentration ($C_{max,\ CDE}$) of several grams per litre (close to that of CD model) at few nm from the membrane wall. Then its concentration dropped, more or less according to the ionic strength, due to the electrostatic exclusion of the free solute by the fouled charged membrane. The concentration at the membrane surface ranged from several grams per litre (uncharged or poorly charged protein) to bulk concentration (1 g/l, no polarisation) and even to zero (highly charged solute) [26].

Table 3
Lysozyme transmission with a modified membrane (Carbosep M1-PEI) versus the ionic strength of KCl at pH 7[a]

I (mol l^{-1})	0.001	0.004	0.0010	0.020	0.050	0.100
Transmission	0.13	0.29	0.61	0.69	0.73	0.73
J (μm s^{-1})	19	19	20	20	21	22

[a] UF conditions: Lysozyme 1 g l^{-1}, tangential velocity: 4 m s^{-1}, transmembrane pressure: 2 bar, temperature: 20°C.

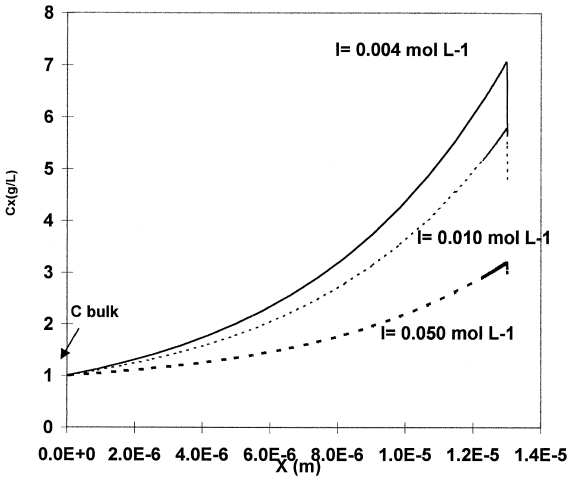

Fig. 5. Lysozyme concentration (C_x) versus the distance X of the membrane wall according to the CDE model (Eq. (8)) for different ionic strengths (KCl) at pH 7. The thickness of the concentration polarisation layer is $d=13$ μm in UF conditions of Table 3.

4.3.1. Application to the UF of protein in single solution

According to the CDE model the transmission of a protein is maximum when the protein is uncharged and lowered when the protein is highly charged.

This point is well evidenced in UF of lysozyme solution in phosphate at various pH and ionic strengths [13]. Phosphate ions are specifically adsorbed by lysozyme and Table 4 shows that observed transmission of lysozyme with the PEI membrane ranged between 1 and 0 according to the physico–chemical environment (constant permeate flux for all experiments).

At pH 9 the transmission versus the ionic strength is featured by an original shape versus the ionic strength, exhibiting a maximum. This maximum was close to the ionic strength range where both the membrane and lysozyme were uncharged. For lower and higher ionic strengths, as both lysozyme and the fouled membrane were charged, transmission decreased due to an additional electrostatic exclusion mechanism due to positives charges at low ionic strength and to negative charges at ionic strength greater than 0.050 mol l^{-1}.

4.3.2. Application to the separation of a model mixture of two proteins

UF of a model mixture of two proteins (50/50 w/w), namely lysozyme (14 400 g mol^{-1}) and lactoferrin (80 000 g mol^{-1}) was performed with variation of the physico–chemical environment in order to enhance the selectivity of the separation. The main objective was to reach a high transmission of lysozyme in the permeate and to retain lactoferrin in the retentate side. According to the CDE model, lysozyme has to be uncharged whereas simultaneously lactoferrin has to be strongly charged for a high electrostatic exclusion mechanism by the charged-fouled-membrane.

Membrane separation ability is given by the selectivity S in the permeate side which is the following ratio:

$S = \mathrm{Tr(lysozyme)/Tr(lactoferrin)}$

Table 4
Comparison of transmission (Tr) with a modified membrane (Carbosep M1-PEI) and sign of electrophoretic mobility (μ) of lysozyme versus the ionic strength of phosphate at pH 4 and 9 (UF conditions as in Table 3)[a]

I (mol l^{-1})	0.001	0.004	0.010	0.020	0.050	0.150
pH=4.0						
Tr	0.00	0.04	0.16	0.38	0.63	0.65
μ	+++	+++	+++	++	++	++
pH=9.0						
Tr	0.42	0.79	0.99	1.0	0.94	0.76
μ	+	+	0	0/−	−	−

[a] Sign symbols according to mobility (SI units) as follows: +++, $\mu<2\times10^{-8}$; ++, $\mu\#1\times10^{-8}$; +, $\mu\#0.5\times10^{-8}$; 0, $\mu<\pm0.5\times10^{-8}$; −, $\mu\#-0.5\times10^{-8}$.

Fig. 6 shows the selectivity of the 300 kDa-PP membrane in NaCl at pH 7 for various ionic strengths in the range 0.001–0.150 mol l^{-1}. Selectivity (S) was roughly constant at about 20, in the whole ionic strength range. The instantaneous purity of lysozyme was 95% in permeate instead of 50% in the feed.

According to their electrophoretic mobilities, the proteins are positively charged at pH 7 in NaCl (Fig. 2). When the ionic strength increases due to NaCl addition, the electrophoretic mobilities of both proteins decrease and the transmission of both proteins increased due to cancellation of the electrostatic exclusion. Nevertheless, no improvement of selectivity was obtained when varying the ionic strength with NaCl as the electrostatic exclusion mechanism occurred for both proteins and was not selective enough to enhance the membrane selectivity.

In phosphate at pH 9, lactoferrin was negatively charged in the whole range of ionic strength whereas lysozyme sign was positive, neutral and negative, respectively (Fig. 2). Accordingly an enhanced selectivity is expected when lysozyme is neutral in the ionic strength range 0.010–0.020 mol l^{-1}. Fig. 6 and Fig. 7 show comparative selectivities of the UF performed with PP membranes. Selectivity of 300 kDa-PP membrane exhibited a maximum close to 120, in phosphate at $I=0.050$ mol l^{-1}, remaining about 70 at $I=0.150$ mol l^{-1} which means that instantaneous purity of lysozyme was greater than 99% in permeate. Selectivity is enhanced in phosphate at pH 9 at $I=0.050$ mol l^{-1} because both lysozyme was uncharged and lactoferrin remaining negatively charged was able to be electrostatically excluded from the membrane [30].

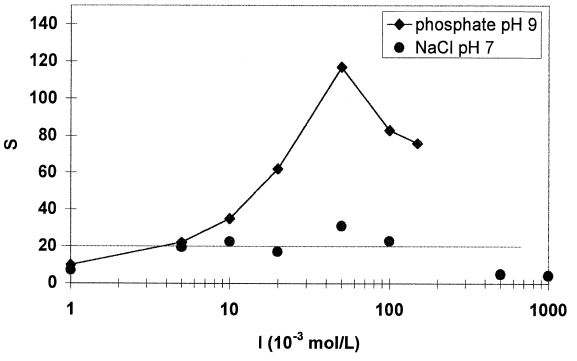

Fig. 6. Influence of the physico-chemical environment on the selectivity (S=Transmission of lysozyme/Transmission of lactoferrin) of Kerasep 300 kDa-PP membrane for a mixture solution of lysozyme and lactoferrin (1:1 g l^{-1}) versus the ionic strength of NaCl at pH 7 (●●) and of phosphate at pH 9 (◆◆). UF conditions: 1 m s^{-1}, 1.5 bar, 12°C, VRR=1.

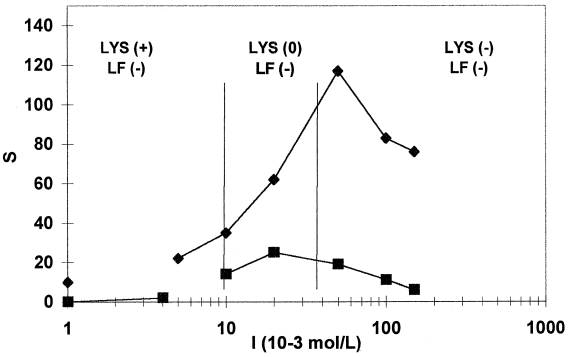

Fig. 7. Comparison of selectivity (S=Transmission of lysozyme/Transmission of lactoferrin) of two PP membranes (◆◆: Kerasep 300 kDa-PP, ■■: M1-PP) for a mixture solution of lysozyme and lactoferrin (1:1 g l^{-1}) versus the ionic strength of phosphate at pH 9. UF conditions: 1 m s^{-1}, 1.5 bar, 12°C, VRR=1.

Fig. 7 shows the selectivity achieved with two PP membranes of different nominal pore radius: 10 nm for M1-PP and 14 nm for Kerasep 300 kDa-PP. As expected, for M1 PP membrane the maximum selectivity was observed close to the ionic strength range for which lysozyme is uncharged. Whereas the mechanism is the expected one, selectivity of the M1-PP membrane was lower than that of the 300 kDa-PP membrane since transmission of lysozyme was not high enough.

The location of the maximum selectivity depends on the operating conditions of the membrane process:

(i) The M1-PP membrane permeability was roughly constant in the ionic strength range 0.010–0.050 mol l^{-1} (not shown) and consequently the maximum was achieved for the expected ionic strength

(ii) The permeability of the 300 kDa-PP membrane increased significantly with the increase of the ionic strength in the ionic strength range (not shown). In that case, the convection term of Eq. (10) is not constant and the maximum of selectivity was shifted

owing to a better transmission of lysozyme due to an increase of the effective pore radius (lower fouling).

As summary, enhanced selectivity was achieved with the simultaneous conditions:

- Uncharged or poorly-charged target solute in the permeate (sieving mechanism)
- Charged enough target solute in the retentate (efficient electrostatic exclusion mechanism)

Direct comparison with SEC should be made: target protein to be recovered in permeate is eluted at its SEC volume and the protein in the retentate is eluted before its expected SEC volume by additional electrostatic exclusion mechanism.

It must be kept in mind that membrane fouling during the UF process acts as a variable pore reducer whereas in liquid chromatography porous volume is constant (hard packing). Consequently maximum of selectivity in UF can be slightly shifted from the expected ionic strength value due to a compromise with decreasing fouling. Selectivity maximum can be enhanced with porous membrane, which became less fouled by increase of ionic strength, because of a better transmission of lysozyme and a remaining low transmission of the protein to be retained. This case corresponds roughly to putative SEC with an increasing porous volume, which increases with ionic strength (soft gels). The target protein to be recovered in permeate would be eluted at its SEC volume. The protein in the retentate would be eluted far from its expected SEC volume. That could be achieved by an increasingly predominant electrostatic exclusion mechanism.

5. Conclusion

Charged solutes are adsorbed on the membrane wall due to the convection flow. The so-called fouled membrane becomes similarly charged as the free solute in the bulk solution as shown by electrophoretic mobility measurements on fouled material particles.

In this study, the occurrence of electrostatic exclusion mechanism in ultrafiltration of charged solutes is shown in addition to sieving mechanism. Low molecular weight charged compounds and proteins are relevant of this mechanism.

(i) Pure electrostatic exclusion is shown for multivalent citrate with zirconia membrane (of solute radius to pore radius as low as 0.02) in the range 0.001–0.010 mol l^{-1}.

(ii) For proteins (lysozyme, lactoferrin) transmissions do not depend on their respective charges towards the initial membrane charge and increase with the ionic strength. The CDE model accounts for electrostatic exclusion mechanism in the close vicinity of the membrane wall. The parameters involved are the electrophoretic mobility of the free protein and the zeta potential of the fouled membrane material. It allows the description of the concentration profile of charged solute in the close vicinity of the membrane wall.

Besides convection and diffusion in the polarisation layer, the management of the electrophoretic migration of solutes allows the improvement of the membrane selectivity (separation ability) via a limited transmission of the protein to be retained.

Electrostatic exclusion mechanism using mobility as operating parameter appears as an efficient tool for enhancement of selectivity of UF membrane separation in a similar way as in size exclusion chromatography when charge effects exclude a charged solute. However, membrane fouling, a severe drawback in ultrafiltration, is not constant with the ionic strength of the bulk solution. Enhanced selectivity can be achieved when the fouling is low by using membranes of large pore radius which provide a high enough transmission of the target solute to be recovered in the permeate side as well as an efficient electrostatic exclusion of the solute to be retained.

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