The electrophoretic membrane contactor: A mass-transfer-based methodology applied to the separation of whey proteins

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A B S T R A C T

In the electrophoretic membrane contactor (EMC), a porous membrane is used to establish a contact across two flowing liquids between which an electrically driven mass transfer takes places. In this work, a methodology is proposed to select the best operating conditions to separate biomolecules in an EMC. Single-solution experiments were coupled with a theoretical approach to predict the influence of the process parameters (pH, membrane MWCO) on the separation factor.This methodology was applied to the separation of whey proteins, α-lactalbumin and β-lactoglobulin, which are known to be difficult to separate. Experiments were first carried out with single synthetic protein solutions at different pH values (4.8, 6 and 8) using cellulose acetate membranes of either 30 or 100 kDa molecular weight cut-off. The experimental work was associated with a theoretical approach to study the mass transfer mechanisms. The parameters used in the model were calculated from the experimental variations of the solute and solvent transfer. The dependence of these parameters on the operating conditions gives the extent of electrostatic repulsion and provides information on the steric effect with respect to separation performance.The model was then used to calculate the separation factor for various operating conditions in order to determine the best ones (pH and membrane) for fractionation. Using the results, fractions enriched in α-lactalbumin and in β-lactoglobulin were obtained at pH 4.8 with the 100 kDa membrane.

1. Introduction

In an electrophoretic membrane contactor (EMC), a porous membrane establishes a contact across two flowing liquids between which mass transfer takes place. The driving force is an electrical field applied perpendicular to the fluid flow. Species are separated on the basis of the difference between their mass flow rates, which can be due to different electrophoretic mobilities, sieving effects or both, depending on the properties of the membrane and the solute. The technique is thus expected to combine the selectivity of membrane filtration and of electrophoresis making it an interesting alternative for the separation of charged biological molecules as it should achieve greater selectivity than conventional membrane filtration while remaining less costly than chromatography [1,2].

Many applications of such systems for the fractionation of proteins or their peptides have been published within the last fifteen years [3,4]. For instance, one apparatus developed by Margolis [5], the Gradiflow, has been used for the extraction of proteins from plasma [6,7], from egg white [8] and from whey [9]. Investigations were also carried out in a three-compartment system [10] to fractionate a mixture of bovine serum albumin and bovine haemoglobin. Galier et al. [11,12] investigated the EMC to separate biomolecules of different sizes and charges such as poly(L-glutamic) acid, α-lactalbumin or bovine haemoglobin. These studies mainly focused on the mass transfer mechanisms involved in EMC. For instance, electrostatic interactions were found to strongly modify separation performance. More recently, Poulin et al. [13,14] evaluated the fractionation of the bio-active peptides of a β-lactoglobulin hydrolysate by using electrodialysis through an ultrafiltration membrane. They investigated the effect of the pH on the migration of basic and acid peptides as well as the influence of the electric field.

However, few works have proposed a global mass transfer approach for the separation of charged biomolecules in mixture.

In this context, the aim of the present paper is to provide a more systematic approach to determine the appropriate operating conditions to separate biomolecules in an EMC. The approach used is based on the knowledge of the mass transfer mechanisms to understand the influence of pH and membrane MWCO as well as the role of electrostatic interactions on the separation efficiency. EMC can

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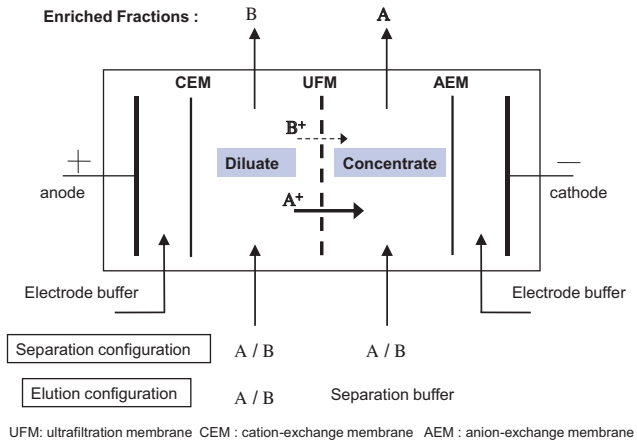

Fig. 1. Schematic drawing of the EMC, separation and elution configurations for a mixture A/B. Target solute: A.

be used in different separation modes and these are firstly discussed and illustrated. Then, the technique is applied to the fractionation of two whey proteins, α-lactalbumin (α-L) and β-lactoglobulin (β-L) which are difficult to separate because of their similar charge and size.

2. Principle of EMC

The principle of EMC, described in previous papers [11,12], is schematically depicted in Fig. 1 for both positively or negatively charged proteins such as those concerned in this work. The separation is achieved through the difference in the mass flow rates of the species. This can be due to their different electrophoretic mobilities and/or sizes, with respect to the membrane, solution (pH) and solute properties.

The voltage across the membrane is applied perpendicular to the flow, and is the only driving force behind the migration through the membrane of the charged components fed in at the inlet.

The compartments in which the outlet concentrations of the target solute are respectively lower and higher than the inlet ones will be further called "diluate" and "concentrate".

As in electrodialysis, the process can be operated in two different ways. Firstly the same solution, containing the species to be separated, can be fed into both compartments. This set-up will be further called the "separation configuration". Secondly, the solution can be fed into only one compartment, the other compartment, the elution one, being fed with the buffer. This will be referred to the "elution configuration".

These two configurations can be used to achieve different objectives, i.e. to favour quantitative or qualitative aspects. Indeed, as far as production is concerned, the separation configuration will be preferable for the separation of proteins that have opposite charges. In contrast, for achieving higher purification, the elution configuration will be preferred, especially for the separation of proteins which have the same charge sign.

3. Theoretical approach

A model was proposed to provide expressions of the solute outlet concentrations as a function of the operating parameters and solute characteristics [11]. In this model, the two phenomena resulting from the voltage, i.e. the electrophoretic migration of the solutes and the electro-osmotic flow of the solution through the membrane, are considered. The following expression of the solute concentration at the outlet of the diluate is derived from the mass balance written for the steady state in the solution and in the membrane, using the Nernst–Planck equation.

$$C_d = C_0\left[1 - \frac{u_{eo}E\tau}{d}\right]^{[\phi\,((u_{mi}/u_{eo})-1)+1]} \quad (1)$$

In this expression, C_0 is the feed solute concentration; C_d is the outlet concentration in the diluate. u_{mi} and u_{eo} are the electrophoretic and electro-osmotic mobilities. These values are both positive for a solute and a membrane carrying the same charge sign (i.e. the electro-migration solute flux and the electro-osmotic solvent flux occur in opposite directions). Inversely, u_{mi} and u_{eo} are positive and negative respectively as the solute and solvent flux occur in the same direction. The electro-osmotic mobility depends on the membrane characteristics (electrical charge, pore size) as well as on those of the electrolyte like pH, ionic strength or ionic composition.

τ is the mean residence time inside the chamber, that is fixed by the flow rate, and d is the compartment thickness.

ϕ is a partition coefficient used to link the solute concentrations inside and outside the membrane. A value of ϕ close to unity means that the membrane/solute interactions are negligible, i.e. that the membrane acts as a "true" contactor. On the other hand, decreasing values of ϕ reveal stronger interactions, the limiting case $\phi = 0$ meaning that the solute is excluded from the membrane. This parameter includes the importance of electrostatic repulsions as well as steric or any other effects.

The outlet solute concentration in the concentrate, C_c, is obtained from the total solute mass balance. As long as the inlet concentrations are identical (separation mode) and equal to C_0, this mass balance is:

$$C_c = 2C_0 - C_d \quad (2)$$

The influence of the membrane on the solute mass transfer is characterised by the value of the partition coefficient ϕ. This value is determined by fitting the experimental variations of the solute concentration versus $E.\tau$ with the ones calculated by Eq. (1). The electric field E, the feed solute concentration C_0 and the mean residence time τ are operating parameters. The electro-osmotic mobility u_{eo} is experimentally determined using Eq. (6) (see below).

In order to estimate the separation efficiency, one can also use another parameter, the separation factor, which is expressed by the target solute (A) concentration factor divided by the concentration factor of the other solute (B):

$$SF = \frac{(C_c/C_c^0)_A}{(C_c/C_c^0)_B} \quad (3)$$

where C_c^0 and C_c are the feed and outlet concentrations in the concentrate, respectively. The separation factor is calculated from the solute concentrations in the compartment enriched with the target solute. A value higher than unity means that separation can be achieved whereas a value equal to unity reveals no selectivity.

4. Separation in EMC

4.1. General approach

EMC selectivity comes from the difference between the mass transfer flow rates of the species through the membrane and can have different origins depending on the characteristics of the solute and of the membrane. It can be due to a difference between electrophoretic mobilities (*charge-based mode*), to a size exclusion effect, due to the respective sizes of the membrane pores and of the solutes (*size-based mode*) or to a combination of both (*charge and size-based mode*).

Consequently, different situations are possible depending on the choice of the buffering pH, which determines the electrophoretic mobilities of the proteins, and the membrane MWCO (Table 1). Two

Table 1
EMC separation.

$u_{mi\,A} \times u_{mi\,B}$	Size and charge	Separation Transferred species	Membrane MWCO and pH
$u_{mi\,A} \times u_{mi\,B} < 0$ Case 1	Opposite sign	*Charge based-mode* A and B	MWCO $\gg$ MW_A and MW_B
$u_{mi\,A} \times u_{mi\,B} > 0$ Case 2	Same sign $MW_A < MW_B$ and $u_{mi\,A} > u_{mi\,B}$	*Charge and size based-mode* A	MWCO $\gg$ MW_A MWCO $\approx$ MW_B pH $\approx$ pI_B
Case 3		*Size based-mode* A	
Case 4	$MW_A < MW_B$ and $u_{mi\,A} < u_{mi\,B}$	*Charge based-mode* B	MWCO $\gg$ MW_A and MW_B pH $\approx$ pI_A

distinct situations are considered for the protein charge: opposite sign ($u_{mi\,A} \times u_{mi\,B} < 0$) and same sign of charge ($u_{mi\,A} \times u_{mi\,B} > 0$). In the following discussion, protein A is the one with the lower molecular weight.

4.2. Influence of pH and membrane MWCO

The difference between the rate of transfer of each species can be improved by choosing appropriate pH and membrane MWCO (see Table 1).

The influence of the membrane MWCO can be ascribed to the variation of the partition coefficient, ϕ. As discussed above, this parameter includes the effect of electrostatic repulsions as well as steric effects. Its value decreases for lower membrane MWCO by increasing the steric effect. The role of the electrostatic interactions will be discussed below. On the other hand, the electrophoretic mobility depends on the pH and tends towards zero for a pH approaching the protein pI.

For proteins with opposite charges ($u_{mi\,A} \times u_{mi\,B} < 0$, case 1, *charge based mode*), the mass transfer of both proteins must be as high as possible to maximize the separation efficiency. Consequently, the membrane MWCO must be higher than the protein molecular weight to avoid any steric effects ($\phi = 1$). Here, the membrane acts as a "true contactor". On the other hand, for proteins with the same charge sign ($u_{mi\,A} \times u_{mi\,B} > 0$), the mass transfer of one protein must be lower than the mass transfer of the other.

In *charge and size-based mode* (case 2) and *size-based mode* (case 3), the protein which has the smallest size (A) is transferred whereas the other (B) must be retained by the membrane. The transfer of the larger protein can be limited by using a membrane MWCO close to its molecular weight as well as by selecting a pH close to its pI. Inversely, in the *charge-based mode* (case 4), the larger protein (B), is transferred whereas the smaller one (A) must be prevented from crossing the membrane. This condition can be reached by using a membrane MWCO high enough to minimize steric effects, but also choosing a pH close to the pI of protein A.

4.3. Influence of electrostatic interactions

It has been demonstrated that mass transfer can be affected by electrostatic interactions taking place at the membrane interface [12]. The effect of the electrostatic interactions on the separation efficiency is evaluated from the calculation of the separation factor SF for different conditions by combining Eqs. (1)–(3). Its value is fixed by the electrophoretic mobility, u_{mi}, and the partition coefficient, ϕ, for each protein, and the electro-osmotic mobility, u_{eo}, which characterises the membrane charge. The numerical values for these parameters (u_{eo}, u_{mi}, ϕ) are typical for protein separation in EMC [11,12].

In case 1 ($u_{mi\,A} \times u_{mi\,B} < 0$, *charge-based mode*), one protein has the same charge sign as the membrane. It might thus be retained by electrostatic repulsion. The influence of increasing interactions, represented by decreasing values for the partition coefficient, is illustrated in Fig. 2. As expected, decreasing separation factors are obtained for decreasing partition coefficients.

In the *charge and size-based mode* (case 2) and *size-based mode* (case 3), the mass transfer of the smaller protein (A) must be as high as possible. Consequently, electrostatic repulsion must be negligible. This is the case for a membrane and a protein having different signs of charge. However, the membrane charge is often fixed by the protein [11,15,16]. Electrostatic repulsion can be high and consequently mass transfer of the smaller protein can decrease. Fig. 3 shows the variation of the separation factor *SF* versus $E{\cdot}\tau$ for different partition coefficients of the smaller protein (A) to illustrate the effect of electrostatic interactions on the separation efficiency. Then, as expected, the increase in electrostatic interactions, i.e. decreasing partition coefficients for protein A, reveals lower values for the separation factor. It can also be observed that in some conditions, the enhancement of electrostatic repulsion is such that selectivity is reduced to zero ($SF = 1$).

In the *charge-based mode* (case 4), the mass transfer of the larger protein (B), which also has the higher electrophoretic mobility, must be as high as possible. Here again, an increase in the electrostatic repulsion also decreases the separation efficiency.

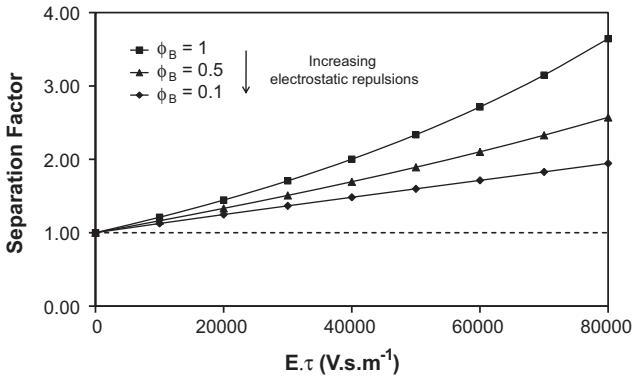

Fig. 2. Variation of the calculated separation factor versus the product of the electric field by the residence time ($E{\cdot}\tau$): influence of electrostatic interactions. Case 1: target protein A; $u_{mi\,A} \times u_{mi\,B} < 0$; $|u_{mi\,A}| = |u_{mi\,B}| = 10^{-8}\,V\,m^2\,s^{-1}$; $\phi_A = 1$; $u_{eo} = 2 \times 10^{-9}\,V\,m^2\,s^{-1}$; membrane and protein B: same sign of charge; separation configuration.

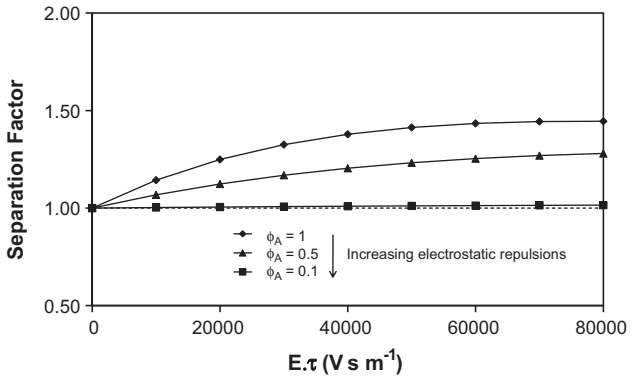

Fig. 3. Variation of the calculated separation factor versus the product of the electric field by the residence time ($E{\cdot}\tau$): influence of electrostatic repulsions on the target protein A. Case 2 and case 3: target protein A, $u_{mi\,A} \times u_{mi\,B} > 0$, $|u_{mi\,A}| = 2 \times 10^{-8}$ V m^2 s^{-1}; $|u_{mi\,B}| = 0.5 \times 10^{-8}$ V m^2 s^{-1}; $\phi_B = 0.5$, $u_{eo} = 2 \times 10^{-9}$ V m^2 s^{-1}, membrane and proteins: same sign of charge, separation configuration.

5. Materials and methods

5.1. Buffer and samples

All chemicals used were of analytical grade. α-Lactalbumin (type III from bovine milk), β-lactoglobulin (from bovine milk) and 2-(N-morpholino ethane sulfonic acid (MES) were purchased from Sigma–Aldrich. Tris(hydroxymethyl)amminomethane (Tris), β-alanine, and acetic acid were from Merck and Histidine from Fluka.

Separation and electrode buffers were Tris-Mes at pH 8.0; Mes-histidine at pH 6.0 and β-alanine-acetic acid at pH 4.8.

The electrical conductivities were 140 μS cm^{-1} and 220 μS cm^{-1} for the separation and electrode buffers respectively. The solutions were prepared by dissolving the appropriate amounts of α-lactalbumin and β-lactoglobulin (α-L and β-L) in the separation buffer. The inlet concentrations were set at 0.1 and 0.2 g.L^{-1} for α-L and β-L, respectively. This concentration ratio ([β-L]/[α-L] = 2) is close to that commonly found in whey (between 2 and 4) [17].

5.2. EMC apparatus and set-up

The experimental set-up has been described in detail in a former paper [11]. The prototype cell used in this work was 17.5 cm long and 2 cm wide, so the membrane active area was 35 cm^2. The thickness of the electrode, diluate and concentrate compartments were 1.0, 0.1 and 0.1 cm, respectively.

Two cellulose acetate membranes (C030F, 30 kDa and C100F, 100 kDa) from Nadir Filtration GmbH (Germany) kindly supplied by Alting (France) were used as the porous membrane. Cellulose acetate was selected because it adsorbs proteins less than other materials such as polyamide or polysulfone.

A cation exchange membrane and an anion exchange membrane were used at the anode and cathode side respectively: Neosepta CMX and AMX (Tokuyama corporation, Japan).

The experiments were carried out in continuous mode. The two separation compartments were continuously supplied by two distinct feed tanks using peristaltic pumps placed at the outlet of the cell. The outlet flow rates were set at constant and equal values. The electrode buffer was circulated in a closed loop from a single tank to the electrode compartments using a gear pump.

The main experimental data, inlet and outlet flow rates, conductivities and pH, current, voltage and temperatures were recorded every 10 min.

5.3. Experimental procedure and operating conditions

All experiments were carried out at ambient temperature (22 ± 3 °C).

The outlet flow rate in the separation chamber and in the electrode compartment was fixed at 100 mL h^{-1} (τ = 230 s) and 5.0 L h^{-1}, respectively.

The experiments were carried out at a constant current, ranging from 10 to 60 mA (i.e. from 3 to 20 A m^{-2}).

The average electric field strength E in the separation chamber was calculated from the following equation:

$$E = \frac{I}{\chi_{avg}\, S} \tag{4}$$

where I is the current, S the membrane area and χ_{avg} the mean electrical conductivity, calculated from the conductivities at the inlet and outlet. For the operating conditions used, the average electric field was between 170 and 800 V m^{-1}.

Since the outlet flow rates were fixed and equal, the electro-osmotic flux J_{eo} was obtained from the measurement of the inlet flow rates in each compartment by the following relationship [11]:

$$J_{eo} = \frac{|Q_c^{inlet} - Q_d^{inlet}|}{2\,S} = \frac{Q_{eo}}{S} \tag{5}$$

Then, the electro-osmotic mobility u_{eo} was deduced from the electro-osmotic flux and of the electric field:

$$J_{eo} = u_{eo} E \tag{6}$$

The experimental variations of the solute concentration versus time (results not shown) show that the steady state was reached after 30–40 min [11]. Therefore, the electro-osmotic flow rate as well as the solute concentration at the outlet of each compartment was measured after 40–60 min. It was also observed that for any set of operating conditions, the temperature increase and pH variation in the concentrate and diluate compartments did not exceed 5 °C, 0.4 and 0.1 pH units, respectively.

5.4. Analytical methods

For single solutions, the concentrations of α-L and β-L were measured by ultraviolet spectroscopy at 280 nm.

For binary solutions, protein concentrations were evaluated by reversed-phase HPLC. The separation was carried out with a PLRP-S column (gel of divinylbenzene polystyrene, 300 Å, 8 μm, 150 mn × 4.6 mn, Polymer Laboratories). The experimental conditions were adapted from the method developed by Resmini et al. [18]. The eluents used were: a milli Q water with 0.1% trifluoroacetic acid (TFA); and B milli Q water-acetonitrile (20:80, v/v) with 0.1% TFA. The flow rate was fixed at 1 mL min^{-1} and the gradient was (% of B): 0–1 min: 46; 1–5 min: 46–53; 5–8 min: 53–58; 8–10 min: 58; 10–13 min: 58–46; 13–16 min: 46. The injection volume was 20 μL and detection was carried out at 210 nm.

5.5. Electrophoretic mobility

The electrophoretic mobilities of α-L and β-L at pH 8 and 6 were estimated from the Henry equation which expresses the electrophoretic mobility as a function of the protein charge and size [19,20]. The estimated value of the electrophoretic mobility of α-L at pH 8 obtained according to this procedure was close to the values measured by capillary electrophoresis in the same buffer (-1.85×10^{-8} m^2 V^{-1} s^{-1}) [11]. These values were also confirmed in the present study by experimental determination with a Zetasizer (Malvern) where a value of -1.6×10^{-8} m^2 V^{-1} s^{-1} was determined at pH 8 in the same buffer (pH and conductivity). The

Table 2
Characteristics of the proteins: molecular weight (M_W), radius (r), calculated charge (z), calculated and experimental isoelectric point pI.

	α-Lactalbumin	β-Lactoglobulin
M_W (kDa)	14.2	18.3 mono (pH ≥ 8) 36.6 dim (pH < 8)
r (nm)	1.9	2.0 (mono) 2.6 (dimer)
z (pH 8)	−5[a]	−10.5[b,*] (monomer)
z (pH 6)	−3.3[a]	−15[b,*] (dimer)
pI cal[a]	4.8	4.4
pI exp[c]	4.2–4.8	5.1–5.4
u_{mi} (pH 8) ($m^2\,V^{-1}\,s^{-1}$)[d]	-1.6×10^{-8}	-2.9×10^{-8}
u_{mi} (pH 6) ($m^2\,V^{-1}\,s^{-1}$)[d]	-1.0×10^{-8}	-2.9×10^{-8}
u_{mi} (pH 4.8) ($m^2\,V^{-1}\,s^{-1}$)[e]	0.1×10^{-8}	0.5×10^{-8}

[a] [21].
[b] Estimated from the procedure described in [20].
[c] [17].
[d] Value calculated from the protein charge.
[e] Measured (Zetasizer).
[*] Average value between isoform A and B of β-L.

error in electrophoretic mobility measurement was estimated to be $\pm0.1\times10^{-8}\,m^2\,V^{-1}\,s^{-1}$.

The estimation of the electrophoretic mobility by the procedure described above seems to be appropriate when the pH is different from the protein isoelectric point (pI) but less so when the pH is close to the pI. At pH 4.8, the electrophoretic mobility of α-L is positive and close to zero. This is in accordance with both calculated and experimental isoelectric points. However, the β-L charge would be respectively positive or negative according to the calculated and experimental isoelectric points. EMC experiments as well as the experimental determination of the electrophoretic mobility with a Zetasizer confirm that β-L is positively charged ($u_{mi\,\beta\text{-L}} = +0.5\times10^{-8}\,m^2\,V^{-1}\,s^{-1}$).

The compiled values of the electrophoretic mobilities given in Table 2 were used in the mathematical model (see Eq. (1)).

6. Results and discussion

6.1. EMC separation: application to the mixture α-L/β-L

Table 2 provides the main characteristics of the proteins, i.e. their molecular weights, radii, charges, isoelectric points (pI) and electrophoretic mobilities.

These two proteins often have the same charge ($u_{mi\,\alpha\text{-L}} \times u_{mi\,\beta\text{-L}} > 0$) due to their close pI. Moreover, the electrophoretic mobility of α-L is always lower than that of β-L because of its lower charge. Then, as discussed above (*see* Section 4.1), separation was achieved in the *size-based mode* (case 3) or in the *charge-based mode* (case 4). In this situation, α-L is protein A (lower size and charge) while β-L represents protein B (higher size and charge).

In the *size-based mode*, α-L must be transferred and β-L retained by the membrane by steric effects and/or electrostatic repulsion. Thus a membrane with a MWCO of 30 kDa, close to the β-L molecular weight, was selected to favour steric effects. Moreover, the retention of β-L by steric effects could be increased by changing the pH. Actually, at ambient temperature, β-L occurs as a dimer over the pH range 3–7 [22–24]. Therefore, two different pH values were used in this study, pH 8 (monomer form) and pH 6 (dimer form), to highlight the influence of the size effect on separation.

In the *charge-based mode*, β-L must be preferentially transferred through the membrane whereas the mass transfer of α-L must be minimum. A membrane with a MWCO of 100 kDa was thus chosen to limit the retention of β-L by steric effect. Two different pH values were selected according to the pI to highlight its influence on the separation efficiency, i.e. one value far from the pI (pH 8) and the other close to α-L pI (pH 4.8).

6.2. Experimental study

The two different approaches for α-L and β-L fractionation and the corresponding pH and membrane MWCO used are summarized in Table 3.

Experiments were first carried out with single protein solutions and different pH values (4.8, 6 and 8) with the two membranes to investigate solvent and protein mass transfer and to estimate separation. The experimental value of the electro-osmotic flux was first used to calculate the electro-osmotic mobility. Then, the partition coefficient ϕ was obtained by fitting the experimental variations of the concentrations versus $E{\cdot}\tau$ with the calculated ones (Eq. (1)).

6.2.1. Electro-osmotic flux

For all the operating conditions used in this study, the electro-osmotic flux was always directed from the anode to the cathode. Consequently, the membrane charge was negative whatever the sign of the protein charge in the buffer solution.

The experimental variations of the electro-osmotic flux were plotted versus the electric field at pH 6 for different solutions (Fig. 4). All operating conditions (pH and membrane MWCO) showed similar trends. The linearity indicates a constant value for the electro-osmotic mobility (see Eq. (6)) and thus of the membrane charge. The electro-osmotic mobilities (Table 4), were obtained from the slope of the curves $J_{eo} = f(E)$. The error in

Table 3
Separation modes for α-L/β-L fractionation: pH and membrane MWCO conditions.

Separation mode Transferred protein	Separation parameter	pH	Membrane MWCO
Size-based mode (case 3) α-L	$MW_{\alpha\text{-L}}/MW_{\beta\text{-L}} = 1.3$ $MW_{\alpha\text{-L}}/MW_{\beta\text{-L}} = 2.6$	8-6	30 kDa
Charge-based mode (case 4) β-L	$u_{mi\,\alpha\text{-L}}/u_{mi\,\beta\text{-L}} = 1.8$ $u_{mi\,\alpha\text{-L}}/u_{mi\,\beta\text{-L}} = 5$	8-4.8	100 kDa

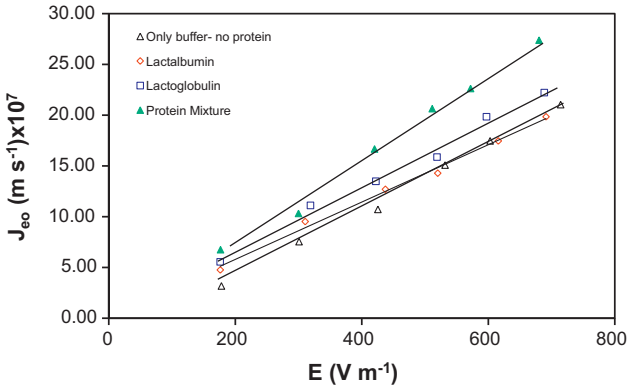

Fig. 4. Variation of the electro-osmotic flux versus the electric field. Operating conditions: buffer: Mes-histidine at pH 6, $Q = 100\,mL\,h^{-1}$, membrane MWCO 30 kDa.

electro-osmotic mobility determination was estimated to be $\pm 0.2 \times 10^{-9}\,m^2\,V^{-1}\,s^{-1}$.

At pH 8 and 6, the electro-osmotic mobility/membrane charge increases in the presence of negatively charged proteins. This has already been discussed in previous papers [11,12]. It is linked to the membrane zeta potential rise when the membrane is put into contact with a solution containing a charged solute [15,16]. The addition of α-L has no measurable influence on the electro-osmotic mobility at pH 8 with the 100 kDa membrane and at pH 6 with the 30 kDa membrane. On the contrary, as far as β-L is present in the buffer, increased values of the electro-osmotic mobility are obtained. These results are in accordance with a previous study, showing a good qualitative correlation between the influence of solute on electro-osmotic flux and the solute charge, which can be linked to its electrophoretic mobility [11]. It seems that the charge of α-L, while different from zero, is not sufficient to change the electro-osmotic mobility, i.e. the membrane charge. On the contrary, an increase is obtained with β-L, which has the highest electrophoretic mobility (see Table 2).

The results obtained at pH 8 with the 30 kDa membrane are slightly different. Indeed, the presence of α-L or β-L increases the electro-osmotic mobility in the same manner. These results can be explained by the lower membrane charge (i.e. electro-osmotic mobility in the buffer). Consequently, the charge of α-L is sufficient to change the electro-osmotic mobility in these conditions.

At pH 4.8, the electro-osmotic mobility was weakly affected by the presence of the proteins which had a low positive charge.

6.2.2. Single-protein mass transfer

The influence of the membrane on the mass transfer of protein is characterised by the value of the partition coefficient ϕ. It was determined by fitting the experimental variations of the solute concentration versus $E{\cdot}\tau$ with those calculated by Eq. (1). The values are reported in Table 5. Typical variations of the corresponding experimental and calculated concentrations are plotted in Figs. 5 and 6. Similar curves were obtained for any operating condition (pH and membrane MWCO).

Table 4
Absolute values of the mean electro-osmotic mobility u_{eo} $(m^2\,V^{-1}\,s^{-1}) \times 10^9$ for different operating conditions and solutions (buffers, buffered single and binary solutions).

Operating conditions	Buffer	α-L	β-L	Mixture α-L/β-L
pH 8 30 kDa	0.8	1.8	1.8	–
pH 6 30 kDa	2.8	2.8	3.4	–
pH 8 100 kDa	1.4	1.4	2	
pH 4.8 100 kDa	1	0.8	0.9	0.7

Table 5
Fitted values of the partition coefficient, ϕ, for single protein solutions.

Operating conditions	α-L	β-L
pH 8 30 kDa	0.6	0.3
pH 6 30 kDa	0.4	0.07
pH 8 100 kDa	0.6	0.45
pH 4.8 100 kDa	1	0.9

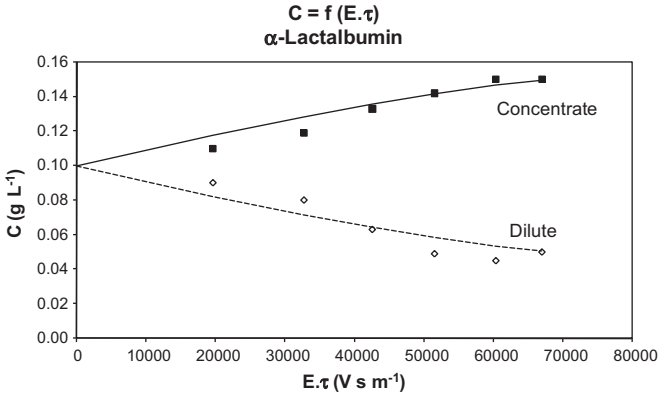

Fig. 5. Comparison between experimental (points) and calculated (curves) outlet concentrations versus the product of the electric field by the residence time ($E{\cdot}\tau$). Operating conditions: single solution of α-L, $C_0 = 0.1\,g\,L^{-1}$; Tris-Mes buffer at pH 8; membrane MWCO 30 kDa; separation configuration.

At pH 4.8 with the 100 kDa membrane, both proteins were almost freely transferred (ϕ was close to one). In this case both membrane and proteins are weakly and oppositely charged, and the results show that the steric effects are negligible.

The partition coefficients of α-L and β-L obtained at pH 8 with the 100 kDa membrane were 0.6 and 0.45, respectively. At this pH, both proteins and membrane were negatively charged. As stated, the steric effects were negligible, so the low values of ϕ mean that transfer α-L and β-L is mainly limited by electrostatic repulsion. These results are in agreement with previous findings [11].

The β-L partition coefficient is lower than that of α-L which indicates stronger electrostatic repulsion between β-L and membrane. This is explained by higher β-L and membrane charges. In fact, the electrophoretic mobility of β-L is higher than that of α-L (Table 2) and in addition, the electro-osmotic mobility obtained with single β-L solution was higher than that measured with a single solution of α-L (Table 4).

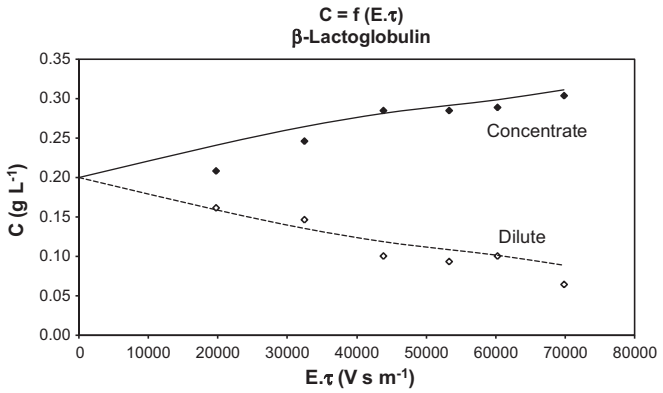

Fig. 6. Comparison between experimental (points) and calculated (curves) outlet concentrations versus the product of the electric field by the residence time ($E{\cdot}\tau$). Operating conditions: single solution of β-L, $C_0 = 0.2\,g\,L^{-1}$; Tris-Mes buffer at pH 8; membrane MWCO 30 kDa; separation configuration.

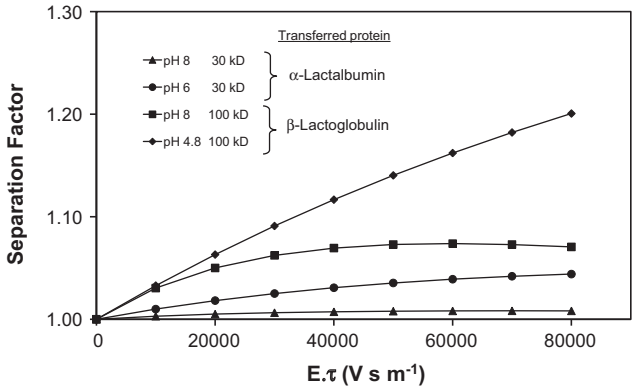

Fig. 7. Estimated separation factor versus the product of the electric field by the residence time ($E{\cdot}\tau$). Operating conditions: mixture: α-L, $C_0 = 0.1\,g\,L^{-1}$ and β-L, $C_0 = 0.2\,g\,L^{-1}$; separation configuration.

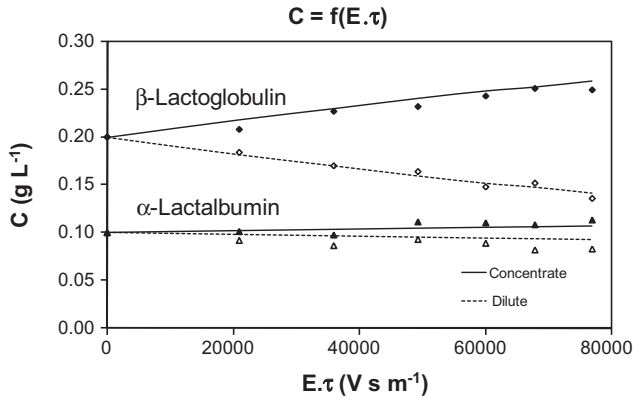

Fig. 8. Comparison between experimental (points) and estimated (curves) outlet concentrations versus the product of the electric field by the residence time ($E{\cdot}\tau$). Parameters obtained from single protein solutions: $\phi_{\alpha\text{-L}} = 1$; $\phi_{\beta\text{-L}} = 0.9$; $u_{eo} = 0.9 \times 10^{-9}\,m^2\,V^{-1}\,s^{-1}$. Operating conditions: mixture: α-L, $C_0 = 0.1\,g\,L^{-1}$ and β-L, $C_0 = 0.2\,g\,L^{-1}$; β-alanine-acetic acid buffer at pH 4.8; membrane MWCO 100 kDa; separation configuration.

The α-L and β-L partition coefficients obtained at pH 8 with the 30 kDa membrane were 0.6 and 0.3 respectively. The 30 kDa membrane was selected to preferentially retain β-L by size effect. The α-L partition coefficient was the same as that obtained with the 100 kDa membrane. Therefore, the transfer of α-L is also governed by electrostatic interactions while steric effects are still negligible. As expected, a lower value for the β-L partition coefficient was obtained at pH 8 compared to that obtained with the 100 kDa membrane. This indicates that with the 30 kDa membrane the transfer of β-L results from the joint effects of charge and size.

As previously mentioned, β-L forms a dimer at pH lower than 7. Consequently one can expect a decrease of β-L transfer due to size effects when changing the pH. This is confirmed by the lower value of the β-L partition coefficient, $\phi_{\beta\text{-L}} = 0.1$, at pH 6.

The partition coefficient of α-L was also lower at pH 6 ($\phi_{\alpha\text{-}L} = 0.4$) than at pH 8 ($\phi_{\alpha\text{-}L} = 0.6$). The α-L transfer decrease indicates stronger electrostatic repulsion due to the increase of the membrane charge since transfer is mainly governed by electrostatic interactions.

6.3. Separation of the protein mixture

Protein separation was evaluated by calculating the separation factor SF, knowing the electrophoretic mobilities of the proteins, the electro-osmotic mobility and the respective partition coefficients obtained from the single-solution experiments (Eqs. (1)–(3)). The value of the electro-osmotic mobility used for the estimation was the value obtained with the protein which had the higher charge since it has been shown that it fixes the membrane charge in binary solutions [12].

Typical variations of the corresponding estimated separation factors are plotted in Fig. 7.

From this figure, the separation factor is always lower than 1.1 with the 30 kDa membrane (*size-based mode*, case 3) since α-L transfer is limited by strong electrostatic repulsions while β-L is not totally retained because of combined electrostatic repulsion and steric effects. Consequently, the separation of the two proteins is impossible in this case.

A higher separation factor was obtained in the *charge-based mode* (*case 4*) with the 100 kDa membrane. As expected, the separation factor increased at pH 4.8, which is close to the pI of the smaller protein, in this case α-L.

Consequently, experimental separation was carried out at pH 4.8 with the 100 kDa membrane to confirm the prediction from the single protein study. The experimental variation of the concentrations versus $E{\cdot}\tau$ is reported in Fig. 8 (points). These experimental concentrations are in agreement with the estimated values (curves).

7. Conclusion

In this work, a global mass transfer approach for the separation of charged biomolecules in EMC was proposed. The methodology is based on the knowledge of the mass transfer mechanisms to understand the influence of the pH, the membrane MWCO as well as the role of the electrostatic interactions on the separation efficiency.

The different separation modes in EMC were discussed and the methodology was illustrated for the fractionation of two whey proteins, α-lactalbumin (α-L) and β-lactoglobulin (β-L) which are difficult to separate because of their similar charge and size.

The experimental work carried out at pH 6 and 8 using the 30 kDa membrane associated with the theoretical approach clearly pointed out the impossibility to fractionate these proteins according to their size (*size-based mode*). Indeed, both proteins possess a high charge which has the same sign as that of the membrane and consequently α-L transfer is limited by strong electrostatic repulsions while β-L is not totally retained by either electrostatic repulsion or steric effect.

The feasibility of using EMC to fractionate α-L and β-L was also evaluated in the *charge-based mode*. In this case, the experiments were performed at pH 8 and 4.8 with a 100 kDa membrane to avoid steric effects. At pH 8 the separation was not achievable because mass transfer of both α-L and β-L was mainly limited by electrostatic repulsion. The results obtained at pH 4.8 confirmed that steric effects were negligible with the 100 kDa membrane and that electrostatic interactions were weak. Consequently, a higher separation efficiency was obtained in the *charge-based mode* with the 100 kDa membrane at pH 4.8 which is close to the pI of one of the proteins, α-L in this case.

Moreover, this study highlights that changing the pH (*charge-based mode*) is more efficient at improving separation than adjusting the membrane MWCO (*size-based mode*) for proteins of the same sign of charge. This is in agreement with the separation of proteins having opposite charges. Indeed, the separation efficiency is maximized by using a membrane MWCO higher than the protein molecular weight to avoid steric effects, i.e. when the membrane acts as a "true contactor".

However, at this stage, the EMC performance was lower than that obtained with other systems mainly because of the strong mass transfer limitations due to electrostatic interactions. Further work is thus still necessary to reduce the impact of these interactions to improve the separation efficiency.

References

[1] G. Bargeman, J. Houwing, I. Recio, G.-H. Koops, C. van der Horst, Electro-membrane filtration for the selective isolation of bioactive peptides from an α_{s2}-casein hydrolysate, Biotechnol. Bioeng. 80 (2002) 599–609.

[2] G. Bargeman, G.H. Koops, J. Houwing, I. Breebaart, H.C. van der Horst, M. Wessling, The development of electro-membrane filtration for the isolation of bioactive peptides: the effect of membrane selection and operating parameters on the transport rate, Desalination 149 (2002) 369–374.

[3] S. Galier, H. Roux-de Balmann, Electrophoretic membrane contactors, Chem. Eng. Res. Des. 83 (2005) 276–284.

[4] M. Aider, D. de Halleux, L. Bazinet, Potential of continuous electrophoresis with and without membranes (CEPM) in the bio-food industry: review, Trends Food Sci. Technol. 19 (2008) 351–362.

[5] J. Margolis, Electrophoretic method for preparative separation of charged molecules in liquids, US Patent No 5.039. 386 (1991).

[6] Z.S. Horvath, G.L. Corthals, C.W. Wrigley, J. Margolis, Multifunctional apparatus for electrokinetic processing of proteins, Electrophoresis 15 (1994) 968–971.

[7] Z.S. Horvath, A.A. Gooley, C.W. Wrigley, J. Margolis, K.L. Williams, Preparative affinity membrane electrophoresis, Electrophoresis 17 (1996) 224–226.

[8] D. Ogle, M. Sheehan, B. Rumbel, T. Gibson, D.B. Rylatt, Design of a new, twelve-channel electrophoretic apparatus based on the Gradiflow technology, J. Chromatogr. A 989 (2003) 63–72.

[9] G.L. Corthals, B.M. Collins, B.C. Mabbutt, K.L. Williams, A.A. Gooley, Purification by reflux electrophoresis of whey and of a recombinant protein expressed in Dictyostelium Discoideum, J. Chromatogr. A 773 (1997) 299–310.

[10] Z. Liu, Z. Huang, J. Chong, H. Yang, F. Ding, N. Yuan, Continuous separation of proteins by multichannel flow electrophoresis, Sep. Sci. Technol. 31 (1996) 1427–1442.

[11] S. Galier, H. Roux-de Balmann, Study of the mass transfer phenomena involved in an electrophoretic membrane contactor, J. Membr. Sci. 194 (2001) 117–133.

[12] S. Galier, H. Roux-de Balmann, Study of biomolecules separation in an electrophoretic membrane contactor, J. Membr. Sci. 241 (2004) 79–87.

[13] J.F. Poulin, J. Amiot, L. Bazinet, Simultaneous separation of acid and basic bioactive peptides by electrodialysis with ultrafiltration membrane, J. Biotechnol. 123 (2006) 314–328.

[14] J.F. Poulin, J. Amiot, L. Bazinet, Improved fractionation by electrodialysis with ultrafiltration membrane: influence of ultrafiltration membrane stacking and electric field strength, J. Membr. Sci. 299 (2007) 83–90.

[15] C. Causserand, M. Nyström, P. Aimar, Study of streaming potential of clean and fouled ultra-filtration membranes, J. Membr. Sci. 134 (1994) 211–222.

[16] K.J. Kim, A.G. Fane, M. Nyström, A. Pihlajamaki, Chemical and electrical characterisation of virgin and protein-fouled polycarbonate track-etched membranes by FTIR and streaming potential measurements, J. Membr. Sci. 134 (1997) 199–208.

[17] L. Pedersen, J. Mollerup, E. Hansen, A. Jungbauer, Whey proteins as a model system for chromatographic separation of proteins, J. Chromatogr. B 790 (2003) 161–173.

[18] P. Resmini, L. Pelligrino, R. Andreini, F. Prati, Determination of soluble milk serum proteins by reverse-phase HPLC (high pressure liquid chromatography), Sci. E Technica Latteriocaseria 40 (1989) 7–23.

[19] R.J. Hunter, Zeta potential in Colloid Science: Principle and Applications, Academic Press Inc., London, 1981.

[20] M. Rabiller-Baudry, A. Bouguen, D. Lucas, B. Chaufer, Physico-chemical characterization of proteins by capillary electrophoresis, J. Chromatogr. B 709 (1998) 23–32.

[21] A. Muller, Process of obtention of height purity α-lactalbumin: elementary steps of the fractionation of whey proteins and cascade set-up, Ph.D. Thesis, National School of Agriculture of Rennes, France, 1996.

[22] A. Muller, G. Daufin, B. Chaufer, Ultrafiltration modes of operation for separating α-lactalbumin from acid casein whey, J. Membr. Sci. 153 (1999) 9–21.

[23] K. Kuwata, M. Hoshino, V. Forge, S. Era, C.A. Batt, Y. Goto, Solution structure and dynamics of bovine b-lactoglobulin A, Protein Sci. 8 (1999) 2541–2545.

[24] S. Bhattacharjee, C. Bhattacharjee, S. Datta, Studies on the fractionation of β-lactoglobulin from casein whey using ultrafiltration and ion-exchange membrane chromatography, J. Membr. Sci. 275 (2006) 141–150.