Electrofiltration of solutions of amino acids or peptides

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Summary — Enhanced transfer according to the isoelectric point (pl) of amino acids and peptides (molecular mass 130–6 000 g mol\(^{-1}\)) was achieved using laboratory filtration in the presence of an electric field. The permeate was enriched with arginine and lysine of negative electrophoretic mobility, while the retentate was enriched with the amino acid characterized by the lowest pl value, aspartic acid. A model which assumes no retention of amino acids by ultrafiltration (UF) membranes describes reasonably well their transmission to the permeate. The peptide mixture permeate was enriched with peptides which were either positively or negatively charged according to the electric field direction. The effect was significant even under low electric field. The model did not quantitatively describe the experimental transmission probably because of significant retention of some peptides by the ultrafiltration membranes. The present study shows that electrofiltration (EF) may be a useful and efficient process for achieving selective separation of charged biological molecules provided that further work is aimed at a better understanding of which mechanisms rule the retention in EF and of the effect of process variables (flux, electric field, conductivity, charge of the molecules).

nomenclature: \( C_p, C_r \), amino acid concentration of permeate, retentate, g l\(^{-1}\); \( E \), electric field, V m\(^{-1}\); \( J \), permeation flux, m\(^3\) m\(^{-2}\) s\(^{-1}\); \( M \), molecular mass; MMCO, molecular mass cut-off; \( N \), Avogadro number \((6.023 \times 10^{23})\); pl, isoelectric pH; \( R_m \), membrane hydraulic resistance, m\(^{-1}\); \( R_f \), hydraulic resistance of fouling layer, m\(^{-1}\); \( T_r \), transmission rate; \( T_P \), transmembrane pressure, Pa; \( r \), molecule radius, m; \( U \), electrophoretic mobility, m\(^2\) s\(^{-1}\) V\(^{-1}\); \( Z \), number of charges; \( e \), charge of electron, C; \( \phi \), sieving coefficient; \( \mu \), dynamic viscosity of feed, Pa s; \( \mu_P \), dynamic viscosity of permeate, Pa s; \( \rho \), density, kg m\(^{-3}\).
Résumé — Électrofiltration de solutions d’acides aminés ou de peptides. Le transfert sélectif d’acides aminés et de peptides (masse molaire : 130-6000 g mol⁻¹) selon leur point isoélectrique était réalisé par filtration sur membrane couplée à l’application d’un champ électrique, E. Le perméat était enrichi en substances possédant une mobilité électrophorétique négative (arginine, lysine, βCN 170-176 ; βCN 177-183) tandis que le rétentat était enrichi en molécules de bas point isoélectrique (acide aspartique). Un modèle décrivant la transmission est proposé. Lorsque la rétention en l’absence de champ électrique est nulle, l’accord avec l’expérience est satisfaisant. Une rétention non nulle en l’absence de champ électrique appliqué ne permet plus de quantifier correctement la transmission trouvée en électrofiltration, EF. Ce travail montre que ce procédé peut être utile et efficace pour réaliser la séparation sélective de molécules biologiques chargées à condition de développer des études approfondies concernant les mécanismes qui régissent la rétention en EF et l’effet des variables opératoires (flux, champ électrique, conductivité, charge des molécules).

électrofiltration / acide aminé / peptide

INTRODUCTION

Selective separation of biologically active peptides obtained by enzymatic hydrolysis of β-casein is not an easy practice because of retention or transmission which could be explained by the respective size of the peptide and of the membrane pores and by electric charge interactions at low ionic strength (Visser et al, 1989; Pouliot and Gauthier, 1990; Nau, 1991; Nau et al, 1993, 1994).

Membrane processes using an electric field have been used to separate ions or macrosolutes according to their charge. Electrodialysis has been proposed to concentrate or to separate a mixture of amino acids at a pH close to their isoelectric pH (Rumeau and Montfort, 1988; Martinez, 1990). A combination of electrodialysis and ultrafiltration was used several years ago to prepare demineralized concentrates of macromolecules (Coca Cola Company, 1975; Ahlgren, 1980). In previous research devoted to electro-ultrafiltration, authors have often studied the effect of the electric field on concentration polarization (Henry et al, 1977; Radovich and Sparks, 1980; Yukawa et al, 1983; Radovich and Behnam, 1983; Bowen and Sabuni, 1987; Visvanathan, 1988; Aimar et al, 1989; Vivoni-Assice, 1989; Moulik and Gupta, 1990). In addition to the expected increase in flux, an improved retention of macromolecules was obtained (Kimura and Nomura, 1982). Moreover, Lentsch (1993) reported an improvement of selectivity based on the electric charge of proteins when using electro-ultrafiltration. Lee and Hong (1987) have proposed a model for the enrichment of one amino acid in the permeate by coupling electrophoresis and dead-end ultrafiltration.

The aim of the present work was to assess the efficiency of an electrophoretic separation through inorganic or organic membranes. Separation of the different amino acids and peptides used in this study was expected owing to their different mobilities. Amino acids were chosen as model molecules since they provided a simpler comparison between experimental and theoretical trends: they were not retained by the membrane, as compared to peptides, which showed complex behaviour in UF (either positive or negative retention).

THEORY

If one assumes that the membrane acts as a diaphragm, no retention is expected (molecular mass cut-off, MMCO > 10 kDa),
and therefore, concentration polarization is negligible. The permeate flux, J, is given by Darcy’s law:

$$J = \frac{TP}{\mu_0 (R_m + R_f)}$$  \hspace{1cm} (1)

where $R_m$ is the clean membrane hydraulic resistance and $R_f$ is the hydraulic resistance due to fouling. The flow due to electro-osmosis is not accounted for in equation 1.

The flux of charged molecule (amino acid, peptide), $J \cdot C_p$ in the permeate is assumed to be the sum of convection and of electrophoretic migration:

$$J \cdot C_p = C_r (J + UE) \phi$$  \hspace{1cm} (2)

where $C_p$ and $C_r$ represent the concentrations in the permeate and in the retentate respectively, $U$ is the apparent electrophoretic mobility at the interface where the pH has an intermediate value along the gradient between the retentate and permeate pH, $E$ is the electric field at the membrane-solution interface and $\phi$ is the sieving coefficient. Given that the membrane carbon support has a good electric conductivity and that the current density is low, the potential is assumed constant throughout the support and the electric field across the carbon support is then negligible. An expression for $C_p$ can be derived from eq (2):

$$C_p = C_r (1 + \frac{UE}{J}) \phi$$  \hspace{1cm} (3)

The transmission, $Tr$, of a species through the membrane is thus:

$$Tr = \frac{C_p}{C_r} = (1 + \frac{UE}{J}) \phi$$  \hspace{1cm} (4)

The mobility depends on size and charge of solutes. An equation has been proposed (Tanford, 1962) which accounts for these parameters:

$$U = \frac{ZE}{6\pi \mu \nu} (m^2 s^{-1} V^{-1})$$  \hspace{1cm} (5)

Where $Ze$ is the electric charge ($Z$, charge number; $\varepsilon = 1.62 \times 10^{-19}$ Cb), $r$ is the radius of the species and $\mu$ the dynamic viscosity of the solution. If one assumes that the solute is spherical an equivalent radius $r$ is estimated as follows:

$$r = \left[ \frac{3Mr}{4\pi \rho N} \right]^{1/3} (N = 6.023 \times 10^{23})$$  \hspace{1cm} (6)

where $M_r$ is the molecular mass and $\rho$ the density. Substituting for $U$ and $r$ in equation (4) gives:

$$Tr = Tr_{UF} \left[ 1 + \frac{E \cdot Z e}{J \cdot \frac{4\pi \rho N}{3Mr}^{1/3}} \right]$$  \hspace{1cm} (7)

where $Tr_{UF} = \phi$ is the experimental transmission.

Equation (7) suggests that the parameters $Ze$ and $M_r^{1/3}$, $E$ and $J$ contribute to the selectivity of the separation. Furthermore, $E/J$ accounts for a competition between convection (proportional to $J$) and electrophoretic migration (proportional to $E$).

**MATERIALS AND METHODS**

**Electro-filtration rig**

Experiments were carried out on a laboratory filtration rig equipped with Carbosep membranes (0.2 m long, 6 mm inner diameter and 0.38 x 10^-2 m² membrane area) kindly provided by TechSep (Miribel, France). The membranes used were an M5 (MMCO = 10 kDa) and an M1 (MMCO = 70 kDa). They were made of a zirconium oxide layer on a microporous carbon support. Additional
experiments were performed using a carbon support (M14 Carbosep Membrane not covered by a zirconium oxide layer) (pore diameter 2 μm) and a 3065 Iris PVDF flat organic membrane (MMCO = 40 kDa).

An electric field under constant potential difference between two electrodes was applied using a DC electric power supply.

With the tubular module equipped with inorganic membranes the anode consisted of a platinum wire of 0.8 mm diameter stretched along the axis of the membrane tube, while the carbon support was used as the cathode. The carbon tube was used as the cathode because it was observed that the ZrO2 layer was damaged when carbon was used as the anode.

With the plate-and-frame EF module equipped with the flat organic membrane, the two flat electrodes made of titanium and the circulating solution of 0.1 mol l⁻¹ NaCl in the electrode compartments were separated from the retentate and permeate by using electrodialysis membranes. It was then possible to keep the retentate pH constant at pH 7.2 throughout the run. The distance between the electrodes was 50 mm. In all electrofiltration (EF) runs the average electric field ranged between 0 and 9 600 V m⁻¹.

A transmembrane pressure (TP) was set between a few millibars and 1.0 bar depending on the membrane in order to get permeation flux in the range of 10⁻⁵ to 10⁻³ l m⁻² h⁻¹. Pressure was measured by a gauge located at the membrane tube outlet. The pressure was representative of the average pressure in the retentate side owing to low pressure drop along the membrane module (0.02 bar). The permeate was not recycled, and it was accounted for in the concentration calculation.

Solution temperature was maintained at 25 ± 0.1°C. With the tubular membranes tangential flow rate was 1 m s⁻¹ (Re = 6 500) monitored by an electromagnetic flowmeter (Altoflux, Krohne, Romans, France; 1% accuracy). Each experimental feed was first ultrafiltered and the results served as a reference for the EF run.

Feed

Amino acid solution

Two liters of an aqueous solution of amino acids (AA) mixture were used for each experiment (concentrations are given in table I). The amino acids (Sigma Chemical Co, St Louis, MO, USA) are soluble at pH 7.5 at 25°C. The initial pH was adjusted to 7.5 by 0.1 N NaOH. However, during EF runs with the inorganic membranes, the pH of the permeate and the retentate changed, so that a pH gradient existed across the membrane. An UF run (without applied electric field) carried out with an amino acid solution adjusted to pH 4.5 by 0.1 N HCl, and a permeate solution at pH 12.0, representative of final EF pH, showed that the membrane selectivity was not altered by these pH variations.

The conductivity of a solution of 3 amino acids (arginine, aspartic acid and leucine) was 260 μS cm⁻¹, compared to 700 μS cm⁻¹ with a 7 amino acids solution.

Peptide solution

The physicochemical characteristics of the peptides from a casein trypsic hydrolysate at pH 7.0 (Nau et al, 1993) and at a conductivity of 830–1 500 μS cm⁻¹, are given in table I. The net charge number, Z for any experimental pH was calculated from the number of positive and negative amino acids in the peptide (Skoog and Wichman, 1986).

Analytical methods

Amino acids analysis was performed by ion chromatography (2.5% accuracy) using a Beckman analyser (Spackman et al, 1958). Peptide analysis (5% accuracy) was performed using an FPLC (C18 column, pepRPC, Pharmacia, Uppsala, Sweden). The elution gradient was: buffer A (0.02 mol l⁻¹ Na phosphate, pH 6.75) buffer B (0.02 mol l⁻¹ Na phosphate (40%); acetonitrile (60%); pH 8.25).

Expression of results

Amino acid and peptide concentrations in the permeate, (Cp) and in the retentate, (Cr) were measured as areas of the chromatogram peaks. Transmission, Tr, was calculated according to: \( Tr = \frac{C_p}{C_r} \) (experimental error, 10%).
Table 1. Characteristics of amino acids and of peptides.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Molecular mass (Da)</th>
<th>pl</th>
<th>Total hydrophobicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>133.1</td>
<td>2.98</td>
<td>0.54</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>181.2</td>
<td>5.63</td>
<td>2.85</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>165.1</td>
<td>5.91</td>
<td>2.00</td>
</tr>
<tr>
<td>Leucine</td>
<td>131.2</td>
<td>6.04</td>
<td>2.40</td>
</tr>
<tr>
<td>Histidine</td>
<td>155.2</td>
<td>7.64</td>
<td>?</td>
</tr>
<tr>
<td>Lysine</td>
<td>146.2</td>
<td>9.47</td>
<td>1.50</td>
</tr>
<tr>
<td>Arginine</td>
<td>174.2</td>
<td>10.76</td>
<td>0.75</td>
</tr>
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</table>

Peptide

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Molecular mass (Da)</th>
<th>pl</th>
<th>Total hydrophobicity</th>
</tr>
</thead>
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<tr>
<td>βCN1–25</td>
<td>3127</td>
<td>2.00</td>
<td>21.05</td>
</tr>
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<td>βCN33–48</td>
<td>2064</td>
<td>3.47</td>
<td>7.00</td>
</tr>
<tr>
<td>βCN1–25 b</td>
<td>2803</td>
<td>3.91</td>
<td>21.05</td>
</tr>
<tr>
<td>βCN184–202</td>
<td>2187</td>
<td>4.11</td>
<td>29.65</td>
</tr>
<tr>
<td>βCN114–169</td>
<td>6363</td>
<td>5.18</td>
<td>73.85</td>
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<td>αCN194–199</td>
<td>748</td>
<td>5.75</td>
<td>10.20</td>
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<td>βCN203–209</td>
<td>742</td>
<td>6.05</td>
<td>15.45</td>
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<td>βCN100–105</td>
<td>646</td>
<td>6.97</td>
<td>6.90</td>
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<td>βCN108–113</td>
<td>748</td>
<td>6.97</td>
<td>10.65</td>
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<td>αCN29–32</td>
<td>490</td>
<td>7.30</td>
<td>6.95</td>
</tr>
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<td>βCN49–97</td>
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<td>7.79</td>
<td>75.55</td>
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<td>9.85</td>
<td>21.45</td>
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<td>βCN177–183</td>
<td>830</td>
<td>10.00</td>
<td>11.25</td>
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<tr>
<td>βCN170–176</td>
<td>780</td>
<td>10.10</td>
<td>12.50</td>
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a Calculated from Bigelow (1967)
b Dephosphorylated

Cleaning procedure

Before any run the rig with the membrane was cleaned using the following procedure: NaOCl containing 1 g l⁻¹ active chlorine after adjustment of pH to 11 with 3.0 mol l⁻¹ NaOH, 15 min; 5 min rinsing with water; 0.05 mol l⁻¹ HNO₃, 15 min; final rinsing with water for 5 min. The initial water flux (Jw) was recorded after 15 min of distilled water UF. Cleaning, rinsing and water flux procedure were performed at 25°C with a transmembrane pressure of 1.0 bar with a tangential flow rate of 1 m s⁻¹, using 0.2 μm filtered tap water.

RESULTS

Amino acids

During ultrafiltration the amino acid concentration in the retentate and in the permeate was almost similar and very close to that of the initial solution, regardless of the pH of the retentate (4.0 or 7.5) and of the permeate (7.5 or 12.0). The transmission of every AA was almost 1.0, indicating no significant adsorption or retention.

During EF, permeation fluxes were ~ 3% higher than those measured for ultrafiltration and varied a little from one run to another. In the absence of pressure and in the presence of an electric field a solvent shift towards the permeate side due to electrosmosis was observed.

The composition of the streams produced by EF were significantly modified as compared to the initial solution: the arginine concentration in the permeate was higher, whereas aspartic acid and leucine concentrations were lower as illustrated by a transmission of Arg > 1 and of Asp and Leu < 1 (figs 1, 2). Lysine behaved the same way as arginine. Tyrosine, phenylalanine and histidine behaved the same way as leucine. According to pl values given in table 1, the interfacial pH is between 7.6 and 9.7. On the other hand, the concentration of aspartic acid in the retentate increased with time, while that of arginine and lysine decreased. Moreover, the retentate pH exhibited a sharp decrease during the first 10 min, from 7.5 to 5.0, and then decreased slowly down to 4.0, while that of the permeate increased rapidly up to 12.0–13.0.
 Whereas in UF AA losses were lower than the detection sensitivity, they were significant in some EF runs. They ranged around 5% in most runs and for most amino acids, except for Asp (17% after 8 h) and for Tyr (30% after 1 h).

Figure 2 shows the experimental transmission versus the predicted transmission calculated according to eq (7).

Peptides

With Carbosep membranes, peptide transmission in UF varied in the range 0.01 to 0.95 with M1 and M5 membranes. It was significantly affected by the electric field particularly for alkaline peptides βCN (177–183) and βCN (170–176) (Tr > 10 with the M1 membrane) (table II). The results are outlined in figures 3 to 6, which represent the experimental transmission in EF, Tr_{exp} versus the transmission calculated according to eq (7), Tr_{cal}. When using an M14 carbon support with no electrical field the transmission was approximately 1.0 with all the peptides irrespective to their size and charge.
Table II. Transmission of peptides of a casein hydrolysate in filtration, F and electrofiltration, EF (calculated after 90 min with M14, M1, M5 and 60 min with 3065) at 25°C.

Transmission des peptides d’un hydrolysat trypsique de caséine en filtration, F et en électrofiltration, EF (calculée après 90 min pour M14, M1, M5 et 60 min pour 3065) à 25°C.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>$M_r$</th>
<th>$pI$</th>
<th>$T_F$</th>
<th>$T_{EF}$</th>
<th>$T_F$</th>
<th>$T_{EF}$</th>
<th>$T_F$</th>
<th>$T_{EF}$</th>
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<tbody>
<tr>
<td>βCN1-25</td>
<td>3127</td>
<td>2.00</td>
<td>1.01</td>
<td>0.72</td>
<td>0.18</td>
<td>1.11</td>
<td>0.13</td>
<td>1.73</td>
<td>-</td>
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<td>βCN33-48</td>
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<td>-</td>
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<tr>
<td>βCN1-25 *</td>
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<td>3.91</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>βCN184-202</td>
<td>2187</td>
<td>4.11</td>
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<td>0.88</td>
<td>-</td>
<td>-</td>
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<td>10.90</td>
<td>0.32</td>
<td>7.39</td>
<td>0.33</td>
<td>0.00</td>
</tr>
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</table>

$E$ (V m$^{-1}$) 0 330 0 9600 0 9000 0 9680
$J$ (l h$^{-1}$ m$^{-2}$) 66.3 12.4 24.8 3.2 9.4 6.7 33 55
pH 7.5 7.5 6.85 4.6 6.85 4.6 7.2 7.0
$r^2$ 0.68 0.81 0.95 0.27

$r^2$, linear regression coefficient of $T_{exp} = aT_{cal} + b$. * Dephosphorylated.

DISCUSSION

Amino acids

Ultrafiltration of amino acid solutions at constant pH (7.5) using a Carbosep M5 membrane is not selective at all as expected because of the low molecular mass of amino acids (< 200 Da) relative to the MMCO of the membrane (10 kDa).

Results obtained in UF were similar irrespective of the presence of a pH gradient. Therefore, it may be concluded that the selectivity of the EF process is actually due to the electric field.
to its electric field as well as the difference in electrophoretic mobility. Consequently, the EF process applied to a multiple amino acid solution led to the production of selectively enriched fractions: an arginine and lysine enriched permeate and an aspartic acid enriched retentate.

The transmission of arginine ($T_r = 1.5 - 15$) is to be compared to 0.7–0.95 for isoleucine, reported by Kimura and Tamano (1984) with a charged polysulfone membrane. Aspartic acid transmission was similar in both reports, 0.2–0.3. The permeation fluxes (30 l h$^{-1}$ m$^{-2}$) of the present experiments were higher than those reported by Lee and Hong (1987) (7.5 l h$^{-1}$ m$^{-2}$ in EF) and Kimura and Tamano (1984) (3–30 l h$^{-1}$ m$^{-2}$ with charged UF membranes).

Changes in pH of the permeate and retentate are due to water electrolysis and losses of amino acids can be explained by adsorption and anodic oxidation of amino acids (Vijh and Conway, 1967; Bogdanovskaya et al., 1986).

The use of hydraulic separation of the electrodes from the permeate and from the retentate (Radovich and Sparks, 1980; Radovich and Behnam, 1983; Lee and Hong, 1988; Aimar et al., 1989; Vivoni-Assice, 1989) did not entirely avoid these undesirable phenomena (Kerhervé et al., 1991). In figure 2

![Fig 4](image1)

**Fig 4.** Experimental transmission, $T_{exp}$ versus the predicted transmission calculated according to eq (7); $T_{cal}$: peptides; M1 membrane.

$T_{exp} = 0.08 T_{cal} - 1.33, r^2 = 0.81.$

![Fig 5](image2)

**Fig 5.** Experimental transmission, $T_{exp}$ versus the predicted transmission calculated according to eq (7); $T_{cal}$: peptides; M5, membrane (calculated without taking into account βCN 49-97 and βCN 100-105).

Transmission expérimentale, $T_{exp}$ en fonction de la transmission calculée selon l'eq (7); $T_{cal}$: peptides, membrane M5 (calculée sans prendre en compte βCN 49-97 et βCN 100-105).

$T_{exp} = 0.32 T_{cal} + 0.57, r^2 = 0.95$

![Fig 6](image3)

**Fig 6.** Experimental transmission $T_{exp}$ versus the predicted transmission calculated according to eq (7); $T_{cal}$: peptides; 3065 membrane.

$T_{exp} = 0.09 T_{cal} + 0.82, r^2 = 0.27.$
the results have been plotted according to equation (7). The data can be divided into two groups: (Arg, Lys and Asp) and the other AAs. The transmission of Arg, Lys and Asp seems to be controlled by: (i) electrophoretic migration; (ii) flux; (iii) the pH of the experiment. Nonetheless with Arg and Lys the model as described by eq (7) did not fit tightly \( r^2 = 0.75 \). One of the assumptions which could explain this is that we did not take into account the effect of the variation of conductivity of the retentate (increase of proton concentration, modification of composition, etc) which occurred during EF. For other amino acids, the pl of which is close to 5.8 or to 7.6 (run at pH 7.5), the electrophoretic migration did not control the transmission. For those of which the pl is close to the pH of the run, a transmission of around 1 was measured, as in standard UF. Corrections are to be found for Phe, Tyr and Leu which do not fit the model satisfactorily, since they are predominant under the zwitterion form (as it is expected from their pl and pK values) at the final retentate pH value of 4.15. A minimum \((pH - pl)\) difference should be assessed for each amino acid, which would allow it to be either retained or transmitted into the permeate. At last, provided that there is no rejection in UF due to well chosen membrane and operating conditions, it can be expected that in EF: i) the higher E/J and/or the higher \((pH - pl)\), the higher the transmission; and ii) the retentate pH should be controlled at an appropriate value between the pl values of the two groups of charged molecules which are to be separated.

**Peptides**

The major difference with respect to amino acid separation is the retention of peptides in UF. It was explained by a size exclusion phenomenon with a high ionic strength solution combined with electric interactions in lower ionic strength solutions (Nau et al, 1993; Kerhervé et al, 1993). In EF, transmission is tremendously enhanced for the peptides characterized by pl higher than the pH of the solution, \( \beta CN \ (170-176) \) and \( \beta CN \ (177-183) \), as shown with amino acids.

Accelerated transfer of peptides due to electrophoretic migration in the reverse direction was confirmed using a plate-and-frame module: alkaline pl peptides, \( \beta CN \ (170-176) \) and \( \beta CN \ (177-183) \) did migrate towards the anode as expected and their transmission was 0.00.

Further experiments using electrically polarized carbon support of an M14 membrane demonstrated that the transfer of peptides of various pl (\( \beta CN \ (1-25) \ pl = 2 \) or 3.91 according to its phosphoryl group number; \( \beta CN \ (33-48) \ pl = 4 \); \( \beta CN \ (177-183) \ pl = 9 \) was significant, even with an electric field as low as 330 V m\(^{-1}\) and it changed migration orientation by reversing polarity. The results suggested that such a process would enable the selective separation of peptides according to their charges, whether positive or negative, either in the permeate or in the retentate.

However, hydrophilic characteristics may affect peptide behaviour under an electric field as with \( \beta CN \ 49-97 \) and \( \beta CN \ 114-169 \), which are highly hydrophobic and are retained much more than they are expected owing to their molecular mass and charge.

The model according to eq (7) was satisfactory since it forecasted whether the peptide transmission is larger than 1 (enrichment in the permeate) or close to 0 (enrichment in the retentate). Nonetheless, the model is far from allowing the determination of quantitative results. Again the solution pH conductivity and its variation during EF process have to be accounted for, and we should consider that under an applied electric field, the value of \( T_{r, \text{UF}} \) (measured with \( E = 0 \)) might induce discrepancies since several physico-chemical conditions and phenomena would not be alike: concentration polarisation, electric or hydrophobic interactions (peptide-peptide; peptide-mem-
brane; etc) which depend on ionic strength and pH (Nau et al, 1995). This would account for a better agreement when TrUF is lower (support of M14 compared to 3065; M1 and at last M5) and the electric field smaller.

CONCLUSION

Provided that there is no retention by the membrane, the main transport phenomena controlling the EF separation of small charged molecules, such as amino acids or peptides, are convection and electrophoretic mobility at the membrane-fluid interface. In this region, and because the membrane itself was used as an electrode, the pH was altered by water electrolysis. When the pl was close to the interfacial pH (± 2), almost no selectivity due to the electrical field was observed. Otherwise, a selective enrichment, both in retentate and permeate, was obtained and was fairly modelled by a single equation.

Larger molecules such as peptides when bearing significant positive charges (pl higher than 9.00 in a solution of pH around 7.0), were actively transmitted or retained due to their high electrophoretic mobility, resulting in permeate or retentate enrichment according to the direction of the electric field as referred to UF convection. Nevertheless, the above model was inadequate for quantitative account for peptide filtration experiments likely because of peptide-membrane interactions (sieving, hydrophobic and electrostatic attraction or repulsion).

The present study shows that EF may be a useful and efficient process for achieving selective separation of charged biological molecules, for example βCN 170–176. Improvements are needed regarding the EF module (insulation of electrodes in order to be able to control the pH value), process variables (lower electric field with regard to filtration flux), membrane (adequate rejection during filtration, conductive material) and modelling, which should take into account more accurately the relative characteristics of the membrane and the molecules to be separated and their interactions.

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