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ESIEX – electrical swing ion exchange: pH control for biomolecule purifications or separations

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A B S T R A C T

Due to their amphoteric properties, biomolecules such as amino acids, peptides and proteins are separated as a function of their charges. A classical method to separate these biomolecules is column ion exchange with pH steps. The proper pH is usually obtained in a chemical way with different buffers of variable dilutions, and this is the source of large amount of effluents. The objective of this work is to study the possibility to control the pH in an electrodeionization (EDI) process without using buffer solutions.

EDI process is a hybrid technique combining electrodialysis and ion exchange (IEX). In EDI, three types of phenomena are likely to generate protons and hydroxyl ions and to play a role in pH: formation by water oxidation or reduction at electrodes, water splitting and IEX. Two parameters play a role in these processes: the choice of current density and the nature of IEX resins (anion, cation or mixed).

To purify a molecule, it is for instance possible to:

–keep key molecule in feed compartment and generate the other species migration: the choice of resin type and of current density must allow maintaining the pH at isoelectric point of key molecule

–generate selective migration of key molecule through an ion exchange membrane: pH must allow this molecule to have the required charge

–fix selectively key molecule on ion exchange resin. The experimental method realized in the present work specifically aims at identifying the coupling of processes for a dipeptide fixation on an ion exchange resin placed in one compartment of electrodialysis with bipolar membrane system.

Keywords: Ion exchange; Electrodeionization; Amino acids; Peptides; Bipolar membrane

1. Introduction

In amino acids and peptides production industry, the most used methods are fermentation and protein hydrolysis. These production steps are followed by separation or purification steps. Because of their amphoteric properties, these molecules are separated as a function of their charges. The classical method to purify or separate these molecules is column ion exchange using pH changes. Different buffers of variable dilutions are classically used to obtain the required pH, which is in a chemical way, and this is the original source of large amount of effluents. The objective of this work is to investigate different methods and to study

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Table 1
Properties of used ion exchange membranes

Name	Company	Type	Thickness (mm)	Resistance (ohm × cm^2)
AMX	NEOSEPTA	Strong anionic	0.12–0.18	2.0–3.5
CMX	NEOSEPTA	Strong cationic	0.14–0.2	1.8–3.8
BP-1E	NEOSEPTA	Bipolar	0.22	–

the possibility to allow controlling pH in an electrodeionization (EDI) process without using buffer solutions.

2. Processes influencing the pH in EDI

In EDI, three types of phenomena are likely to generate protons and hydroxyl ions, and so to play a role in pH: formation by water oxidation or reduction at electrodes, water splitting and ion exchange (IEX). We will describe these phenomena by presenting successively conventional electrodialysis (ED), electrodialysis with bipolar membrane (EDBM) and EDI.

The properties of ion exchangers (membranes and resins) used in this study are given in Tables 1 and 2. The experimental cell used is home made. This cell is adjustable and can include two to seven compartments, formed by independent blocks. The efficient area of membranes is 40 cm^2. The thickness of central compartments is 15 mm. The electrode compartments contain platinised titanium electrode in form of a grid (mesh = 3 mm) of a surface of 60 cm^2 and a thickness of 15 mm.

2.1. Conventional electrodialysis (ED)

When working in ED beyond limiting current density, water splits on the surface of ion exchange membrane on dilute side, where ion concentration is depleted during operation, to provide additional protons and hydroxyl ions to transport electrical current [1]. As a result, there are an acidification on cathode side and an alkalization on anode side of membrane. It should be noted that these pH changes risk damaging ion exchange membrane in an irreversible way. In ED, we usually work below limiting current density.

2.2. Electrodialysis with bipolar membrane (EDBM)

A bipolar membrane is composed of two IEX layers: one is cationic, the other is anionic. Bipolar membrane allow amplifying water splitting phenomenon at the interface of two ion exchange layers, according to a mechanism of protonation–deprotonation accelerated by the presence of a catalyst. Thanks to the particularity of these membranes to produce protons and hydroxyl ions by water splitting under the influence of electrical field, EDBM is an effective process to acidify or alkalize solutions avoiding using chemicals and minimizing effluent generation. It is widely used in fruit juices and others solutions treatment in food industry. Several operations of purification, of pH stabilization or acid conversion, without using or producing effluents, are feasible [2–5].

To study the possibilities of pH control with this system, an experimental work has been carried out with a cell illustrated in Fig. 1. This cell is composed of five compartments. A bipolar membrane is placed between compartment N°3 and N°4. Under electrical current, it produces protons in compartment N°3 and hydroxyl ions in compartment N°4. pH changes in these two compartments are monitored over time until steady state for a given current density. Experiments have been performed at different current densities. Salts solutions circulate in single pass in all the compartments.

pH changes at steady state as a function of current density are shown in Fig. 2. pH in compartment N°4 increases as a function of current density because of an alkalization of solutions by bipolar membrane, while pH in compartment N°3 decreases because of an acidification of solutions. From these pH values, molar flux of protons and hydroxyl ions are calculated

Table 2
Properties of used ion exchange resins

Name	Matrix	Porosity	Type	Capacity (meq/mL)	Specific gravity (g/L)	Maximum temperature (°C)
AMBERLITE IRA-900	Styrene DVB	Macroreticular	Anion (strong base)	1.0	670	60 (OH) 77 (Cl)
AMBERLITE 200	Styrene DVB	Macroreticular	Cation (strong acid)	1.75	800	145

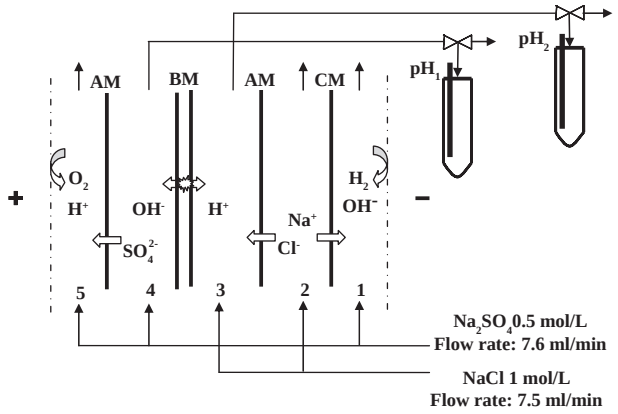

Fig. 1. Experimental electrodialysis cell with bipolar membrane. (CM: cationic membrane, AM: anionic membrane, BM: bipolar membrane, –: electrodes).

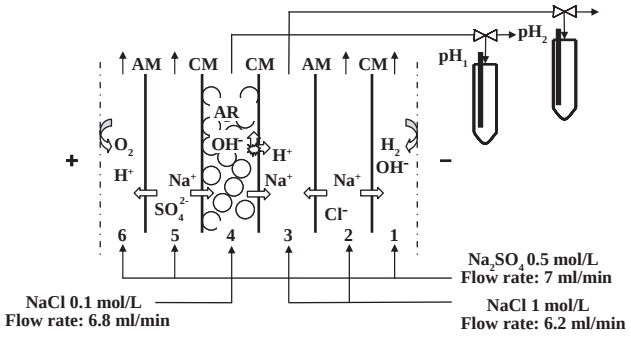

Fig. 3. Experimental system coupling electrodialysis and ion exchange (CM: cationic membrane, AM: anionic membrane, AR: anion exchange resin).

and represented as a function of current density in the same figure. We can see that molar flux of protons and hydroxyl ions have almost the same values when current density is below 0.035 A/cm^2. At higher current density molar flux of hydroxyl ions is less than that of protons. The reason is that some hydroxyl ions migrate through anionic membrane. Also shown are the theoretical values of molar flux calculated by the ratio of current and Faraday constant (96,500 C/eq). We can see that the molar flux obtained experimentally is a bit less than the theoretical values.

2.3. EDI

2.3.1. Water splitting

As seen previously, in conventional electrodialysis, water splitting is generated by too low ion concentration to transport current. It seems that water splitting is promoted by the contact of ion exchangers with opposed charges, phenomenon used in bipolar membrane.

The idea is to see if this phenomenon can be developed by realizing a contact between a homopolar membrane and ion exchange resins.

To understand these phenomena, an experimental study has been performed. The experimental set-up is illustrated in Fig. 3. The cell is composed of six compartments. An anion exchange resin bed has been placed in one compartment (N°4) of ED system and in contact with two cationic membranes. Salts solutions circulate in single pass in all the compartments. We should note that the concentration of solution in compartment N°4 is ten times less than the others to enhance water splitting. Water splitting occurs on contact points of anion exchange resin and cationic membrane and produces hydroxyl ions and protons. Protons migrate through cationic membrane and lead to a pH drop in compartment N°3. pH changes in compartment N°3 and N°4 are monitored over time for each current density until steady state.

A classical method has been used to measure limiting current density: it consists in drawing the cell resistance (calculated as U/I) as a function of the inverse of current 1/I. The particular appearance of curve allows determining the value of limiting current density from the intersection of two tangents. Fig. 4 shows the comparison of three different systems: ED with anion exchange resin (noted CM + AR) and without ion exchange resin (noted CM), and electrodialysis with bipolar membrane (noted BM). If we compare CM + AR system and CM system, we can see that limiting current density is lower in the case of CM + AR, that is to say, water splitting occurs easier in CM + AR system. For BM system, the curve doesn't have the same appearance. So we can not read limiting current

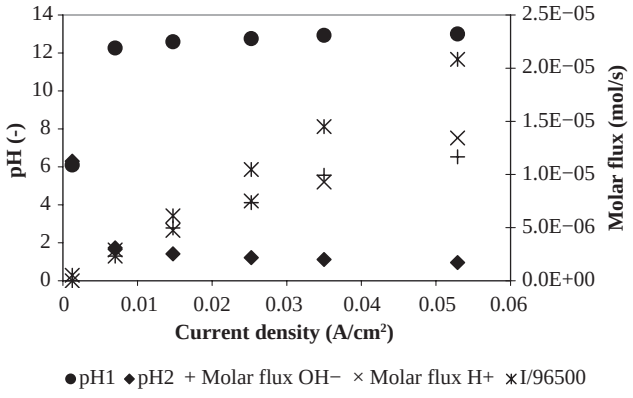

Fig. 2. pH variations at steady state and molar flux of OH^- and H^+ ions as a function of current density using electrodialysis with bipolar membrane cell.

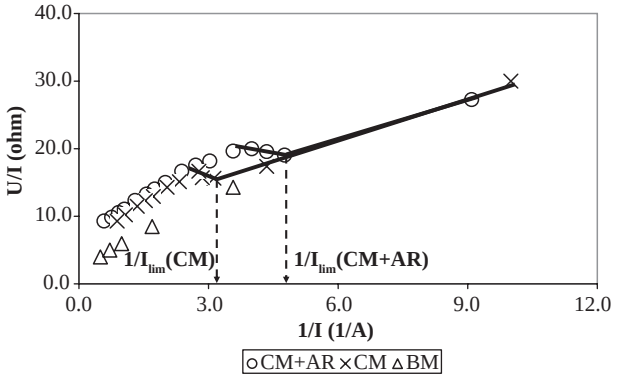

Fig. 4. Determination of limiting current density with different systems: electrodialysis and anion exchange resin, conventional electrodialysis, electrodialysis with bipolar membrane (CM: cationic membrane, BM: bipolar membrane, AR: anion exchange resin).

density for this system. The reason may be that water splits with a very low current density and that we can not observe it with our equipment.

Fig. 5 illustrates obtained pH values at steady state in compartments N°3 and 4 as a function of current density. This figure compares the results obtained in presence (noted CM + AR) and in absence (noted CM) of anion resin bed. As expected, we observe a pH increase in compartment N°4 and a pH decrease in compartment N°3 for both of the cases. However, if we compare pH changes in compartment N°4 for the two cases, we can see that pH increases more quickly and higher with the presence of anion resin bed. The same phenomenon has been observed in compartment N°3. We can conclude that, for the same current density, the presence of anion resin bed generates a stronger alkalization or acidification, so a stronger water splitting.

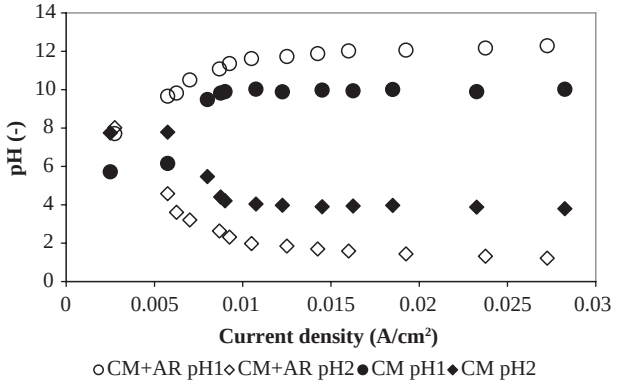

Fig. 5. pH variation as a function of current density at steady state with coupling system of electrodialysis and ion exchange comparing to that of conventional electrodialysis system (AR: anion resin, CM: cationic membrane).

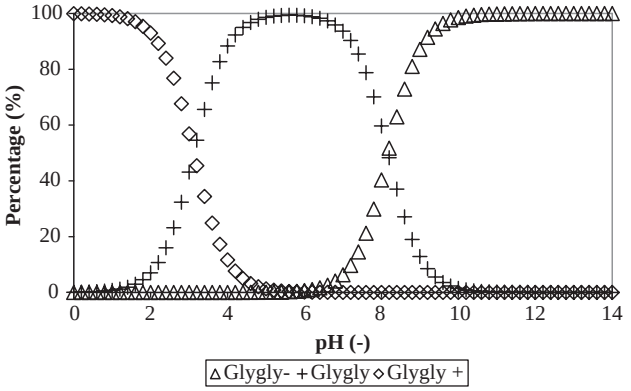

Fig. 6. Distribution of different species of Glycylglycine as a function of pH.

2.3.2. IEX

IEX, used alone, can modify the pH of solution by the following ways: anion resin initially under hydroxyl form, release OH^- during IEX, whereas cation resin, initially under proton form release H^+; It is therefore well known that a percolation of saline solution, on one or the other type of resin, will modify the pH of solution.

We should note that once resin bed is saturated we have to regenerate it, while in EDI, resin bed is regenerated by water splitting in electroregeneration regime [6].

3. pH control for biomolecules purification or separation

3.1. 1st case: key molecule remains in feed compartment while the other species migrate

To avoid the migration of key molecule, pH should be maintained at isoelectric point of this molecule. For this goal the parameters are the choice of current density and the type of ion exchange resin.

Eliseeva et al. [7] have, on this principle, realized the demineralization of a basic amino acid solution. They have used a mixed resin bed and studied the influence of ratio of anion resin to cation resin on pH value as a function of current density. They have observed that for a given current density pH increases when the fraction of anion resin increases, and for a given ratio of anion resin to cation resin pH increases with current density.

The choice of resins ratio and of current density allowed them to adjust pH value to the isoelectric point

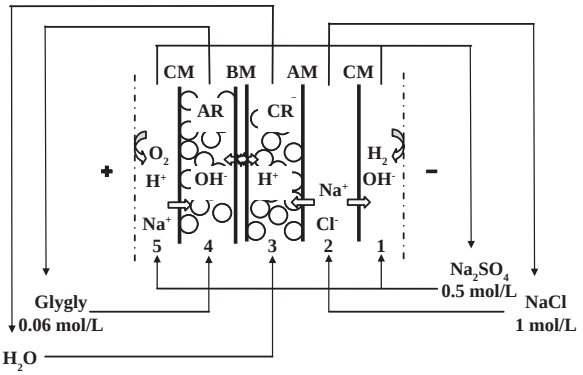

Fig. 7. Schema of EDBM/IEX coupling cell for dipeptide fixation (CM: cationic membrane, AM: anionic membrane, BM: bipolar membrane, AR: anion exchange resin, CR: cation exchange resin).

of amino acid. During the operation, demineralization is realized with a low loss of key product. The range of pH is controlled from 8.5 to 10.

3.2. 2nd case: key molecule migrates selectively through ion exchange membrane

Grib et al. [8] have studied extraction of amino acid with an EDBM system. Amino acids are introduced in their zwitterionic form. Hydroxyl ions produced by bipolar membrane allow amino acids to convert to their anionic form. Thus they can migrate through an anionic membrane. The authors have shown that initial pH and the value of current density must be optimized in order to have the required concentration of hydroxyl ions: this concentration must be sufficient to convert all the amino acids to their anionic form, but must not be too high to lead to a transport competition between amino acids and hydroxyl ions, which have a higher mobility.

3.3. 3rd case: key molecule is selectively fixed on ion exchange resin bed

In order to identify the coupling of processes, we have studied experimentally pH changes when a dipeptide is fixed on an ion exchange resin bed, filled in one compartment of an ED system with bipolar membrane. This study will be presented in the next part.

4. Experimental study of pH changes of a dipeptide solution in an EDBM/IEX coupling system

4.1. Method and materials

The experimental cell is composed of five compartments (Fig. 7). Compartment N°3 is filled with a cation exchange resin while compartment N°4 is filled with an anion exchange resin. During experiments a 0.5 mol/L Na_2SO_4 solution circulates in electrode compartment N°1 and N°5 and a 1 mol/L salt solution circulates in compartment N°2. Demineralised water circulates in compartment N°3. The dipeptide Glycylglycine (Glygly) has been selected for this study. The species distribution as a function of pH is illustrated in Fig. 6. Properties are given in Table 3. A 0.06 mol/L Glygly solution circulates with a flow rate of 7 ml/min in compartment N°4. All the solutions circulate in batch mode. A pH probe allows monitoring pH changes of Glygly solution over time. Some samples have been collected over time to measure Glygly concentration by spectrophotometry at 214 nm.

4.2. Results and discussion

Fig. 8 shows the results for the pH and concentration changes of Glygly solution as a function of time for different values of current density. Generally, an increase of pH and a decrease of concentration have been observed. Two current densities have been used: 0.0125 A/cm^2 and 0.025 A/cm^2, that is currents of 0.5 A and 1 A respectively. It can be observed that when current density is increased pH increases more quickly. The distribution of different species of Glygly corresponding to pH over time is illustrated in Fig. 9 in the case of 0.5 A.

The initial pH of Glygly solution is about 4.5. At this value Glygly is almost under neutral form (Glygly), as seen in Fig. 9. During experiment, pH increases over time due to two phenomena: the generation of hydroxyl ions by bipolar membrane and the release of hydroxyl ions initially fixed on ion exchange resin bed during IEX.

Table 3
Properties of Glycylglycine

Dipeptide	M	pK_C	pK_N	pI	Formula
Glycylglycine	132.1	3.12	8.17	5.71	$NH_3^+CH_2CONHCH_2COO^-$

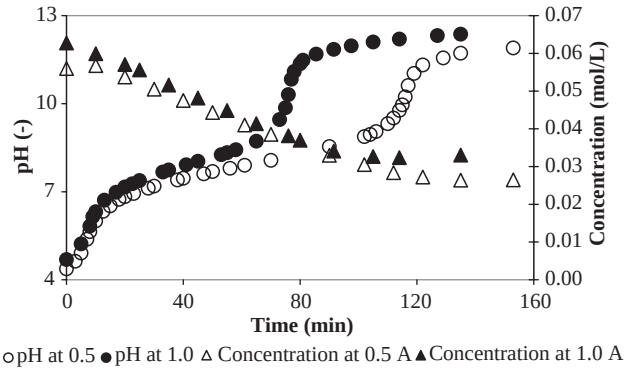

Fig. 8. pH and concentration changes of Glygly solution as a function of time during its fixation with the EDBM/IEX cell.

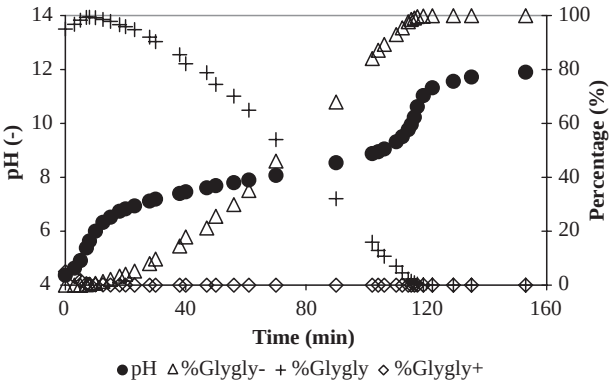

Fig. 9. Distribution of the different species of Glygly and corresponding pH values as a function of time in the case of $I = 0.5$ A during Glygly fixation with the EDBM/IEX cell.

Hydroxyl ions produced react firstly with Glygly in solution to form its anionic species. When the concentrations of neutral form (Glygly) and of anionic form ($Glygly^-$) are closed, we can observe a pH plateau with a value whose order of magnitude is pK_N of Glygly, that is to say, about 8.

When almost all the Glygly is in its anionic form, hydroxyl ions produced by the two phenomena mentioned above lead to a pH increase until more than 12.

During experiment, the anionic form ($Glygly^-$) fixes on ion exchange resin bed as soon as it is transformed from its neutral form (Glygly). So the global concentration of Glygly in solution decreases over time as shown in Fig. 8. When the hydroxyl ions produced are no more used for the transformation of Glygly to $Glygly^-$, they compete with $Glygly^-$ for fixation on ion exchange resin bed. As a result, we can observe in Fig. 8 that the global concentration of Glygly in solution changes little at the end whatever the current densities. Because of the alkalization of solution a great part of resin remains in OH^- form.

5. Conclusion and perspectives

IEX and electrodialysis with bipolar membrane allow both of them releasing or producing H^+/OH^- ions, and so playing a role in pH. With the coupling of these two processes, a competition occurs between ion transport and equilibrium displacement. The operating parameters, like current density, initial pH and type of ion exchange resin, are linked.

If it is possible to adjust these parameters to obtain a required pH value, we should also note that, in the present study of H^+/OH^- ions generation we did not take into account the notion of buffering capacity, which is important and essential for biomolecules purification or separation. The prospects of this work are to study the potential of different systems, for example, use of weak ion exchange resin, to obtain and take advantage of pH buffering capacity.

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