MEMBRANES IN THE BIOBASED ECONOMY

ELECTRODIALYSIS OF AMINO ACIDS FOR THE PRODUCTION OF BIOCHEMICALS

OLGA KATTAN

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Membranes in the biobased economy

Electrodialysis of amino acids for the production of biochemicals

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DISSERTATION

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Olga María Kattan Readi

born on April 14th, 1981
in San Salvador, El Salvador

This thesis has been approved by:

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“Si 7 veces tropecé por inexperto, imprudente o descuidado,
70 veces 7 me levantaré por perseverante, convencido y apasionado”
Ricardo Velázquez Parquer

A mi abuelo, Erich Werner Jokisch.
Porque la fuerza y la voluntad de no rendirse se llevan dentro.
Porque sonriendo se logran grandes cosas, especialmente dentro de uno mismo.
Porque la rectitud y honestidad nunca deben faltar.
Por su ejemplo.

A mi madre, Margarita Jokisch.

Porque la entrega es incondicional.

Porque la admiración es incomparable.

Porque la gratitud es indescriptible.

Por su amor.

A mis hermanas, Celina y Michelle.

Porque nuestra amistad es única.

Porque su lugar en mi vida es insustituible.

Por darle a mis días un sentido diferente.

Por su apoyo.

A Leticia Mendoza.

Porque la vida no siempre es fácil.

Porque el apoyo de quien nos quiere nunca falta.

Porque la fe en Dios nos mantiene en pie.

Por su amistad.

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1
Introduction

ABSTRACT

The aim of this research is to investigate the potential of membrane technology for the development of a novel process route for the production of biobased chemical intermediates from cheap and renewable protein sources. This chapter gives an introduction to the biobased economy and explores membrane processes that have been studied or have the potential to be applied in the biorefinery concept. More specifically, it reviews the application of electrodialysis for the isolation of biomolecules with focus on amino acids and presents an overview of the currently used methods for the production thereof. At the end, the scope and outline of this thesis are presented.

1.1 Industrial sustainability: Towards the biobased economy

There is no doubt that in the 21st century the transition from an economy that is mainly based on fossil feedstocks towards a biobased economy is needed [1]. The biobased economy can be defined as "the technological development that leads to a significant replacement of fossil fuels by biomass in the production of pharmaceuticals, chemicals, materials, transportation fuels, electricity and heat" [2]. Here technological development obviously refers to the biorefinery concept and it falls within the definition of industrial sustainability [3].

The World Commission on Environmental and Development defines sustainable development as "a process of change in which the exploitation of resources, the direction of investments, the orientation of technological development and institutional change are all in harmony and enhance both current and future potential to meet human needs and aspirations, that is meeting the needs of the present without compromising the ability of future generations to meet their own needs" [4].

Within this concept, more and more attention is paid to orient the technological development towards novel process routes for biobased products [5]. Different biomass sources, their potential and applications have been studied in depth in the past years [6]. For example, the conversion of microalgae to biofuel [7, 8], the application of waste-to-energy technologies where waste streams can be converted into valuable biofuels [9] and the potential of plants such as *Jatropha Curcas* [10-12] to be used as lignocellulosic biomass for the production of biofuels and commodity chemicals [6, 13, 14]. Moreover, on the way towards process intensification, attention is paid to the integration of different potential technologies for sustainable biobased production routes [15].

The way is still long, broad and uncertain. Biobased feedstocks certainly have an enormous potential as sources for industrial chemical intermediates while already existing technologies are successfully applied in conventional refineries. How efficient this and new technologies can be for processing of biobased feedstocks, and, on the other hand, how much the potential of biobased feedstocks would increase if appropriate new technologies are developed, requires investigation. As Lord Kelvin said, "to measure is to know".

1.2 The potential of biobased feedstocks for the production of biochemicals: Towards the biorefinery concept

While fuels and energy can be obtained from renewable resources such as wind, water and sun, chemicals need to start from a carbon source, which can be found in biomass. However, careful evaluation of the potential biomass sources is required to evaluate the potential of biobased feedstocks for the production of biochemicals. For example, byproduct streams, like dried distiller's grains with solubles (DDGS) or vinasse, byproducts of the bioethanol production from maize or grain and from sugar beets or sugarcane, respectively [16] have a relatively high residual protein content. Such feedstocks can be used as cheap amino acid sources and further converted into industrial chemical intermediates.

Different chemicals can be produced from different amino acids. For example, 1,5 – pentanediamine (PDA) can be produced from lysine (Lys) while ethanolamine (Etn), an industrial product used as an intermediate in the herbicide, textile, metal, detergent, plastics, and personal care products industries can be produced from serine (Ser) [17].

The most abundant amino acid in most biobased feedstocks is glutamic acid (Glu) [16]. Glu can be used for the production of γ-aminobutyric acid (GABA), an intermediate for the production of N-methylpyrrolidone (NMP) [17, 18]. For example, the Glu potential in maize and wheat DDGS is 1.8 Mton per year. NMP is worth about 3000 €/ton and its worldwide demand is 100 – 150 kton per year, an amount that could be met considering its production from Glu [16]. Amino acids available in biobased feedstocks, however, are present as a mixture and need to be isolated for the production of specific chemicals. To achieve their isolation, the development of energy efficient separations is needed.

1.3 Membrane technology in the biobased economy: Electrodialysis and its application in amino acid separation

Membrane processes are known to be energy efficient and environmentally friendly, what makes them perfect candidates for industrial sustainability. Membranes offer the selective and efficient transport of specific components and can also improve the performance of reactive processes. One of the greatest advantages of membrane technology is its potential towards process intensification.

Conventional separation processes in industry can be replaced by membrane separations. Usually membranes show a decrease in equipment size (i.e. capital costs), energy savings, safety increase, lower environmental impact and a higher raw materials exploitation [19]. These advantages are without doubt of benefit in biorefineries as well.

Membrane technologies can be applied in a biorefinery in different areas, such as for separation and purification of molecules from biomass, removal of fermentation inhibitors, enzyme recovery from hydrolysis processes, in membrane bioreactors for bioenergy, for the production of chemicals, bioethanol, bio-oil and biodiesel, bioethanol dehydration and last but not least, algae harvesting [20]. For example, combining catalysis, membrane technology and reactor engineering in the development of a bioreactor for biodiesel production overcomes the limitations of the conventional methods, like supercritical technology, such as wastewater generation and high energy consumption [21].

Ultrafiltration and nanofiltration as pretreatment and separation units find their applications in the production of biobased chemicals such as lactic acid and amino acids [22-24]. Hybrid membrane processes have also demonstrated their potential in the biorefinery concept. Recently, Ecker et al. published an overview of the first results of the pilot plant *Green Biorefinery Upper Austria*, where a lactic acid and an amino acid enriched solution are obtained from grass silage juice using a hybrid membrane system [25].

Especially interesting for the recovery of biomolecules is electrodialysis (ED), a membrane process that uses a potential difference as driving force to separate ions from solution. In this process, ions migrate from one compartment (*feed*) through an ion exchange membrane, to another compartment (*receiving*) under an applied electrical potential difference.

The ion exchange membranes (IEMs) contain either fixed positive groups (anion exchange membranes, AEM) or negatively charged groups (cation exchange membranes, CEM). They allow the transport of ions of opposite charge (counter ions), either negatively charged ions (anions) or positively charged ions (cations), respectively, while retaining ions with the same charge (co-ions) [26]. Figure 1.1 shows a schematic representation of the electrodialysis process.

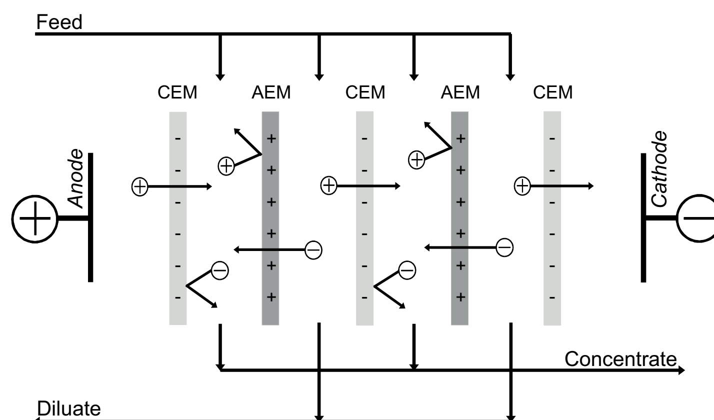

Figure 1.1. Schematic representation of an electrodialysis process.

Especially interesting is the application of electrodialysis for amino acid separation. Amino acids are zwitterionic molecules whose charge is determined by the surrounding pH. The pH at which a particular molecule carries no net charge is the isoelectric point (pI). Electro-membrane processes use an electric field as driving force for the separation and as such can be applied for the isolation of amino acids with different charge behavior [27-41]. The fractionation of a mixture of amino acids with almost identical charge behavior represents a challenge. Enzymatic modification can be applied to specifically modify an amino acid to obtain new molecules with pronounced differences in the iso-electric points and consequently a different charge behavior [18, 42], enabling the further isolation with electrodialysis. Moreover, this approach offers the possibility for process intensification, where simultaneous reaction and further separation take place in one step.

In electrodialysis processes, operation at the highest possible current density is desired to have the maximum ion flux per unit membrane area. Concentration polarization that results from the difference in the transport numbers of the ions in the solution and in the selective membrane restrict the highest applicable current density, and this value is known as the limiting current density (i_{lim}). The limiting current density can be calculated based on Equation 1.1 [43]:

$$i_{lim} = \frac{FD}{\bar{t}_{+} - t_{+}} \cdot \frac{C^b}{\delta} \qquad \text{Eq. 1.1}$$

With i_{lim} the limiting current density [cm^2/mA], F the Faraday constant [96485 A · s/mol], D the diffusion coefficient of the specific ion [m^2/s], C^b the bulk solution concentration of the ion [mol/m^3], $\bar{t}_{+}$ and t_{+} are the transport numbers of the ion in the membrane [-] and in the solution [-], respectively, and δ the boundary layer thickness [m]. Equation 1.1 shows that the

limiting current density will increase with an increase in bulk solution concentration (C^b), an increase in the salt diffusion coefficient (D), a decrease in the transport number through the membrane ($\bar{t}_+$) and a decrease in boundary layer thickness (δ). The i_{lim} can be therefore influenced by changing, for example, the boundary layer thickness, which is mainly determined by the viscosity of the solution, the flow rate of the solution, the cell geometry and the membrane orientation, parameters that all correspond to hydrodynamic conditions. A typical procedure for the determination of the i_{lim} are the recording of current – voltage curves. These curves can be obtained using a six compartment electrodialysis cell with a stack configuration as shown in Figure 1.2.

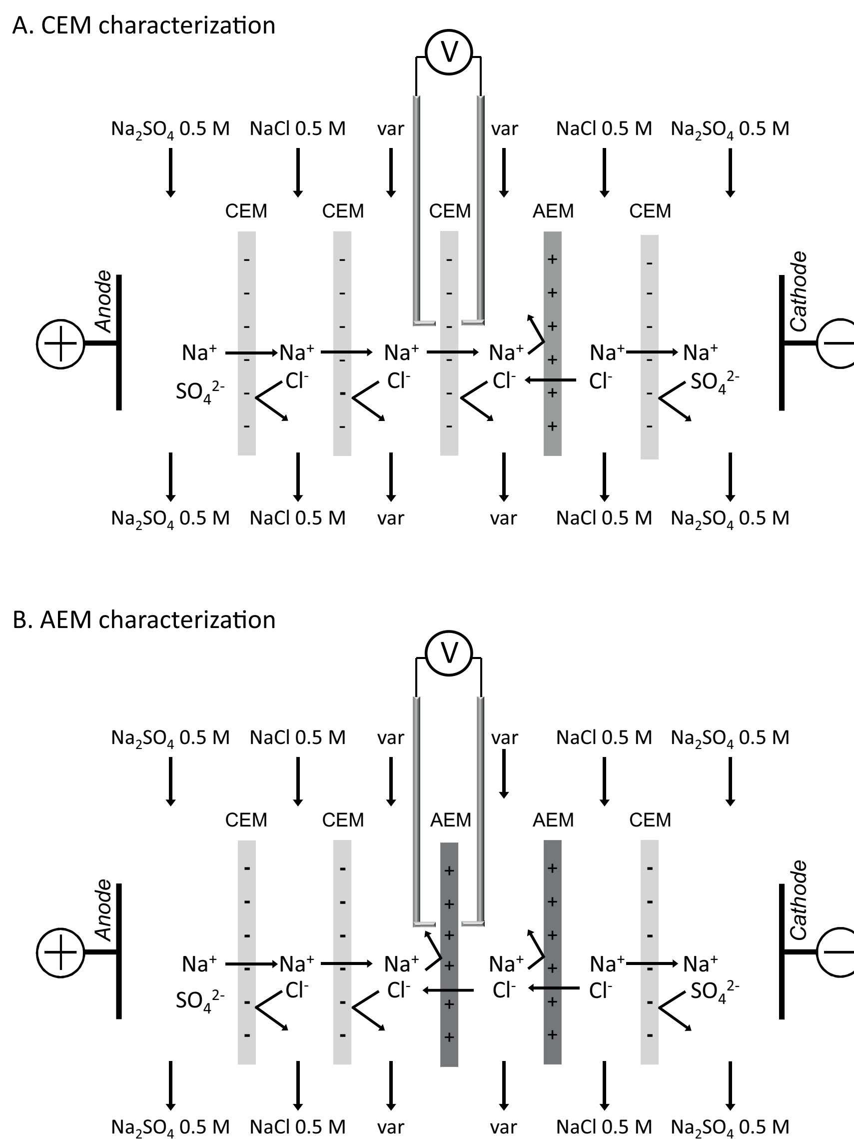

Figure 1.2. Schematic representation of a six compartment measurement module for characterization of ion exchange membranes based on current – voltage curves for determination of the limiting current density [43].

The specific membrane under investigation is the membrane in the middle (CEM in Figure 1.2a and AEM in Figure 1.2b) As can be seen in Figure 1.2, the stack is equipped with auxiliary membranes to prevent the transport of water dissociation products (produced at the working electrodes) towards the compartments adjacent to the membrane under investigation. As electrode rinsing solutions Na_2SO_4 0.5 M is used. NaCl 0.5 M is circulated in compartments 2 and 5 [43]. For the measurement of the limiting current density NaCl solutions of varying concentration (indicated as *var* in Figure 1.2) or the solution used for the specific application is circulated. Figure 1.3 shows a typical current – voltage curve for a monopolar IEM.

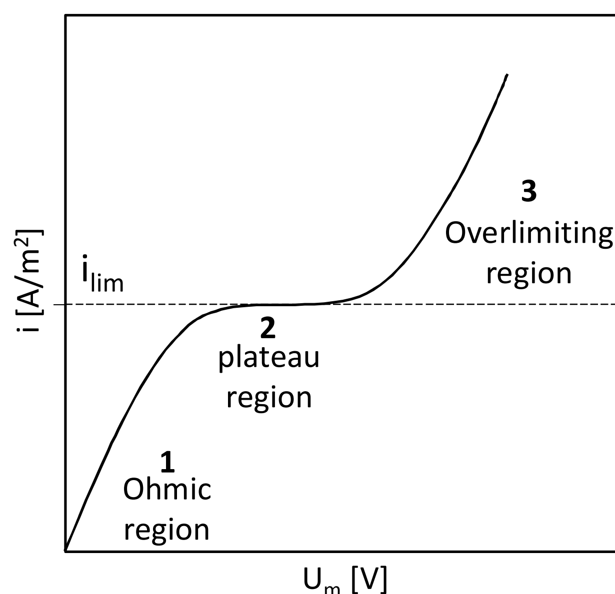

Figure 1.3. Typical current – voltage curve of monopolar membranes.

In Figure 1.3 three regions can be identified. At low current densities ions are available in the boundary layer of the membrane to transport the current from one compartment to another. Ohm's law is valid and therefore these region is called the Ohmic region. Here an increase in voltage causes a linear increase in current (Figure 1.3, region 1). The ions migrate faster through the membrane than from the bulk solution towards the boundary layer. Because of this the ion concentration at the membrane surface decreases until it reaches zero. The current density that corresponds to this point is the so-called limiting current density (i_{lim}). A further increase in voltage over the system does not cause the current to increase further due to the lack of ions to transport the current. Consequently, the current reaches a plateau while the voltage further increases (Figure 1.3, region 2). A further increase in voltage leads to the production of H^+ and OH^- from water splitting what provides the necessary ions to transport the current. This region is called the overlimiting region (Figure 1.3, region 3).

If operation in the overlimiting region takes place, the current will be used not only to transport the target amino acids but also for water splitting. This will not only decrease the current efficiency but influence the pH of the adjacent solutions as well, which is in some cases undesired. Especially for the separation of amino acids, a constant pH of the solutions is of utmost importance due to the sensitivity of the charge of the amino acids with respect to pH. If an amino acid is present in the feed in its negative form and the aim is to isolate it through an anion exchange membrane, the produced H^+ from water splitting will combine with the negative amino acid. The amino acid will then be neutral and this phenomenon, known as barrier effect, will limit the maximum amino acid recovery. This will be discussed in more detail in Chapter 2 of this thesis.

To minimize the effect of pH changes occurring during the electrodialysis process external pH control, such as acid/base dosing or the use of a buffer can be applied (discussed in more depth in Chapter 3 of this thesis). Another approach considered is the use of bipolar membrane electrodialysis (BPM-ED).

A bipolar membrane consists of an anion and a cation selective layer [44]. Between these two ion exchange layers, a transition region exists. When an electrical potential is applied over a monopolar (AEM or CEM) membrane, ions are removed from this transition region similar to a desalination process with electrodialysis (Figure 1.4a). Desalination continues until the concentration of ions approaches zero. At this point the ions to transport the current need to be generated via water splitting, a process that results in the production of H^+ and OH^- (Figure 1.4b). The function of a bipolar membrane is based on this principle. Two ion exchange layers of opposite charge are joined together. At first, ions are removed from the transition region (Figure 1.4c). Once this region is depleted from ions, further transport of current happens due to the generation of protons and hydroxyl ions that migrate through the cation or anion exchange layer of the bipolar membrane, respectively (Figure 1.4c).

Bipolar membranes can also be characterized based on current – voltage curves as described previously for monopolar ion exchange membranes. The bipolar membrane is placed in the middle between two electrodes for the determination of the potential difference across the membrane (Figure 1.2) [45]. At the same time bipolar membranes are also placed adjacent to the membrane under investigation.

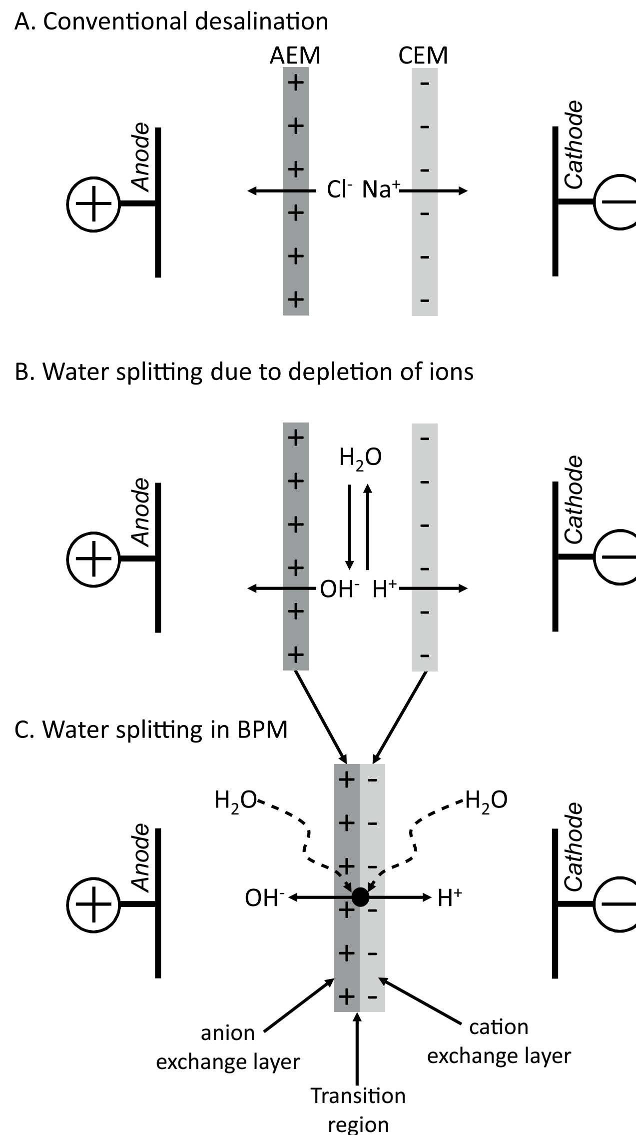

Figure 1.4. Schematic drawing of the principle of water splitting in a bipolar membrane (BPM). a) Ion transport in conventional desalination, b) water splitting in conventional electrodialysis due to the depletion of ions, c) water splitting in bipolar membrane electrodialysis (BPM-ED). Adapted from [44, 46].

Figure 1.5 visualizes that the current – voltage curve of a BPM shows different characteristic parts. In the beginning the current is only transported by salt ions (J_{M+}, J_{X-}). This happens below the first limiting current density, i_{lim_1}, as indicated in Figure 1.5. At this current density a very high resistance is obtained (increase in voltage) due to the removal of salt ions from the transition region of the BPM. As i_{lim_1} is a measure for the selectivity of the BPM towards ion leakage, its magnitude can be used to compare different bipolar membranes regarding their co-ion leakage [45].

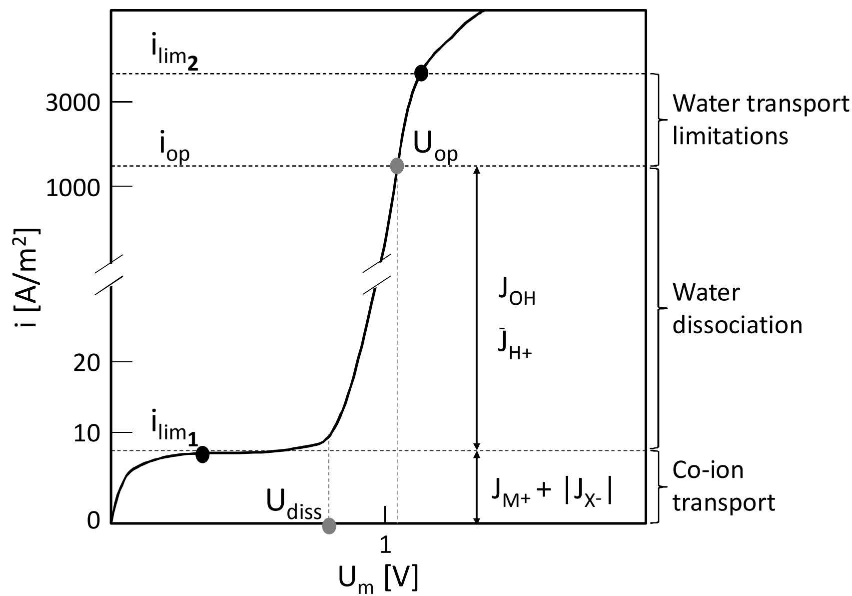

Figure 1.5. Schematic representation of a current – voltage curve of a bipolar membrane (BPM) in a salt solution M^+X^- [45].

Above i_{lim_1} water splitting occurs at the corresponding voltage for water dissociation, U_{diss}, and H^+ and OH^- are produced to transport the current (J_{H+}, J_{OH-}). With further increase in the voltage i_{lim_2} is reached (Figure 1.5). Above this value the water splitting efficiency is higher than the water transport towards the bipolar membrane interface so that the water consumed for the production of H^+ and OH^- cannot be replenished and the membrane dries out [47].

Conventionally, electrodialysis with bipolar membranes is applied when the generation of H^+ and OH^- is advantageous, for example, for the production of acids and bases from the corresponding salt [48-64]. In those cases, a high water splitting efficiency is desired. To achieve this, the operating current density, i_{op}, is chosen as high as possible. In this way, ion transport is also reduced [46]. A novel application of bipolar membrane electrodialysis, which does not necessarily require a high water splitting efficiency, is discussed in more detail in Chapter 4 of this thesis.

1.4 Scope and outline of the thesis

Alternatively to the conventional refinery, where chemicals are produced from fossil feedstocks, this thesis describes the use of amino acids present in biomass to produce biobased chemical intermediates. Amino acids already have the required functionalities (i. e. –N and –O), resulting in less process steps, lower energy consumption and less CO_2 emissions (Figure 1.6).

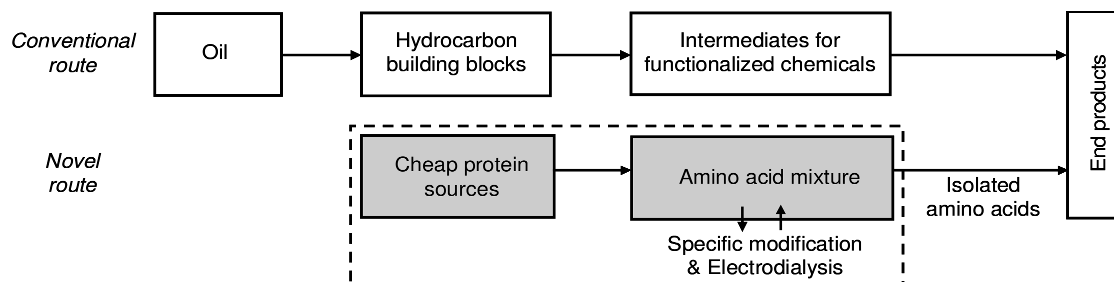

Figure 1.6. Conventional and novel route for the production of functionalized chemical intermediates.

The aim of this study is to explore and investigate the potential of ED in biorefinery processes and more specifically for the separation of amino acids. In this approach, enzymatic modification of specific amino acids is combined with electrodialysis. Figure 1.7 summarizes the research outline.

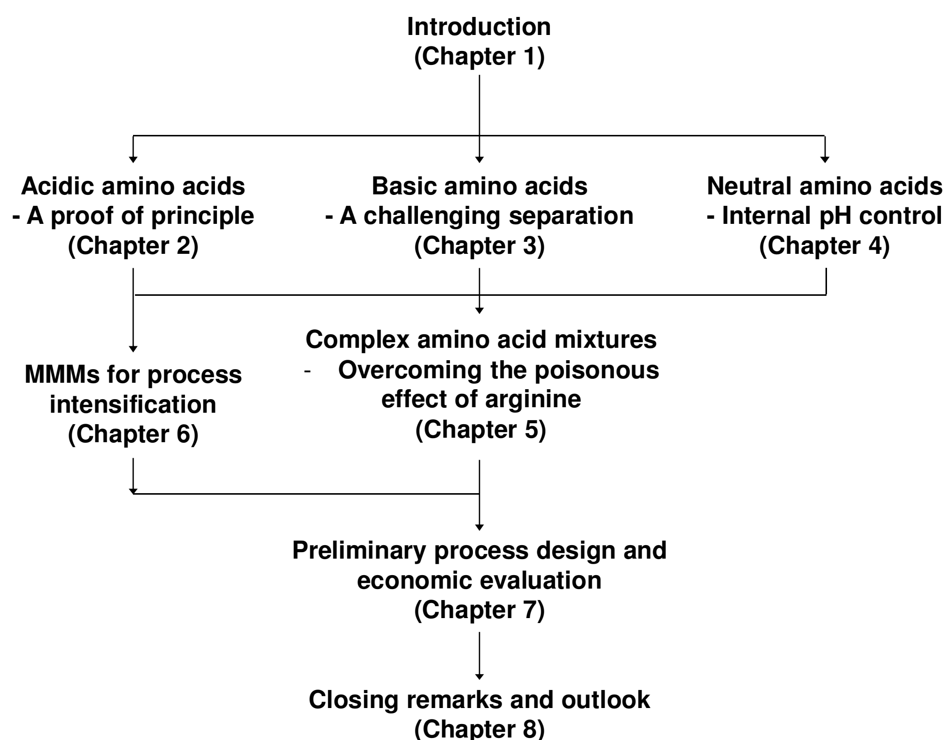

Figure 1.7. Research outline.

Chapter 2 shows the proof of principle of this novel concept. It explores, to the best of our knowledge, for the first time, the combination of electrodialysis and enzymatic modification for the isolation of target amino acids. It introduces important definitions in the electrodialysis process such as limiting current density, recovery, retention, flux, current efficiency and energy consumption. As model system the acidic amino acids, Glu and Asp, are selected. First, the

separation of Glu and Asp together using electrodialysis is investigated. Next, the enzymatic decarboxylation of Glu into GABA is considered. This conversion does not only lead to an interesting chemical intermediate, but also introduces significant differences in the charge behavior of the obtained product and the unconverted Asp. Furthermore, it discusses in depth the ion transport based on the overall performance of the process and the maximum amino acid recovery obtainable for the specific system.

Chapter 3 focuses on the isolation of the basic amino acids, Lys and Arg. Here the enzymatic modification of Lys into PDA is considered and its further isolation from Arg is studied. This separation is more challenging due to the high sensitivity of the charge of the amino acids towards small variations in pH. Besides investigating the influence of pH for the specific separation, it compares the process performance of the separation with and without external pH control (acid/base dosing) and the use of a buffer.

Chapter 4 presents a novel concept for internal pH control using a segmented bipolar membrane (sBPM). The concept is applied to the separation of Etn, the modification product of Ser, and a neutral amino acid, alanine (Ala). During this separation of positively charged Etn from neutral Ala at neutral pH, the pH in the feed stream decreases, causing Ala to get a slightly positive charge, thus compromising the product purity. The effect of using a sBPM, containing both, monopolar areas for ion transport and bipolar areas for enhanced water splitting to control the pH and the overall process performance is investigated.

From the results obtained in Chapter 4, the limitations of the existing commercially available membranes for the separation of positively charged Arg become obvious. Arg shows a strong poisoning effect on the process performance. **Chapter 5** compares the performance of electrodialysis for the separation of positively charged Arg using three different types of membranes: 1) commercially availailable cation exchange membranes, 2) electrodialysis with ultrafiltration membranes and 3) with tailor made cation exchange SPEEK membranes to overcome the poisonous effect of Arg on the electrodialysis process performance.

Chapter 6 focuses on the preparation of mixed matrix membranes as a platform for enzymatic reaction with the final aim to investigate the integration of electrodialysis and enzymatic modification in one single operation. This chapters deals with the preparation of mechanically stable mixed matrix membranes containing an enzyme carrier for enzyme immobilization. It

presents the characterization of the prepared membranes and evaluates their performance for the enzymatic decarboxylation of Glu into GABA. Finally, it shows the feasibility of the integration of enzymatic conversion and the further separation of amino acids using electrodialysis.

Chapter 7 studies the economic feasibility of the suggested approach based on the separation of the acidic amino acids. A first separation with electrodialysis, followed by enzymatic conversion and a second electrodialysis stage for further separation are considered with a fixed purge of 10%. Amino acid flux and enzymatic conversion are based on experimental results. The sensitivity analysis is performed based on membrane cost and amino acid flux. This chapter shows the increase in amino acid flux and decrease in membrane cost that are needed to reach economic feasibility.

Chapter 8 summarizes this work and evaluates the potential of this approach. It provides an outlook on the future of membranes in the biobased economy.

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2
Electrodialysis of acidic amino acids - A proof of principle

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ABSTRACT

Electrodialysis using commercially available ion exchange membranes was applied for the isolation of L-glutamic acid (Glu) and L-aspartic acid (Asp) from a mixture of amino acids. Based on the differences in their isoelectric points, Glu and Asp, being negatively charged at neutral pH, can be separated from neutral and basic amino acids. Outstanding recoveries for Glu and Asp of around 90% and 83%, respectively, were obtained. The further separation of Glu from Asp with electrodialysis is enabled with an enzymatic modification step where Glu is converted into γ-aminobutyric acid (GABA) with the enzyme glutamic acid α-decarboxylase (GAD) as the catalyst. Negatively charged Asp is separated from uncharged GABA at neutral pH conditions with a current efficiency of 70% and a recovery of 90%. Higher current efficiencies and lower energy consumption can be obtained when adjusting the current in time. This opens the route to successful isolation of amino acids for biorefinery applications using an integrated process of enzymatic conversion and separation with electrodialysis.

2.1 Introduction

The depletion of fossil fuels, the increasing oil prices and emissions of CO_2, urge the chemical industry to find alternative routes for the production of functionalized chemicals. This, combined with the need of green alternatives for energy and fuels, is the driving force for the emerging sustainable technology based on renewable resources, which aims to shift the conventional refinery towards biorefinery concepts. With the appropriate conversion and separation technologies, a significant amount of biomass feedstocks can be used for the production of bioenergy, biofuels and biochemicals [1]. For example, amino acids obtained from cheap protein sources (e.g. side streams from the production of biotransportation fuels from rapeseed oil) can be used in a biorefinery to produce chemicals from biomass as the amino acids already have the required functionalities (i. e. –N and –O). This results in less process steps, lower energy consumption and less CO_2 emissions. However, the amino acids in the feedstock are present as a mixture and need to be isolated for further conversion.

Amino acids are zwitterionic molecules whose charge is influenced by the surrounding pH. For instance, the acidic amino acids, glutamic acid (Glu) and aspartic acid (Asp), have a negative charge at neutral pH (Figure 3.1) but can become positively charged at low pH or negatively charged at high pH. The enzymatic decarboxylation of glutamic acid gives γ-aminobutyric acid (GABA) as a product, which is neutral over a larger pH region, like other amino acids such as glutamine (Gln), as can be seen in Figure 3.1.

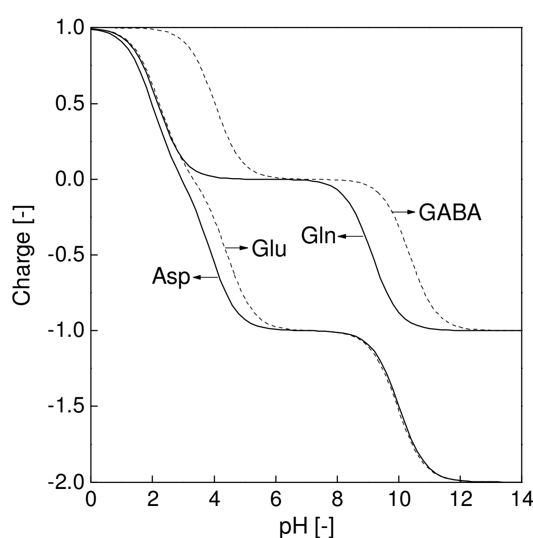

Figure 2.1. Charge behavior of Glu and Asp (acidic amino acids), Gln (neutral amino acid) and GABA (modification product of Glu) with respect to pH.

Electrodialysis is a promising technique for the isolation and separation of the various amino acids based on their differences in charge behavior as a function of pH. Nevertheless, some amino acids have similar isoelectric points (pH at which the charge is zero), but also an almost identical charge behavior with respect to pH (Figure 2.1, e.g. Asp and Glu). In order to separate those amino acids further, one may choose the help of an amino acid specific chemical conversion. Enzymatic reactions can be amino acid specific and produce molecules with a charge behavior different from the original one (e.g. Glu and GABA).

The novelty of this investigation, after achieving the separation of Glu and Asp together with electrodialysis, is the combination of the enzymatic modification of glutamic acid into γ-aminobutyric acid (GABA) allowing the isolation of aspartic acid from glutamic acid with electrodialysis. GABA, besides being a valuable product used in the food industry, can also be used for the production of industrial chemicals such as the monomer N-vinylpyrrolidone (NVP) [1, 2]. In this way, the isolation of single amino acids (and/or their modification products), like Asp and GABA, is achieved in this research.

This work demonstrates that a carefully chosen process combination of electrodialysis with enzymatic conversion allows the isolation of one derived amino acid while producing a valuable biorefinery product. The paper addresses strategies to maximize product recovery by process parameters such as current density and pH.

2.2 Theoretical background

Electrodialysis (ED) is an electro-membrane process that uses an electrical potential difference over the membrane as driving force for the selective extraction of ions from solutions. ED is widely used for the production of e.g. table salt and organic acids [4].

During the process, ions migrate from one compartment (*feed*) through an ion exchange membrane, to another compartment (*receiving*) under an applied electrical potential. Commonly used membranes for electrodialysis are ion exchange membranes (IEM), which contain either fixed positive groups (anion exchange membrane, AEM) or fixed negative groups (cation exchange membrane, CEM) and that are selective for either negatively or positively charged ions, respectively. If an ionic solution gets into contact with an IEM, ions with the opposite charge as the fixed ions in the IEM (counter ions) can go through the membrane while ions with the same

charge (co-ions) will be retained. This principle is also known as Donnan exclusion [5]. Electrodialysis can also be used in biorefinery applications to separate, e.g. amino acids (zwitterions) from a biobased feed (Figure 2.2) since they can be positive, neutral or negative depending on the surrounding pH.

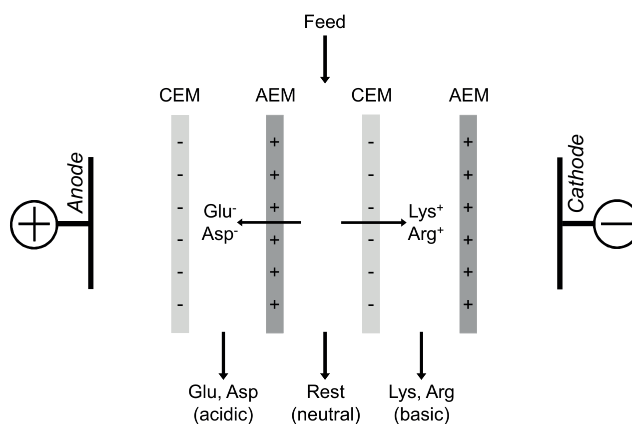

Figure 2.2. Schematic representation of the electrodialysis process for the separation of amino acids.

The charge behavior of the amino acids depends on their specific iso-electric point (pI), the pH at which a particular molecule carries no net charge. Table 2.1 shows a comparison of the differences in the isoelectric points, and the charge at neutral pH, of two acidic amino acids, aspartic acid (Asp) and glutamic acid (Glu), two basic amino acids, lysine (Lys) and arginine (Arg), and three neutral ones.

Table 2.1. Comparison between different amino acids, their iso-electric points and their charge at neutral pH.

Amino Acid	pI	Average Mass [Da]	Side Chain Charge (pH =7)
Aspartic acid	2.85	133.10	Negative
Glutamic acid	3.15	147.13	Negative
Lysine	9.60	146.19	Positive
Arginine	10.76	174.20	Positive
Alanine	6.01	89.09	Neutral
Glycine	6.06	75.07	Neutral
Tryptophan	5.89	204.23	Neutral

In principle, ED should be able to separate amino acids as long as there is a difference in their corresponding isoelectric points or electrophoretic mobility. Nevertheless, the amino acids are divided into three different groups according to their charge behavior. Such is the case for glutamic acid and aspartic acid. Both show the same charge at a specific pH, making it

impossible to isolate them from each other with electrodialysis. Therefore, the charge behavior of one of them needs to be modified to allow further separation.

To date, several researchers have focused on the application of electrodialysis for the separation of different amino acids, for example, the recovery of L-tryptophan from crystallization wastewater [6], the separation of proline [7], the isolation of tyrosine from amino acid mixtures [8] and the separation of lysine, methionine and glutamic acid [9]. Although the separation of Asp and/or Glu from a mixture of amino acids is possible [10 - 12], the separation of Glu from Asp using the conversion product of one of the components to establish the isolation of the single amino acids has not yet been studied to the best of our knowledge.

Kumar et al. [11] carried out electrodialysis experiments of charged Glu (Glu^-) obtaining a recovery of 85%, a satisfactory current efficiency of 60.5% and an energy consumption of 5.38 kWh/kg. The same study also reports low recoveries (<20%), low current efficiencies (<15%) and an energy consumption higher than 19 kWh/kg for the electrodialysis of negatively charged lysine (Lys^-). Based on these results, Kumar et al. also carried out experiments for the separation of Glu^- from a mixture containing also Lys^+. The reported values of Glu^- recovery, current efficiency and energy consumption are 85%, 65.5% and 12.9 kWh/kg, respectively. For this research, non-commercial ion exchange membranes made of sulfonated poly ether sulfone (SPES) were used [11]. Another study carried out by Sandeaux et al. [12] focused on the extraction of different fractions of amino acids from protein hydrolysates, where chicken poultry, ox blood and human hair were used as raw materials. The recoveries obtained for Asp and Glu (acidic fraction) were around 98% and 88%, respectively [12].

When looking at the isoelectric point of Asp and Glu (2.85 and 3.15, respectively), it becomes clear that isolating one from the other with electrodialysis is an ambitious challenge due to their similar charge behavior with pH. Therefore, enzymatic modification of either Asp or Glu is suggested. Lammens et al. succeeded in converting Glu into γ-aminobutyric acid (GABA) with the enzyme glutamic acid α-decarboxylase (GAD) as the catalyst [2]. GABA has not only a neutral charge in the pH range where Asp is negative, but it is also a valuable product used in the food industry and can also be used for the production of the monomer N-vinylpyrrolidone (NVP) and other industrial chemicals [2, 3].

Only little has been reported in literature regarding the separation of GABA/Asp mixtures. To the best of our knowledge, only Habe et al. performed an investigation on the separation of GABA from Glu [13]. Batch electrodialysis experiments were carried out at a pH of 3, where GABA is positively charged ($GABA^{+}$) while Glu has no net charge (Glu^{0}). For these experiments, membrane cartridges from ASTOM corp. were used, with an effective membrane area of 550 cm^2. The initial feed concentration was 416 mM and 136 mM for GABA and Glu, respectively. No other salt ions with higher electrophoretic mobility that could compete with the amino acid ions were present. The separation of $GABA^{+}$ from Glu^{0} was successful, resulting in a GABA recovery rate of 82 - 89%, a current efficiency of 81 - 85%, and an energy consumption of 0.197 – 0.204 kWh/kg [14]. The high current efficiency and the low energy consumption might be the result of adjusting the voltage in time, avoiding that too high currents were reached and therefore, increasing the efficiency of the current utilization.

The results obtained by Habe et al. [13] indicate that the separation of GABA from Asp is possible. In general, the previous studies report the separation of Asp and/or Glu from other amino acids. If both present, they are separated together from the mixture used as feed. To the best of our knowledge, no data of the isolation of Asp from Glu has been reported in literature.

One of the drawbacks of electrodialysis is the limiting current density. It is desired to operate at the highest possible current density where the maximum ion flux per unit membrane area (limiting current density, LCD) is obtained [14]. At low current densities enough ions are available in the boundary layer of the membrane to transport all current from one compartment to another. In this region, Ohm's law is valid and therefore it is called the ohmic region.

An increase in the current density causes the ion concentration in the boundary layer to decrease. In electrodialysis, the transport of ionic species through ion exchange membranes is controlled by the diffusion in the membrane itself as well as by the diffusion in the film formed by the boundary layer at the solution-membrane interface [15]. In this region, ions migrate faster through the membrane than they migrate from the bulk of the solution towards the membrane, which results in a significant increase of the resistance (voltage) over the system.

At this limiting current density, the amino acid concentration at the membrane surface is zero. After this point the current density starts to increase again. For monovalent ions this is also known as the overlimiting current region and is mainly caused by electro convection, a

hydrodynamic effect that destabilizes the laminar boundary layer [16]. In this region also water splitting occurs. The current is too some degree no longer used for the transport of the target ions only, since their availability at the membrane surface is not sufficient anymore, but is also used to split H_2O into H^+ and OH^- at the membrane surface. The generated H^+ and OH^- can result in a pH change depending on the extent of water splitting. This of course influences the charge behavior of the amino acids in particular at the surface of the membrane, as will be shown later. This phenomenon can be minimized by operating at or below the limiting current density. In this way the maximum amino acid flux is obtained and no energy is wasted by side effects such as water dissociation.

2.3 Experimental

2.3.1 Materials

Amino acid solutions used for limiting current density, as well as for electrodialysis experiments were prepared by using amino acids (L-glutamic acid, L-aspartic acid) in solid state with a purity of 98% or higher obtained from Sigma-Aldrich. GABA, the modification product of L-glutamic acid, was purchased from Fluka with a purity of 98% (purum). The pH of the amino acid solutions was adjusted using NaOH from Merck Chemicals and Reagents. Sodium sulfate solutions (Merck Chemicals and Reagents) with a concentration twice as high as the total amino acid concentration in the feed solution were used as electrode rinsing solutions.

Ion exchange membranes from the type fumasep PEEK reinforced FKB (CEM) and fumasep PEEK reinforced FAB (AEM) were purchased from FumaTech GmbH, Germany. The properties of the membranes are given in Table 2.2 [17].

Table 2.2. Properties of the ion exchange membranes used [17].

Property	FKB-PEEK	FAB-PEEK
Thickness [mm]	0.08 – 0.10	0.10 – 0.13
Elect. resistance [$\Omega \cdot cm^2$]	<4	<1
Selectivity [%]	>98	>96
Ion exchange capacity (IEC) [meq/g]	0.9 – 1.0	1.3

2.3.2 Methods

2.3.2.1 Limiting current density

A four compartment cell was used for the study of the limiting current density [18]. Ion exchange membranes were placed inside the cell between two electrodes (platinized titanium at the anode side, stainless steel at the cathode side), with the membrane under investigation in the middle, where oxidizing (anode) and reduction (cathode) reactions take place.

The current was increased slowly via a power supply (Delta Elektronika) and the voltage across the membrane under investigation was measured between two Haber – Luggin capillaries with a voltage meter. The current density was plotted against the voltage over the membrane. The LCD is graphically determined from this plot. The detailed description of the method used for the determination of the limiting current density is described in detail elsewhere [18].

2.3.2.2 Electrodialysis

Electrodialysis experiments were carried out with a four compartment cell type ED-40 equipped with titanium, iridium plasma coated stainless steel electrodes (FumaTech GmbH). The schematic configuration of the electrodialysis cell is shown in Figure 2.3.

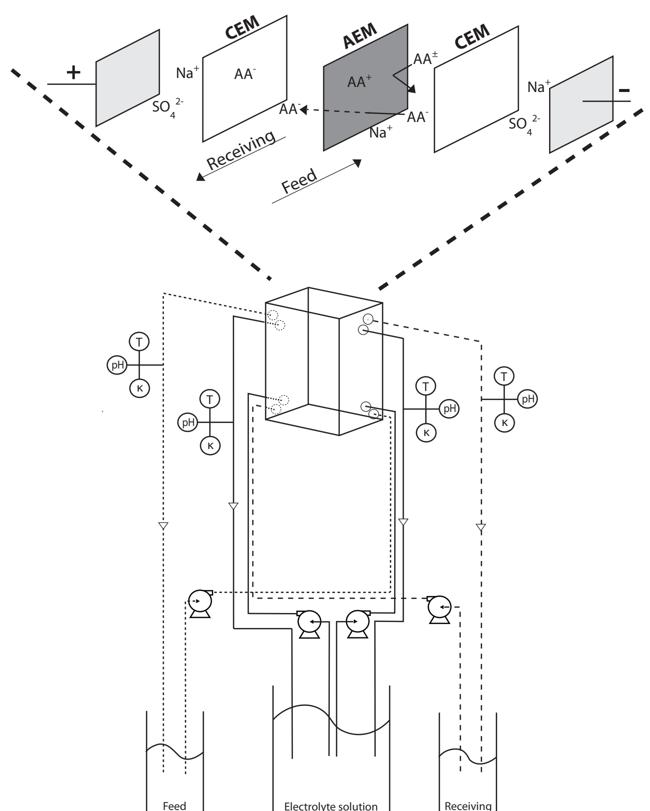

Figure 2.3. Schematic representation of the electrodialysis set-up with a stack of ion exchange membranes.

The configuration of the membrane stack consisted of CEMs on the electrode's side (both, cathode and anode) and an AEM in the middle. The effective membrane area is 36 cm^2. Thick diamond structured welded mesh spacers with a thickness of 475 μm made of PVC/Polyester were placed between the ion exchange membranes in the feed and receiving compartments. Between the outer membranes 900 μm thick diamond structured welded mesh spacers made of polyethylene were used.

The electrodialysis set-up was equipped with a power supply from Delta Elektronika (0 – 30 V; 0 – 5 A) and two Masterflex pumps (Model 7521-25). The experiments were carried out at constant current (i_{lim}) and a flow rate of 25 ml/min.

As feed and receiving streams, 1 L of feed solution with a concentration of 25 mM of each amino acid present and 1 L of MiliQ water were used, respectively. The electrode rinse consisted of 2 L Na_2SO_4 with a concentration twice as high as the total amino acid concentration present in the diluate stream. The electrode rinse solution was feed from one vessel and at the electrodes, the stream was split; half of the stream was fed to the cathode, the other half to the anode. After leaving both electrode compartments, the pH in the cathode stream and the anode stream were determined separately. After that, both streams were merged again and recirculated back to the electrode rinse vessel. The pH, the conductivity and the temperature were monitored with time. Every hour, a sample from each stream was collected and analyzed with U-HPLC [19].

2.3.2.3 Process evaluation

To assess the process the amino acid flux, the recovery, the current efficiency and the power consumption are determined. They are calculated as shown in Eq. 2.1 – 2.4.

Flux

The flux is the amount of moles transported through the effective membrane area per time unit. The flux is calculated according to the following equation.

$$J_i = \frac{V}{A} \cdot \frac{\Delta C}{\Delta t} \qquad \text{Eq. 2.1}$$

Here, V is the total volume of the compartment [m^3], C is the concentration in the compartment [mol/m^3], t is the time [s] and A is the effective membrane area [m^2].

Recovery

The recovery is the fraction of amino acids originally present in the feed compartment that is transported to the receiving compartment, as given in Equation 2.2.

$$R_{aa} = \frac{M_{i,rec,t=t}}{M_{i,feed,t=0}} \cdot 100 = \frac{C_{i,rec,t=t} V_{rec}}{C_{i,feed,t=0} V_{feed}} \cdot 100 \qquad \text{Eq. 2.2}$$

With M the amount of moles of amino acids present in the feed/receiving of component i [mol], V the volume of the feed/receiving compartment [m^3], $C_{i,rec,t}$ the concentration of component i in the receiving at time t [mol/m^3] and $C_{i,feed,t=0}$ the initial feed concentration of component i [mol/m^3].

Current efficiency

The current efficiency is the fraction of the current that is transported by the target ions, in this case, the amino acids. The current efficiency can be calculated using Equation 2.3.

$$\eta = \frac{F z_i V}{I_{applied}} \frac{(C_t - C_0)}{t} \qquad \text{Eq. 2.3}$$

Where F is the Faraday constant [C/mol], z_i is ionic charge [-], V is the volume of the compartment [m^3], C_t and C_0 are the concentrations at time t and at time 0, respectively [mol/m^3], I is the applied current [A] and t is the time [s].

Energy consumption

Energy consumption (E) is the amount of Joules needed to transport one kg of amino acids. It is calculated as follows:

$$E = \frac{I_{applied} \int_0^t U(t)dt}{V_{rec} C_{rec,t}} \qquad \text{Eq. 2.4}$$

With $C_{rec,t}$ the final concentration of that component in the receiving compartment [mol/m^3], V_{rec} the volume of the receiving compartment [m^3], U the voltage dependent on the time [V], $I_{applied}$ the applied current [A] and t the time [s].

2.4 Results and discussion

2.4.1 Limiting current density

To operate ED at maximum current efficiency, the i_{lim} was determined in a four compartment electrodialysis cell for Glu^-, Asp^-, a mixture of Glu^-/Asp^- and a mixture of $Asp^-/GABA^0$. The Current-Voltage curves for the mixtures investigated are presented in Figure 2.4.

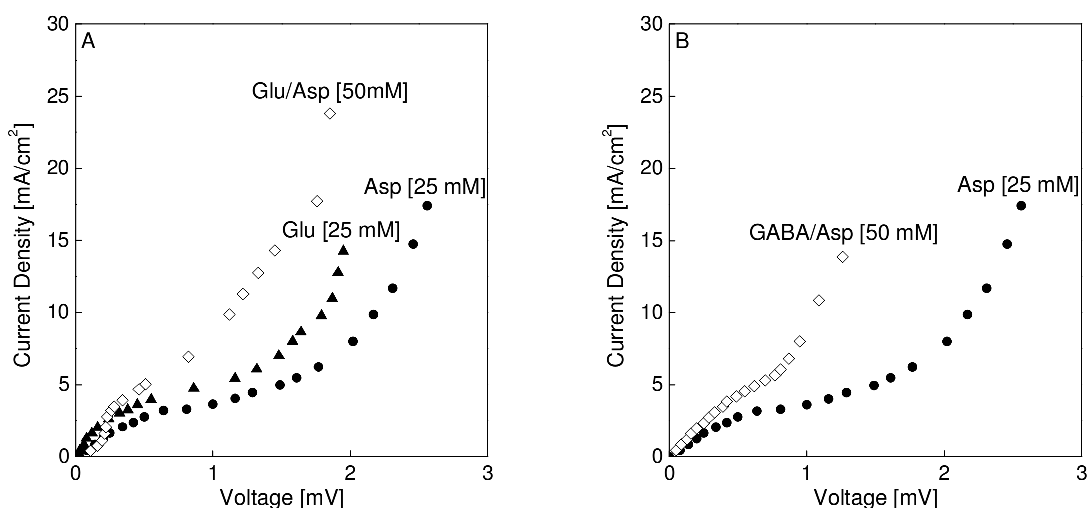

Figure 2.4. a) Current-voltage plot for Asp^-, Glu^- and a mixture thereof; b) Current-voltage curve for Asp^- and a mixture of Asp^- and $GABA^0$. pH of the investigated solutions = 6.0.

Figure 2.4 represents typical current-voltage curves as described in theory. The values of current density for the amino acids and amino acid mixtures investigated (summarized in Table 2.3) are in the same range as the i_{lim} of an standard type anion exchange membrane Neosepta AMX (Tokuyama Soda Inc., Japan) when measured for a solution of NaCl of the same concentration (25 mM), which is expected to have an i_{lim} of around 3.5 mA/cm^2 [18]. The values shown in Table 2.3 were used for the electrodialysis experiments of the different mixtures to guarantee an efficient current utilization minimizing unwanted side effects such as water splitting.

Table 2.3. Limiting current density of single amino acids and amino acid mixtures investigated at pH = 6.0.

Amino acid system	Concentration [mM]	i_{lim} [mA/cm^2]
Glu^-	25	2.53
Asp^-	25	2.42
Glu^-/Asp^-	25/25	3.58
$Asp^-/GABA^0$	25/25	3.05

2.4.2 Electrodialysis

The enzymatic modification of L-glutamic acid (Glu), a decarboxylation reaction, has been proven by Lammens et al. [6]. The enzyme glutamic acid α-decarboxylase (GAD) was immobilized in two different ways, by covalent binding to Eupergit and by gel entrapment in calcium alginate. Here we assume that the process leads to sufficiently large conversion leading to a mixture of Asp and GABA. Hence, the experiments presented below cover the investigation of the separation of two mixtures consisting of a) Asp^-/Glu^- and b) $Asp^-/GABA^0$. The process is evaluated with respect to recovery, current efficiency, energy consumption and the amino acid flux. Besides the mixture experiments, single amino acid experiments were carried out for Asp^- and Glu^-, called the individual amino acid experiments. A concentration of 25 mM for each of the amino acids was chosen arbitrarily with the only consideration of being far below the solubility limit for Glu and Asp (59 mM and 34 mM at pH = pI and 25℃, respectively). All electrodialysis experiments were performed with the membrane configuration shown in Figure 2.3 and as explained in Section 2.3.2.

2.4.2.1 Electrodialysis of single amino acids

The results of the single amino acid experiments for Asp^- [25 mM] and Glu^- [25 mM] are summarized in Table 2.4. High recoveries and reasonable current efficiencies are obtained for both systems, and these are comparable with the results obtained by Kumar et al. [11]. However, lower energy consumptions and higher amino acid fluxes than the values reported by Kumar et al. [11] are obtained, suggesting that the current used by Kumar et al. [11] was above the limiting current density, causing water splitting to occure and decreasing the current efficiency. Working at i_{lim}, as is the case for the present study, results in lower voltages over the ED stack, which translates in lower energy consumptions needed to transport the amino acids. The higher flux might be the result of using an amino acid concentration in the feed in the present work (25 mM) that was more than two times higher than the one used by Kumar et al. (10 mM) [11].

Table 2.4. Process performance parameters for the recovery of Asp^- and Glu^- using ED. Initial feed pH = 6.0.

Process parameters	Asp^-	Glu^-
Recovery [%]	96.0	86.2
Current efficiency [%]	60.1	57.0
Energy consumption [kWh/kg]	2.23	2.13
Flux [10^{-4} mol/m^{2}s]	1.90	1.80

Samples of the electrode rinse solutions were taken in time and analyzed for amino acid leakage throughout the experiment. No amino acids were found in the electrode rinse solution at any stage of the experiments.

2.4.2.2 Separation of Asp/Glu as a mixture

The results of the isolation of Asp^- and Glu^- as a mixture are presented in Figure 2.5, which shows the concentration behavior for Asp^- and Glu^- during the electrodialysis experiment. As in the individual amino acid experiments, no amino acids were found in the electrode rinse solutions. The performance evaluation of the process is presented in Table 2.5.

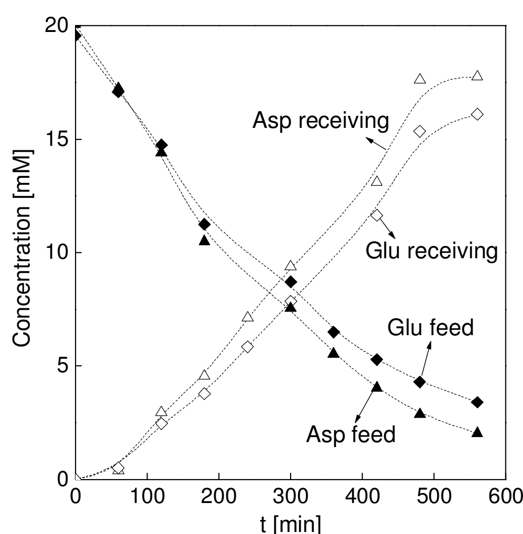

Figure 2.5. Concentration behavior of Glu^- [25 mM] and Asp^- [25 mM] in time during the electrodialysis experiment. Flow rate: 25 ml/min; Initial pH of the feed = 6.0.

Figure 2.5 shows that the concentration of both amino acids in the feed stream increases while it decreases in the receiving stream for both amino acids due to the transport of the amino acids Glu^- and Asp^-. The process performance parameters are summarized in Table 2.5 obtaining for both amino acids a high recovery of over 80% and a current efficiency of 72%. When compared with the values obtained for the individual amino acid experiments (Table 2.4) the recovery is lower, but the current efficiency is higher. This is a consequence of a higher amino acid concentration in the feed and the same operation time. Our results indicate that stopping the experiment earlier, e.g. at t = 500 min, leads to an increase in current efficiency with about 10%. The consequence is a decrease in the recoveries of Glu and Asp of about 4 to 5%. The reason is the too high current density (above the limiting current density) at the end of the experiment, resulting in the use of a significant percentage of the current for water splitting. This is also

reflected in the lower energy consumption for the mixture (Table 2.5) than for the individual amino acid experiments. It can be expected that operating this experiment for longer times will result in higher recoveries but at the same time higher energy consumption and lower current efficiencies.

Table 2.5. Process performance parameters for the separation of Asp^- and Glu^- as a mixture. Initial feed pH = 6.0.

Process performance parameters	Results
Recovery (Asp^-) [%]	89.9
Recovery (Glu^-) [%]	82.6
Current efficiency [%]	71.8
Energy consumption (Asp^-) [kWh/kg]	0.9
Energy consumption (Glu^-) [kWh/kg]	0.8
Flux (Asp^-) [10^{-4} mol/m^{2}s]	1.4
Flux (Glu^-) [10^{-4} mol/m^{2}s]	1.3

The total ion flux for the mixture experiment is higher than the fluxes obtained for the individual amino acid experiments. According to Table 2.5 the total ion flux of Asp and Glu together was $2.69 \cdot 10^{-4}$ mol/m^{2}s. The flux of the individual amino acid experiments was between 1.80 - 1.90 $\cdot$ 10^{-4} mol/m^{2}s, which is almost 1.4 times lower. The higher flux can be explained from the higher current density applied during the mixture experiment. The $i_{applied}$ for the mixture was 3.6 mA/cm^2 (as determined from the limiting current density experiments) and for only Asp^- or Glu^- it is around 2.5 mA/cm^2, which is also 1.4 times lower. This is interpreted as a direct relation between the ion flux and the applied current density.

The results obtained in the present investigation are comparable with the values reported by Kumar et al [11] who evaluated the separation of Glu^- from Lys^+ in terms of Glu^- recovery and current efficiency. Nevertheless, the energy consumption obtained by Kumar et al. [11] was much higher, 12.9 kWh per kg Glu, which is the result of working at constant voltage instead of controlling the current. The voltage used by Kumar et al. is higher than the values used in the present study, while the total amino acid concentration used by Kumar et al. [11] is much lower, suggesting that the experiments were carried out at current densities above i_{lim}.

Kumar et al. [11] also reported a flux ($0.65 \cdot 10^{-4}$ mol/m^{2}s) that is almost twice as low as the flux of Glu^- obtained in this research ($1.28 \cdot 10^{-4}$ mol/m^{2}s). Equation 2.5 indicates that the transport through ion exchange membranes in electrodialysis is dependent on convection, diffusion and

migration [20]. It shows that the flux will decrease when the concentration decreases. Kumar et al. [11] used a lower feed concentration than the one applied in the present investigation, resulting in lower amino acid fluxes.

$$J_i = -D_i \frac{dC_i}{dx} - \frac{z_i F C_i D_i}{RT} \frac{d\varphi}{dx}$$ Eq. 2.5

2.4.2.3 Isolation of Asp from GABA

The results shown in the previous sections prove that negatively charged Asp and negatively charged Glu can be isolated from a feed stream with electrodialysis. Enzymatic conversion of one of them is needed to modify the specific charge behavior, hence, enabling the separation of the two. In the present investigation, the decarboxylation of glutamic acid towards GABA is considered. Electrodialysis experiments of a mixture containing Asp and GABA are carried out at neutral pH. At these pH conditions, Asp is negatively charged while GABA has no net charge.

In Figure 2.6 the concentrations of Asp^- and GABA during an ED experiment of a mixture containing both compounds (Asp and GABA) are plotted against time. The concentration of Asp in the receiving solution increases while it decreases in the feed. No GABA is detected in the receiving compartment which is in agreement with its neutral charge. Consequently the concentration of GABA in the feed remains constant.

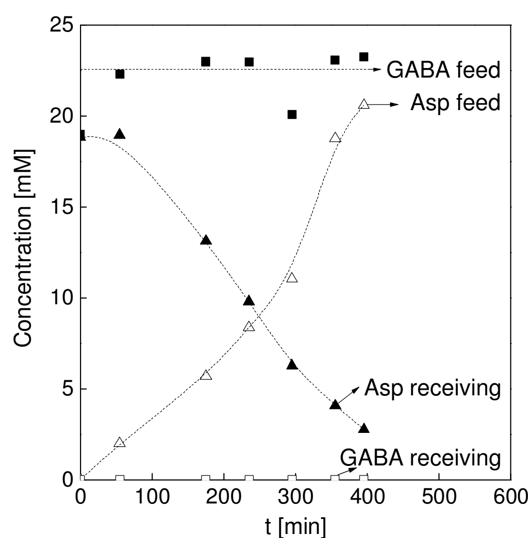

Figure 2.6. Concentration behavior of Asp^- [25 mM] and $GABA^0$ [25 mM] with time during the electrodialysis experiment. Flow rate: 25 ml/min; Initial pH of the feed = 6.0.

A summary of the process performance parameters is shown in Table 2.7. An outstanding recovery of Asp of around 88% is obtained. Being $GABA^0$ not potential sensitive but one of the desired products, the energy consumption for the production of $GABA^0$ has been calculated based on the energy needed for the separation of Asp^- for a better interpretation. No amino acid leakage towards the electrode compartments was found.

Table 2.7. Process performance parameters for the isolation of Asp^- from GABA. Initial pH of the feed = 6.0.

Process parameters	Results
Recovery (Asp^-) [%]	88.1
Current efficiency [%]	73.8
Energy consumption (Asp^-) [kWh/kg]	1.10
Flux (Asp^{-1}) [10^{-4} mol/m^{2}s]	2.34

2.4.2.4 Ion transport

During the ED experiments, the pH, the conductivity, the voltage and the amino acid content were monitored. The behavior of these specific process parameters throughout the experiment reflects the performance of the process. In addition, the pH changes limit the amino acid recovery. In the following paragraphs the overall performance of the process and the maximum recovery of amino acids are discussed in more detail.

Overall performance of the process

Together with the amino acid content, the pH gives a good indication of what happens with the amino acids during the separation process. The results obtained for the separation of Asp^-/Glu^- [25 mM/25 mM] are used as an example.

Figure 2.7a shows the behavior of the pH with time of the 4 different compartments in the ED stack (as indicated in Figure 2.3), being the cathode, anode, receiving and feed stream. The change in the conductivity in the receiving and the feed compartment in time, as well as the behavior of the voltage throughout the experiment, are shown in Figure 2.7b. A detailed discussion of these figures is given in the following paragraphs.

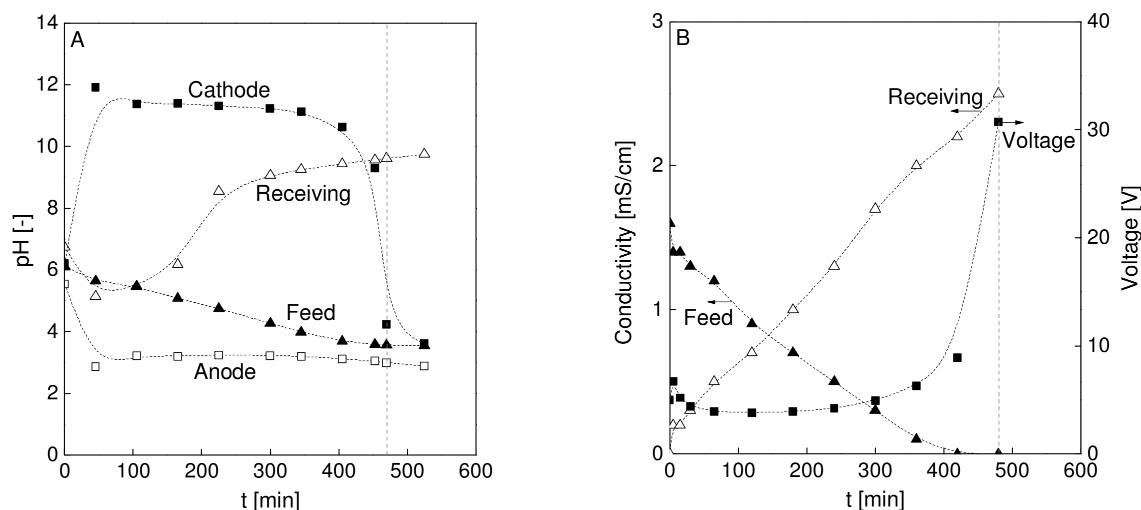

Figure 2.7. Overall process performance of the electrodialysis of Asp⁻/Glu⁻ [25 mM]: a) pH behavior with time during the single electrodialysis experiments. b) 1st y-axis: Conductivity behavior in time during the single electrodialysis experiments. 2nd y-axis: Voltage behavior in time during the electrodialysis experiments. Flow rate: 25 ml/min; Initial pH of the feed = 6.0.

It is important to mention that the electrode rinse solution, captured in one single vessel, splits going on one hand through the cathode and on the other to the anode. Before merging again, the pH measurements are performed. Therefore, different values of pH are reported for the cathode and for the anode.

At the very start of the experiment, the pH in the cathode increases, while it decreases in the anode compartment, as can be seen from Figure 2.7a. This is due to the production of OH^- and H^+ in the cathode and in the anode compartment, respectively. The electrolyte solution is being circulated through both compartments and collected again. The streams coming from both electrode compartments are mixed again before going back to the electrode compartments. This makes that the pH remains constant throughout the further experiment at the outlet of the electrodialysis stack for both streams. At t ~ 500 min, however, a steep decrease in pH can be observed at the cathode side again. This is related to H^+ produced by water splitting in the feed channel. At t ~ 500 min the amino acid concentration in the feed is very low. At this low amino acid concentration, the limiting current density is surpassed and water splitting sets in.

H^+ and OH^- ions are formed as a result of the water splitting. H^+ ions remain in the feed compartment and accumulate at the AEM surface blocking the transport of Asp^- and/or Glu^-. At t ~ 500 min, the pH in the feed decreases to around 4 (indicated in Figure 2.7a). H^+ will react

with Asp^- and/or Glu^- into H_2O and Glu^0 or/and Asp^0 preventing their further transport through the AEM towards the anode due to their lack of charge. Once the feed has been depleted of negatively charged amino acids, the produced H^+ cannot combine with the amino acids anymore and migrates further towards the cathode. This is possible, since the membrane between the feed compartment and the cathode is a cation exchange membrane.

The pH in the anode side, on the other hand, does not increase, since the produced OH^- ions are retained in the receiving compartment by the cation exchange membrane that is located between the receiving and the anode stream. The amino acid content in the receiving solution is high, enabling the recombination of the amino acids present with the OH^- produced by water splitting at the surface of the anion exchange membrane. The charge of the amino acids in the receiving compartment goes from -1 to -2 and a slight increase in the pH can be observed. To maintain electroneutrality, positive ions will migrate from the electrolyte compartment to the receiving compartment, resulting in an increase in conductivity in this stream as shown in Figure 2.7b. The lack of ions in the feed causes the resistance (voltage) of the solution to increase at t ~500 min (Figure 2.7b). The transport pathways for the amino acids, protons and hydroxyl ions are shown in Figure 2.8.

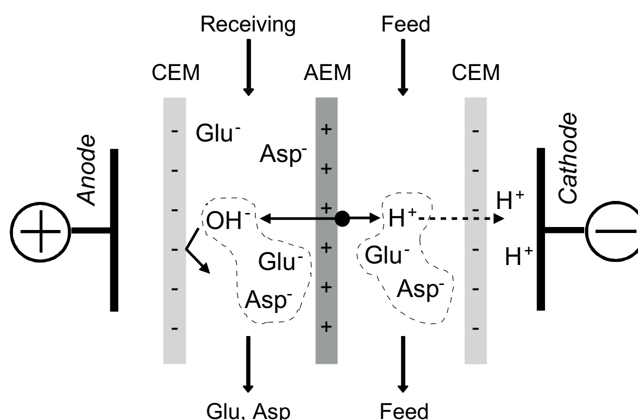

Figure 2.8. Schematic representation of the water splitting phenomena and ion transport during the electrodialysis of a mixture containing Glu^- and Asp^- at neutral pH.

The pH behavior in the feed and the receiving compartment is of high importance for the understanding of the ED process since it may limit the recovery of amino acids. Figure 2.7a. shows that the pH at the beginning of the experiment increases in the receiving compartment while it decreases in the feed and it then stabilizes for both streams. Krol [18] studied the water dissociation on monopolar ion exchange membranes, reporting a decrease of the pH at the cathode side of the membrane, which corresponds to the *feed* in the present study, and an

increase at the anode side of the membrane, which corresponds to the *receiving* stream for this case, for both, anion and cation exchange membranes [17]. The changes in pH were significantly more pronounced for the anion exchange membranes [17], which should be the case for the present study as well, since the transport of Asp^- and/or Glu^- takes place through the AEM. However, for the special case of amino acids, the pH changes are not so pronounced at the initial stage of water splitting due to protonation and deprotonation reactions of the amino acids present in both streams. This is explained in more detail in the following paragraphs.

Maximum recovery of amino acids

For a better understanding of how the charge of the amino acids present in the different compartments behaves with the change in pH throughout the electrodialysis experiments, graphs that show the pH behavior throughout the experiment and the relation between the pH and the charge of the amino acids were prepared. The results for the electrodialysis of Asp^- [25 mM] are shown in Figure 2.9, which consists of two x-axis, where the main axis (bottom) shows the time [min], representing the duration of the electrodialysis experiment. The secondary x-axis (top) shows the charge [-] of the amino acids present in the mixture used for the specific experiment. The y-axis represents the pH.

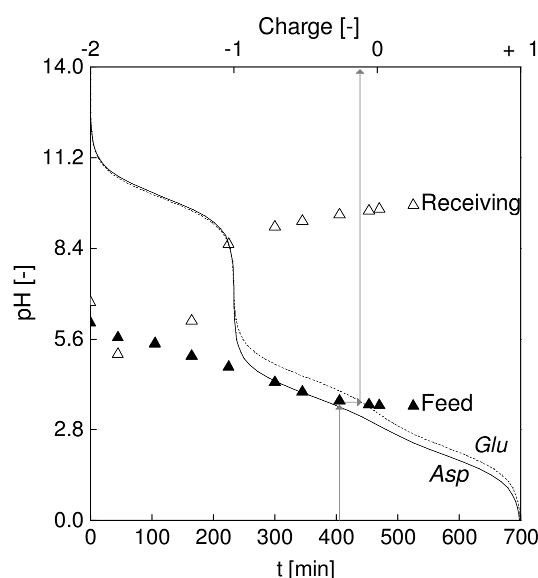

Figure 2.9. 1st x-axis: pH plotted against time for Asp^-/Glu^- [25 mM/25 mM] (Feed:▲, Receiving: Δ) during single amino acid experiments; 2nd x-axis: pH plotted against charge for Asp and Glu. Flow rate: 25 ml/min; Initial pH of the feed = 6.0.

For example, at the end of the experiment, the pH in the feed is ~ 3.6 (horizontal line). At this pH, the charge of both amino acids present, Glu and Asp, is between -0.5 and 0, as indicated in Figure 2.9 with the vertical lines. In this way, the charge of the amino acids present in the feed and the receiving streams during the experiment can be estimated based on the pH changes in these compartments. The pH in both compartments changes significantly throughout the experiment. Due to the depletion of ions in the feed, additional ions need to be generated to transport the current. Water splitting takes place, especially on the AEM surface, producing OH^- and H^+ ions. The H^+ ions remain in the feed compartment causing the pH to decrease down to ~ 3.6, neutralizing the negatively charged amino acids as long as they are available in the feed, avoiding their further migration. OH^- formed on the AEM migrates to the receiving stream causing the pH to increase from 6 to 10 (Figure 2.9). OH^- promotes the further deprotonation of Glu^- and Asp^- already present in this stream causing their charge to go from -1 to -2, as can be seen in Figure 2.9. This does not influence the process since these amino acids will remain in the receiving stream in any case. The latter does have a negative effect on the process in the case of positive amino acids present in this stream that are expected to migrate in a positive form. In that case the OH^- formed will neutralize these avoiding their further transport as cations. As reported elsewhere this phenomenon is called the barrier effect (Figure 2.10) [20, 21].

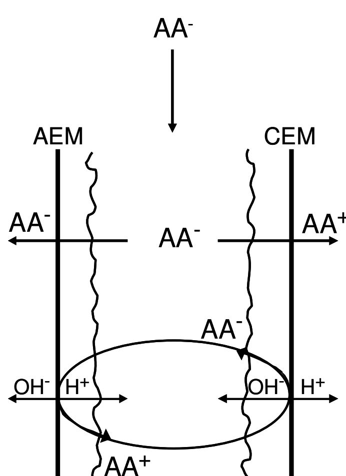

Figure 2.10. Schematic representation of the barrier effect for the electrodialysis of amino acids [21].

Similar behavior is observed for the separation of Asp^- from GABA (Figure 2.11), except that the pH of the feed stays more or less constant compared to Figure 2.9. This is caused by GABA that is still present in the feed and has a neutral charge. Similar results are obtained for all other electrodialysis experiments as well.

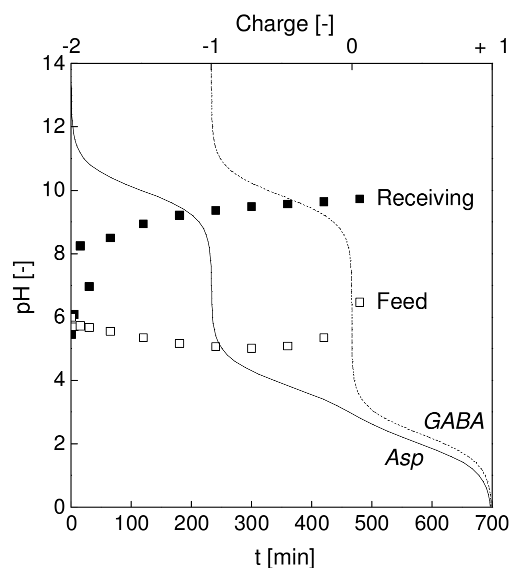

Figure 2.11. 1st x-axis: pH against time for $Asp^-/GABA^0$ [25 mM/25 mM]; 2nd x-axis: pH against charge of Asp and GABA. Flow rate: 25 ml/min; Initial pH of the feed = 6.0.

For the specific application investigated in the present study, the pH change occurs sufficiently late enough in the electrodialysis process (also shown in the increase in resistance/voltage) to achieve high recoveries. At the end of the experiment, the concentration of amino acids in the feed is sufficiently low.

To minimize the barrier effect caused by water splitting, the limiting current density needs to be increased. One way of doing so could be increasing the flow rate in time. An increase in the flow rate will increase the mass transfer, hence, increasing the limiting current density [15]. The Reynolds number is increased resulting in a decrease in the boundary layer thickness. However, this is limited by the maximum flow rate of the electrodialysis stack and increasing the pumping speed also increases the energy consumption. Another possibility is to adjust the current in time to guarantee continuous operation below i_{lim}, similar to the procedure described in the work of Habe et al. [14] in the present study.

2.5 Conclusions

The integration of enzymatic conversion and electrodialysis is an interesting and promising technology to isolate single amino acids from amino acid mixtures with similar iso-electric point for biorefinery applications. For all experiments, high recoveries (~90%) at high current efficiencies and low energy consumptions were achieved, leading to pure product streams. The

occurrence of the barrier effect influences the charge behavior of the amino acids, preventing even higher recoveries. Therefore, it is suggested to decrease the $i_{applied}$ in time in order to operate below i_{lim}, minimizing the barrier effect, hence, increasing the current efficiency and decreasing the energy consumption.

The positive results obtained from this investigation open the route to successful isolation of amino acids for biorefinery applications using an integrated process of enzymatic conversion and separation with electrodialysis.

2.6 Acknowledgements

This work is a GSPT project performed in close collaboration with Wageningen University (NL), which investigates the specific modification of the amino acids. Susan Witte is especially acknowledged for the amino acid analysis.

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3

Electrodialysis of basic amino acids - A challenging separation

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ABSTRACT

Amino acids from biobased feeds are an interesting feedstock for the production of biobased chemicals from cheap protein sources, as amino acids already have the required functionalities. Amino acids are zwitterionic molecules whose charge is determined by the surrounding pH. This makes the use of an electrical field as driving force for their separation, as in membrane electrodialysis (ED), attractive. Electrodialysis with commercially available ion exchange membranes was applied for the isolation of the basic amino acids L-Lysine (Lys) and L-Arginine (Arg) as a mixture and further separation of Arg from 1,5 pentanediamine (PDA), which is obtained from the enzymatic conversion of Lys. Electrodialysis experiments for the separation of PDA from Arg are carried out at three different pH's: 12.5 ($PDA^{0}/Arg^{-0.5}$), 10.9 ($PDA^{+0.5}/Arg^{0}$) and 10.0 ($PDA^{+1.5}/Arg^{0}$). Due to the sensitivity of the charge of the amino acids with respect to pH changes, experiments at constant pH (using acid or base dosing or using a buffer in the feed solution) are carried out as well. Control of the pH significantly improves the performance of the process. The separation of PDA from Arg at pH = 10.0, when PDA has an average net charge of +1.5, resulted in the highest recovery (63%) at the highest current efficiency (83%) and significantly low energy consumption (3 kWh/kg). Depending on the conditions, pure streams of amino acids, either Arg (at pH = 12.5) or PDA (pH = 10.9) could be obtained, which shows the strength of the concept of enzymatic conversion combined with electrodialysis for the fractionation of amino acids for biorefinery applications.

3.1 Introduction

The depletion of fossil fuels, the increasing oil prices and the emission of CO_2 rise the need for green alternatives for the production of energy, fuels and chemicals. Emerging sustainable technologies based on renewable resources promote the shift of conventional refineries towards biorefinery concepts. It is well known that a significant amount of biomass feedstocks can be used for the production of such bioenergy, biofuels and biobased chemicals (chemicals produced from biobased feeds) [1]. An interesting feedstock for the production of biobased chemicals is amino acids that can be obtained from cheap protein sources (e.g. side streams from the production of biotransportation fuels from rapeseed oil), as amino acids already have the functionalities (i. e. –N and –O) required for the production of chemicals. In such feeds, the amino acids are usually present as a mixture and need to be isolated for further processing. However, this requires the development of appropriate conversion and separation technologies necessary for large scale, economically viable processes.

Amino acids are zwitterionic molecules whose charge is determined by the surrounding pH. Table 3.1 shows an overview of the average net charge of specific amino acids at neutral pH based on the corresponding isoelectric points (pI, pH at which a particular molecule carries no net charge). The behavior of two basic amino acids, lysine (Lys) and arginine (Arg), two acidic amino acids, glutamic acid (Glu) and aspartic acid (Asp), and three neutral ones is shown.

Table 3.1. Isoelectric points, average mass and average net charge at neutral pH of different amino acids.

Amino acid	pI	Average mass [Da]	Side chain charge (pH =7)
Arginine	10.76	174.20	Positive
Lysine	9.60	146.19	Positive
Aspartic acid	2.85	133.10	Negative
Glutamic acid	3.15	147.13	Negative
Alanine	6.01	89.09	Neutral
Glycine	6.06	75.07	Neutral
Tryptophan	5.89	204.23	Neutral

Because of the charge behavior of amino acids, electrodialysis (ED) is an attractive technology to isolate amino acids for further processing into bulk or specialty chemicals. ED is an electro-membrane process that uses an electrical potential difference over the membrane as driving force for the selective extraction of ions from solutions. ED is widely used for the production of e.g.

table salt and organic acids [2] and can be also used in biorefinery applications to separate e.g. amino acids [3] as long as there is a difference in charge behavior with respect to pH. A schematic representation of the separation of the different amino acids depending on their charge is shown in Figure 3.1.

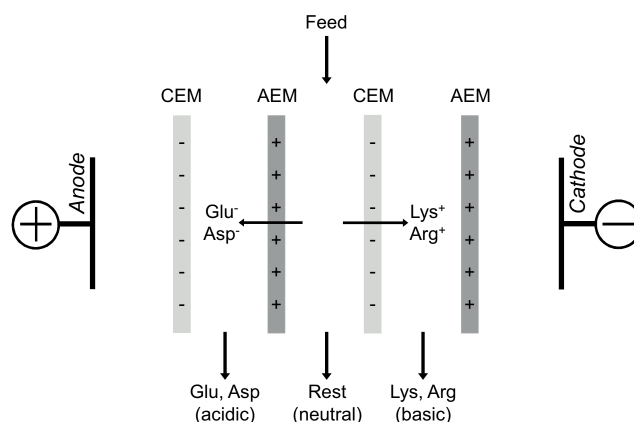

Figure 3.1. Schematic representation of electrodialysis for the separation of amino acids.

To the best of our knowledge, one of the first reports on the separation of amino acids with electrodialysis was done by Martinez et al. where they study the separation of alanine over a wide range of pH (1.4 – 11) [4]. Further studies on the applicability of electro-membrane processes for the separation of amino acids with different charge behavior has been published elsewhere [5 – 8].

Next to the approach presented in Figure 3.1, work has been done on the application of electrodialysis with ultrafiltration membranes (EDUF) for the selective separation of organic charged compounds [9 – 12]. This technology is not only interesting and successful for the isolation of biomolecules with a molecular weight higher than 500 Da [9], but might also have some benefits in the transport rate during the separation of smaller molecules such as amino acids [10] due to the significantly large MWCO of the membrane with respect to the target molecules [11]. This approach might be especially interesting for the separation of amino acids with relatively large molecular weight such as arginine (Table 3.1).

Besides the relatively large molecular weight, other challenges can be expected for the separation of mixtures containing arginine. Sandeaux et al. studied the performance of a cation exchange membrane (CMV Selemion) in the presence of positively charged arginine reporting the poisoning of the membrane by increasing its resistance (from 2 $\Omega \cdot cm^2$ to 80 $\Omega \cdot cm^2$ when the

concentration of arginine in the membrane increased from 0 mmol/g to 1.5 mmol/g) [13]. Fares et al. completed this study determining the individual fluxes of sodium and arginine counter ions and chloride co-ions concluding that the presence of arginine also decreased the membrane permselectivity, thus, adversely affecting the process economics [14]. These observations, together with the relatively high molecular weight of arginine (Table 3.1), increase the potential of EDUF for this specific separation, presented in Chapter 5 of this thesis. In the present study, the proof of principle is based on the separation of lysine alone.

Isolation of e.g. a single basic amino acid from the group of basic amino acids is more complex, as some amino acids have almost identical charge behavior. This is the case for the basic amino acids Lys and Arg (Table 3.1). The separation of one from the other using electrodialysis is an ambitious challenge as their charge dependence on the pH of the solution is very similar. One possibility to achieve separation is the enzymatic modification of either Arg or Lys to obtain new molecules with pronounced differences in the pI's and consequently in the charge behavior of the amino acids, enhancing the successful fractionation of one of the two aa's. Teng et al. studied the enzymatic decarboxylation of Lys into 1,5-pentanediamine (PDA) with the enzyme lysine decarboxylase (LDC) as the catalyst [15]. The enzyme lysine decarboxylase is amino acid specific; it does not convert Arg nor is inhibited by its presence in the mixture [15]. Arg is an interesting feedstock that can be used as raw material for the production of 1,4-butanediamine and urea, both finding their application in the plastics and fertilizer industry [16, 17]. PDA (aliphatic diamine), on the other hand, is a valuable feedstock for the production of nylon [18, 19] and has a completely different charge behavior when compared to the basic amino acids Lys (pI = 9.60) and Arg (pI = 10.76). Consequently, enzymatic modification of Lys into PDA allows the isolation of these two valuable amino acids into separate fractions. The applicability of this approach has been demonstrated to be successful for the acidic amino acids Glu and Asp [20, 21].

Figure 3.2 shows the charge behavior of the basic amino acids, Lys and Arg, the modification product of Lys, PDA and glutamine (Gln) as an example of the neutral amino acids. The charge behavior with pH of the different amino acids of interest and modification products thereof is calculated using the software CurTiPot (Version 3.3.2 for MS-ExcelR _ 1997–2007). It contains a database of dissociation constants of different substances and offers a tool to calculate the average protonation over the full pH range. As such, the average net charge of the amino acids can be calculated based on the charge of the species when fully deprotonated.

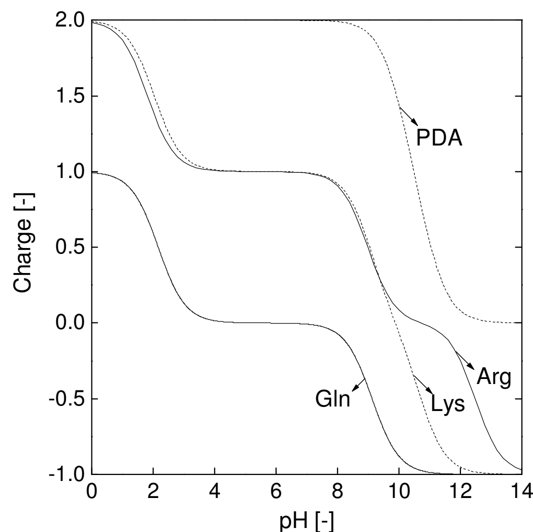

Figure 3.2. Charge behavior of Lys and Arg (acidic amino acids), Gln (neutral amino acid) and PDA (modification product of Lys) with pH.

The isolation of Arg from Lys (or its modification product) with electrodialysis occurs at basic pH, as can be seen in Figure 3.2. In this region, small changes in pH cause significant changes in the average net charge of Lys, Arg and PDA. To minimize water splitting, which will produce protons and hydroxyl ions and consequently induce changes in pH in the different ED compartments [20], operation at or below the limiting current density (LCD) is of utmost importance to obtain the maximum ion flux per unit membrane area [22]. In this way, high recoveries at high current efficiencies and low energy consumptions can be obtained and current and energy are not wasted for side effects such as water dissociation.

Since pH changes may also occur as a result of the transport of the amino acids from one compartment to another (as is the case for other ions present in the solution, e.g. electrode rinse solutions) special attention needs to be dedicated to study the influence of methods to control the pH. To our knowledge, a comparison of the process performance with no pH control and with different ways of pH control (at different values of pH) to overcome the limitations of the fractionation of the specific amino acid mixture has not been reported in literature yet.

The novelty of the present work is the combination of the enzymatic decarboxylation of Lys into 1,5-pentanediamine (PDA) with the separation using electrodialysis, where we especially focus on the effect of the pH on the separation to allow the further isolation of Lys from Arg. Electrodialysis experiments for the separation of PDA from Arg are carried out at two different

pH's: 12.5 ($PDA^0/Arg^{-0.5}$) and 10.9 ($PDA^{+0.5}/Arg^0$). Due to the sensitivity of the average net charge of the amino acids with respect to pH changes, experiments at constant pH are carried out, using pH adjustment by acid or base dosing or experiments where a buffer is used in the feed solution. Furthermore, the separation of PDA from Arg at pH = 10.0, when PDA has a significant positive average net charge of +1.5, and that at pH = 10.9, when PDA has an average net charge of +0.5, is investigated to determine the influence of the magnitude of the charge. The process performance for the isolation of PDA from Arg at these different conditions is evaluated, resulting in an enhanced isolation of single basic amino acids for further conversion into biobased chemicals.

The objectives of the present study are (1) to prove that the combination of electrodialysis and enzymatic conversion for further isolation of pure product streams containing either one amino acid or a modification product thereof is suitable for the basic amino acids, (2) to compare the transport of charged arginine with the transport of charged PDA and (3) to compare the process performance at different pH values (at different average net charges of either Arg or PDA) with no pH control, which serves as the base case, and with two different ways of external pH control determining the best operating conditions for the separation of Arg from PDA to be used as a starting point on further research on the separation of real mixtures integrating enzymatic conversion and electrodialysis.

3.2 Experimental

3.2.1 Materials

Amino acid solutions used for the determination of the limiting current density, as well as for electrodialysis experiments, were prepared using amino acids (Lys, Arg, Gln) in solid state with a purity of 98% or higher obtained from Sigma-Aldrich. PDA, the modification product of Lys, was purchased from Fluka with a purity of 98% (purum). Sodium sulfate solutions (Merck Chemicals and Reagents) with a concentration twice as high as the total amino acid concentration in the feed solution were used as electrode rinsing solutions. For the pH control, 1 M solutions of NaOH and HCl (both from Merck Chemicals and Reagents) were prepared. The buffer solutions were prepared as follows: for the experiments at pH = 10.9, 6.6 ml 0.1 M NaOH were added to 1 L 0.5 M disodium hydrogen phosphate and made up to 2 L with distilled water. For the experiments at pH = 12.5, 40.8 ml 0.2 M NaOH were added to 50 ml 0.2 KCl and made up to 2

L with distilled water. Ion exchange membranes (CEM: Fumasep PEEK reinforced FKB, AEM: Fumasep PEEK reinforced FAB) were purchased from FumaTech GmbH, Germany [20, 23].

3.2.2 Methods

3.2.2.1 Limiting current density

A 4-compartment cell was used to determine the limiting current density [24]. Ion exchange membranes were placed inside the cell between two electrodes (platinized titanium at the anode side, stainless steel at the cathode side), with the membrane under investigation in the middle. The current was increased slowly via a power supply (Delta Elektronika) and the voltage over the membrane between two capillaries close to the membrane was measured with a voltage meter. The current density was plotted against the voltage over the membrane. A detailed description of the method used to determine the limiting current density is given elsewhere [24]. The limiting current density was determined for different amino acids. Furthermore, the limiting current density of solutions with different concentrations of Arg at pH = 12.5 was determined to show the dependence of the LCD on the concentration.

3.2.2.2 Electrodialysis

Electrodialysis experiments (for the separation of Lys from Gln as proof of principle and for the separation of PDA from Arg) were carried out using a 4-compartment cell type ED-40 equipped with titanium/iridium plasma coated stainless steel electrodes (FumaTech GmbH) and an effective membrane area of 36 cm^2. Thick diamond structured welded mesh spacers with a thickness of 475 μm made of PVC/Polyester were placed between the ion exchange membranes in the feed and receiving compartments, while 900 μm thick diamond structured welded mesh spacers made of polyethylene were used for the electrode compartments.

Two different membrane configurations were used for the experiments: for the separation of positively charged amino acids the stack of membranes consisted of a CEM on the anode side (positive electrode), followed by another CEM in the middle and an AEM at the cathode side (negatively charged electrode) to avoid further migration of the positively charged amino acids towards the cathode (Figure 3.3a); for the separation of negatively charged amino acids the stack of membranes consisted of CEMs on the electrode sides (both cathode and anode) and an AEM in the middle (see Figure 3.3b).

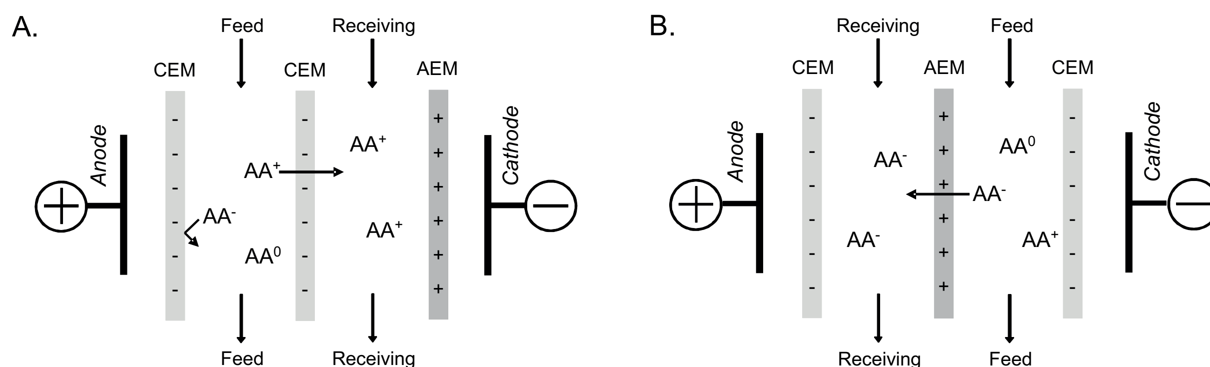

Figure 3.3. Schematic representation of the membrane configuration used in the electrodialysis measurements for the separation of a) positively charged amino acids, and b) negatively charged amino acids.

As feed and receiving streams, 1 L of feed solution with a concentration of 25 mM of each amino acid present and 1 L of MilliQ water were used, respectively. The electrode rinse solution consisted of 2 L Na_2SO_4 with a concentration twice as high as the total amino acid concentration present in the diluate stream and was circulated through both electrode compartments and collected again in one vessel. The pH, the conductivity and the temperature were monitored in time. A sample of each stream was collected and analyzed every hour using U-HPLC [15]. Previous to the electrodialysis experiments for the separation of PDA from Arg, the limiting current density of the charged amino acids at the specific pH and feed composition was determined. Electrodialysis experiments for the isolation of PDA and Arg were performed at 80% of the determined limiting current density.

In addition to electrodialysis experiments without pH control, experiments at constant pH were carried out for comparison. Constant pH was achieved using two different methods: 1) pH adjustment, where either an acid or a base was dosed to the feed solution to either lower or increase the pH when needed, or 2) using a suitable buffer to maintain the pH of the feed solution also containing the target amino acids. The pH was controlled using an αlpha-pH800 pH/ORP controller (EUTECH Instruments). Two Masterflex C/L pumps were connected to the pH controller. When the pH in the feed solution increased above the set value, the pH controller activated the first pump, which dosed the acid to the vessel containing the feed solution. When the pH dropped below the desired value the second pump was activated pumping base into the feed. 0.1M HCl and 0.1 NaOH were used as acid and base, respectively. As buffering systems Na_2HPO_4/NaOH and KCl/NaOH were used for pH = 10.9 and pH = 12.5, respectively. Although KCl/NaOH is not precisely a buffer since NaOH is not the conjugate acid for water, it provides a buffering effect where OH^- may serve as reservoir against the acidification

of the feed compartment due to the migration of basic amino acids. In this work KCl/NaOH will be referred as a buffer to make a clear distinction between the experiments with acid/base dosing and the experiments where a solution is used to provide a buffering effect.

All electrodialysis experiments performed in this work are summarized in Table 3.2. The separation of Lys from Gln was carried out as proof of principle.

Table 3.2. Summary of electrodialysis experiments.

Amino acid mixture	pH	No pH control	pH adjustment	Buffer
Lys^{+1}/Gln^{0}	6.0	X	---	---
$PDA^{0}/Arg^{-0.5}$	12.5	X	X	X
$PDA^{+0.5}/Arg^{0}$	10.9	X	X	X
$PDA^{+1.5}/Arg^{0}$	10.0	X	---	---

3.2.2.3 Process evaluation

To assess the process, the amino acid flux, the recovery, the current efficiency and the power consumption are determined as explained in our previous work [20]. In addition, the retention of the amino acid (or the modification product thereof) in the feed stream is calculated as explained elsewhere [25]. This process parameter indicates the purity of the product (receiving stream). All process parameters are calculated based on the real concentration of amino acids present in the receiving and the feed and the overall current and voltage difference applied. For the experiments with pH adjustment, where NaOH or HCl is added in time to control the pH, the concentration in the feed streams has been corrected for the volume increase.

3.3 Results and discussion

3.3.1 Limiting current density

To allow the operation of ED at maximum current efficiency, the limiting current density (i_{lim}) was determined in a four-compartment electrodialysis cell for the mixtures $Arg^{0}/PDA^{+1.5}$ (pH = 10.0), $Arg^{0}/PDA^{+0.5}$ (pH = 10.9) and $Arg^{-0.5}/PDA^{0}$ (pH = 12.5). The values obtained are close to 3.0 mA/cm^2 (Table 3.3) and are in agreement with the limiting current densities determined for Glu^{-} and Asp^{-} at the same concentrations [20]. The results are shown in Figure 3.4.

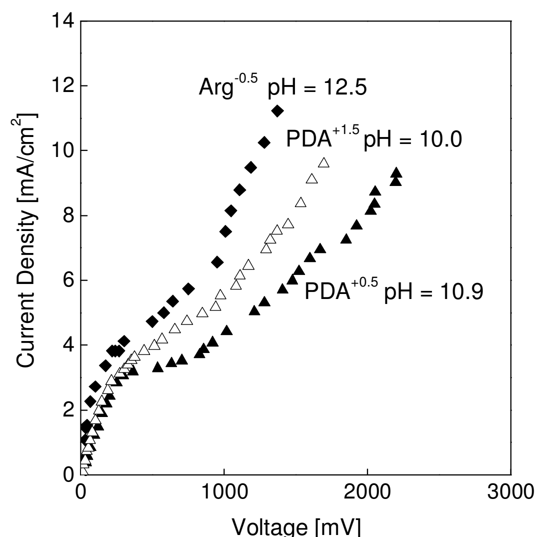

Figure 3.4. Current-voltage plot for PDA$^{+0.5}$ (25 mM, pH = 10.9), PDA$^{+1.5}$ (25 mM, pH = 10.0), Arg$^{-0.5}$ (25 mM, pH = 12.5).

For Arg$^{-0.5}$ at pH = 12.5 the effect of the amino acid concentration on the limiting current density is investigated. Four different concentrations are tested (25, 50, 75 and 100 mM) and a linear relation is obtained between the limiting current density and the amino acid concentration in the bulk solution (Figure 3.5). It is important to specify that it cannot be guaranteed that all the ampholytes are in ionic form. However, under the assumption that for all tested concentrations the same ratio of charged ampholyte is present due to the addition of the same ratio of base for titration, the statement on the linear relation between concentration and limiting current density is still valid. Krol et al. obtained similar results for the limiting current density for NaCl solutions at different concentrations, where a scatter in the overlimiting region was also observed for all experiments, most probable due to hydrodynamic instabilities occurring in this region [23].

The results shown in Figure 3.5 are in agreement with the linear relation between the limiting current density and the concentration in the bulk solution assuming all other parameters (D, δ, t^m and t^s) remain constant [23]. PDA$^{+1.5}$, PDA$^{+0.5}$ and Arg$^{-0.5}$ (same concentration, 25 mM) show a limiting current density of ~ 3 mA/cm^2. The variations between the three different mixtures are not significant and within the experimental error. These results are in agreement with the determined limiting current density for the acidic amino acids, Glu, Arg and GABA [20]. All electrodialysis experiments were operated at 80% of the value of the resulting limiting current density.

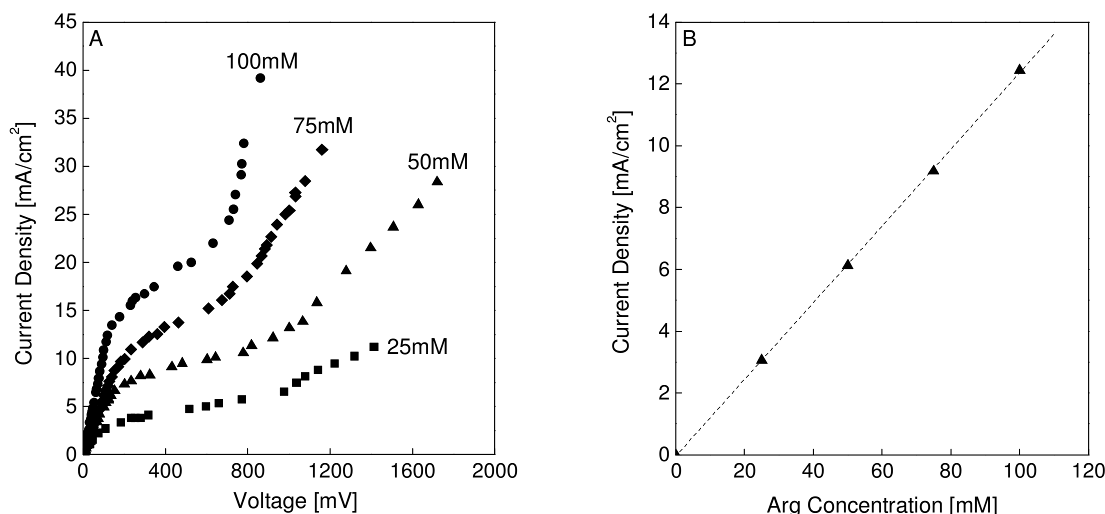

Figure 3.5. a) Current-Voltage plot for different concentrations of Arg $^{-0.5}$ at pH = 12.5; b) Relation between limiting current density and amino acid concentration for Arg $^{-0.5}$ at pH = 12.5.

Having an effective membrane area of 36 cm^2 and an initial concentration in the feed of 25 mM (charged amino acid), the electrodialysis was operated at a current of 0.09A. The determined limiting current densities are summarized in Table 3.3.

Table 3.3. Limiting current density of the single amino acids at the corresponding pH.

Amino acid	pH	Concentration [mM]	LCD [mA/cm²]
$PDA^{+1.5}$	10.0	25	3.00
$PDA^{+0.5}$	10.9	25	2.98
$Arg^{-0.5}$	12.5	25	3.06
		50	6.13
		75	9.18
		100	12.44

The experimental pH was reached by adding the specific molar mass of base (NaOH) or acid (HCl) to reach the desired average net charge of the amino acid under investigation. This means that the concentration of counter ions (ions with opposite charge to the fixed charge of the ion exchange membrane) to be transported through the membrane to be tested does not increase but the concentration of co-ions (ions with the same charge to the fixed charge of the ion exchange membrane) does. Krol et al. studied the limiting current density on monopolar ion exchange membranes for different salt solutions and concluded that the concentration of counter ions, as well as its nature, does have an effect on the limiting current density [24]. However, to the best of our knowledge, no data are reported on the influence of the concentration or nature of the co-

ions present in the solution. When we compare the results obtained in this work with those obtained for the acidic amino acids [20], it cannot be directly concluded that an increase in co-ion concentration does not significantly influence the limiting current density obtained for each amino acid under investigation. However, the possible resulting variations in the diffusion coefficient, transport numbers and boundary layer thickness - parameters that have a direct effect on the limiting current density [24] - seem to cancel each other leading to the maybe apparent lack of influence of the co-ion concentration on the limiting current density.

3.3.2 Electrodialysis

Teng et al. studied the enzymatic modification (decarboxylation) of L-Lysine (Lys) into 1,5-pentanediamine (PDA) with the enzyme lysine decarboxylase (LDC) [15]. In this work we assume that this reaction leads to sufficiently high conversions resulting in a mixture of Arg and PDA. Hence, the experiments presented below cover the investigation of 1) the separation of Lys^+ from uncharged Gln at neutral pH conditions as proof of principle, and 2) the separation of Arg from PDA, the modification product of Lysine, at different pH conditions with and without pH control (pH adjustment, buffer). The process is evaluated with respect to amino acid recovery, current efficiency, energy consumption, amino acid flux and retention (product purity). A concentration of 25 mM for each of the different amino acids was chosen arbitrarily with the only consideration of being far below the solubility limit of Arg and Lys. All electrodialysis experiments were performed three times with the membrane configurations shown in Figure 3.3 depending on the average net charge of the target amino acids and as explained in Section 3.2.2.

3.3.2.1 Separation of Lys^+ from uncharged Gln

The results of the separation of Lys^+ from uncharged Gln (Figure 3.6) are in agreement with previous results [7, 8], indicating that it is possible to separate positively charged amino acids from neutral (uncharged) amino acids at neutral pH. The concentration of Lys^{+1} in the receiving stream increases, while it decreases in the feed. No Gln was found in the receiving stream: as expected Gln was completely retained by the membrane and remained in the feed compartment.

Electrodialysis experiments for the separation of Lys from Gln were intended to serve as proof of principle for the separation of basic amino acids only. These experiments resulted in Lys recoveries of 70% at high current efficiencies and low energy consumptions of around 70% and 1.4 kWh/kg respectively.

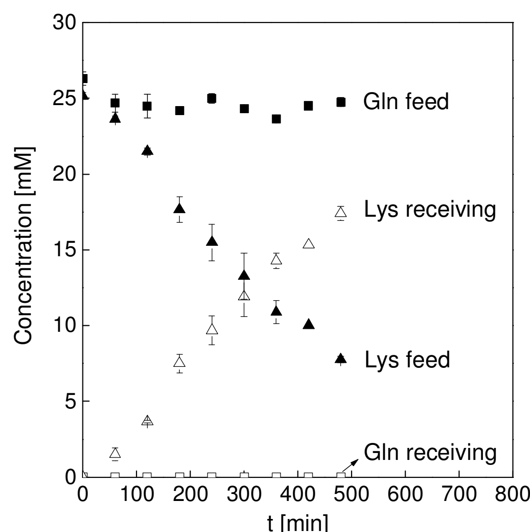

Figure 3.6. Concentration behavior of Lys and Gln in feed and receiving stream during the electrodialysis of Lys^{+1} [25 mM] and Gln^{0} [25 mM] at pH = 6.0 and a flow rate of 25 ml/min.

Experiments were stopped when the pH in the feed was above 8, the pH at which Lys starts losing its positive average net charge (Figure 3.2). Continuation of the experiment would lead to higher product recoveries, but combined with a decrease in the current efficiency and an increase in the energy consumption of the process. Adjustment of the current in time could result in an overall improvement of the process. However, the focus of the present investigation is on the isolation of Arg from PDA and not on the enhancement of the separation of the basic amino acids as a mixture.

3.3.2.2 Separation of Arg from PDA

Electrodialysis experiments for the separation of Arg from PDA were carried out at two different pH's with and without pH control (Table 3.2). The results are discussed in the next paragraphs.

Influence of the average net charge of the amino acids

As a first approach, two different pH conditions were selected based on the average net charge of the amino acids present in the mixture, PDA and Arg. At pH = 12.5, Arg has an average net charge of around -0.5 while PDA is slightly positively charged (Figure 3.2). This difference in average net charge at pH = 12.5 of PDA and Arg suggests the possible isolation of one from the other at these pH conditions. The same behavior, but inverted, can be found at a pH of around 10.9. At this pH (close to the isoelectric point of Arg) PDA has an average net charge of around +0.5 while Arg is slightly negatively charged, suggesting that separation at these conditions can

also take place. To determine and understand the deleterious effects that relatively small pH changes could have on the process performance, an overview of the charge behavior of both amino acids in the surroundings of pH = 12.5 (pH = 12.5 and decreasing) and pH =10.9 (pH = 10.9 and increasing) calculated with the software CurTiPot as explained in the introduction is given in Table 3.4.

Table 3.4. Overview of the average net charge of Arg and PDA in the surroundings of the selected working pH's.

pH = 12.5			pH = 10.9		
pH	Arg	PDA	pH	Arg	PDA
12.2	-0.344	0.052	10.8	-0.005	0.706
12.4	-0.454	0.033	11.0	-0.022	0.535
12.6	-0.568	0.021	11.2	-0.044	0.389

Electrodialysis experiments at pH = 12.5 for the separation of $Arg^{-0.5}$ from PDA^{0} resulted, after 8 hours of operation, in an amino acid flux of $6 \cdot 10^{-5}$ $mol/m^2 \cdot s$, low Arg recovery (25%), low current efficiency (12%) and relatively high energy consumption (3.5 kWh/kg). The retention of PDA in the feed compartment was found to be 99.9%, meaning that a rather pure product was obtained nevertheless. Longer operation times resulted in higher recoveries but lower product purity. On the other hand, shorter operation times resulted in higher product purity but lower recovery. As an example, the results at t = 7 h, t = 8 h and t = 10 h are summarized in Table 3.5.

Table 3.5. Process parameters in time of the electrodialysis of $Arg^{-0.5}/PDA^{0}$ at pH = 12.5.

Time [h]	7	8	10
Flux [10^{-5} mol/m^2s]	6.08 ± 1.29	6.12 ± 1.16	6.80 ± 0.17
Recovery [%]	23.06 ± 3.91	25.01 ± 3.64	41.16 ± 0.83
Current efficiency [%]	11.74 ± 1.50	11.81 ± 1.24	13.12 ± 0.32
Energy consumption [kWh/kg]	2.85 ± 0.48	3.51 ± 0.54	4.97 ± 0.51
Retention [%]	99.98 ± 0.03	99.86 ± 0.04	99.08 ± 0.03

The decrease in flux and current efficiency and the increase in the energy consumption in time can be explained by the pH changes in the feed compartment in time, as can be seen in Figure 3.7. It consists of two x-axes, where the main axis (bottom) shows the time [min], representing the duration of the electrodialysis experiment. The second x-axis (top) shows the average net charge [-] of the amino acids present in the mixture to be separated.

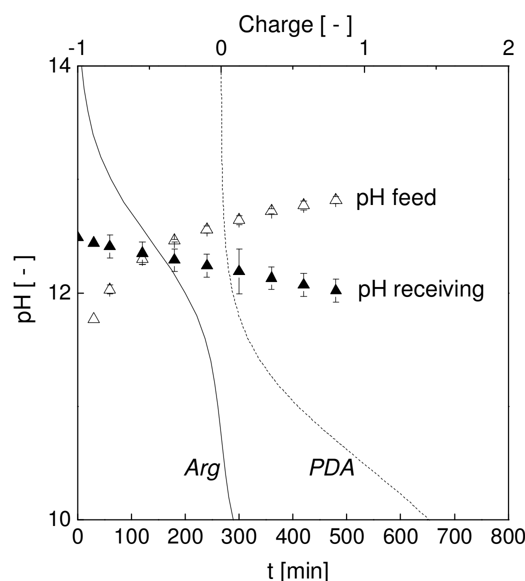

Figure 3.7. 1st x-axis: pH plotted against time for $PDA^{0}/Arg^{-0.5}$ [25 mM/25 mM] during electrodialysis experiments; 2nd x-axis: pH plotted against average net charge for PDA and Arg. Flow rate: 25 ml/min; Initial pH of the feed = 12.5.

The pH [-] is plotted on the y-axis. After 8 hours of operation (t = 480 min in Figure 3.7) the pH in the feed decreased down to 12. At this pH, the average net charge of PDA starts increasing slowly, while the average net charge of Arg goes to zero (Figure 3.7). The pH in the feed decreased to around 11.8, causing the average net charge of Arg to go from around -0.5 to around -0.2. At this point, the ions in the feed to be transported are scarce and energy is used for water splitting instead of being used for the transport of amino acids, as reported previously [20]. When comparing the electrodialysis of $PDA^{0}/Arg^{-0.5}$ at pH = 12.5 with the electrodialysis of $PDA^{+0.5}/Arg^{0}$ at pH = 10.9, an improvement in the separation performance can be observed. The results after 8 h of operation of both different experiments are summarized in Table 3.6.

Table 3.6. Process parameters in time of the electrodialysis of $Arg^{-0.5}/PDA^{0}$ at pH = 12.5 and the electrodialysis of $PDA^{+0.5}/Arg^{0}$ at pH = 10.9. Operation time = 8 h.

Amino acid mixture	$PDA^{0}/Arg^{-0.5}$	$PDA^{+0.5}/Arg^{0}$
pH	12.5	10.9
Flux [10^{-5} mol/m^{2}s]	6.12 ± 1.16	9.92 ± 0.86
Recovery [%]	25.01 ± 3.64	50.34 ± 2.88
Current efficiency [%]	11.81 ± 1.24	19.15 ± 0.17
Energy consumption [kWh/kg]	3.51 ± 0.54	3.55 ± 0.02
Retention [%]	99.86 ± 0.04	100.0 ± 0.00

Electrodialysis of $PDA^{+0.5}/Arg^{0}$ at pH = 10.9 results in complete retention of Arg in the feed compartment, leading to an extremely high purity product (PDA). This can be explained based on the permselectivity of the membranes used. The separation of negatively charged Arg at pH = 12.5 takes place using anion exchange membranes (AEM), while the separation of positively charged PDA uses cation exchange membranes (CEM). Higher retention of co-ions is observed for the latter case, hence, when the amino acids are transported through CEMs. The permselectivity of cation exchange membranes is in general higher than that of anion exchange membranes, as is the case in our research: FKB-PEEK (CEM) and FAB-PEEK (AEM) membranes have a permselectivity of 98% for K^+ over Cl^- and 96% for Cl^- over K^+, respectively [20, 24].

The lower average net charge of the amino acids present in the feed has an influence on the energy consumption as well. At the beginning of the process, the feed solution used for the electrodialysis experiments at pH = 12.5 has a higher conductivity (6.2 mS/cm) compared to the feed solution used when working at pH = 10.9 (2.3 mS/cm). This is due to the increased ion concentration, since NaOH was added to adjust the pH. OH^- ions react with the amino acids but Na^+ is still present in the solution. The higher initial conductivity of the feed solution allows lower initial voltages to reach the set current (i.e. 7.0 V at pH = 10.9 and 5.6 V at pH = 12.5). However, during the experiment, the applied voltage increases significantly for the experiment at pH = 12.5, while it remains almost constant for the experiment at pH = 10.9, as shown in Figure 3.8. The reason for this is that other ions present in the solution are transported while at the same time the average net charge of the amino acids decreases. The lack of ions to be transported leads to increased voltages. This results in a higher energy consumption for the experiment at pH = 12.5, as the energy consumption is directly related to the voltage difference over the membranes [20].

The presence of other charged species used to control the pH might negatively influence the efficiency of the process as especially smaller ions can also be transported under the influence of an electrical field, making the process less efficient. To investigate this effect, experiments with pH control using acid or base dosing or the use of a buffer were carried out at pH = 10.9 and pH = 12.5. The results are discussed in the following paragraphs.

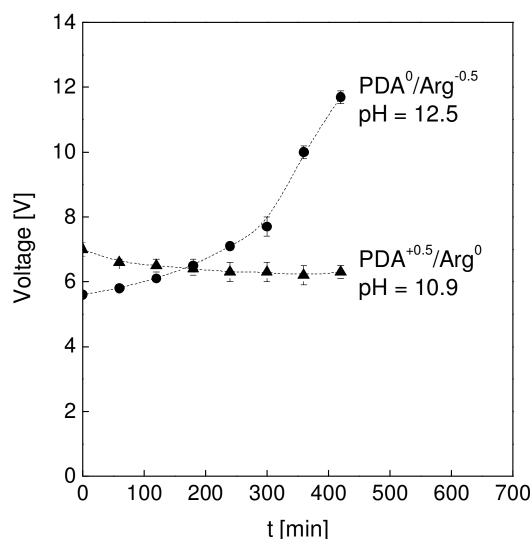

Figure 3.8. Behavior of the voltage during the electrodialysis experiments of $PDA^{+0.5}/Arg^{0}$ at pH = 10.9 and $PDA^{0}/Arg^{-0.5}$ at pH = 12.5.

pH control for enhanced process performance: pH adjustment and the use of a buffer

Electrodialysis experiments at constant pH are carried out using two different methods to control the pH: 1) the adjustment of the pH in time by the addition of an acid (0.1M HCl) or a base (0.1M NaOH) when the pH drops or increases below or above the set value, and 2) using a buffer for the feed solution (Na_2HPO_4/NaOH for pH = 10.9, KCl/NaOH for pH = 12.5). The change in PDA concentration in time for the feed and the receiving streams for the experiments at constant pH (pH adjustment, buffer) are compared with the electrodialysis experiments without pH control (see Figure 3.9). As expected, the use of a buffer in the feed solution does not enhance the separation, but deteriorates the process. This is due to the addition of small ions that compete with the target amino acids being also transported towards the receiving stream and the change of electrophoretic mobility of the amino acids due to the increased ionic strength of the solution as it has been studied and reported for other molecules [26].

In the end, this two effects govern over the constant average net charge of the target amino acids throughout the experiment reducing –rather than increasing- the recovery of the target molecules. On the other hand, the use of pH adjustment, although leading to similar recoveries for the separation of $PDA^{+0.5}$ from Arg^{0} as in experiments without pH control, shows a significant improvement over the system without pH control in terms of current efficiency and energy consumption.

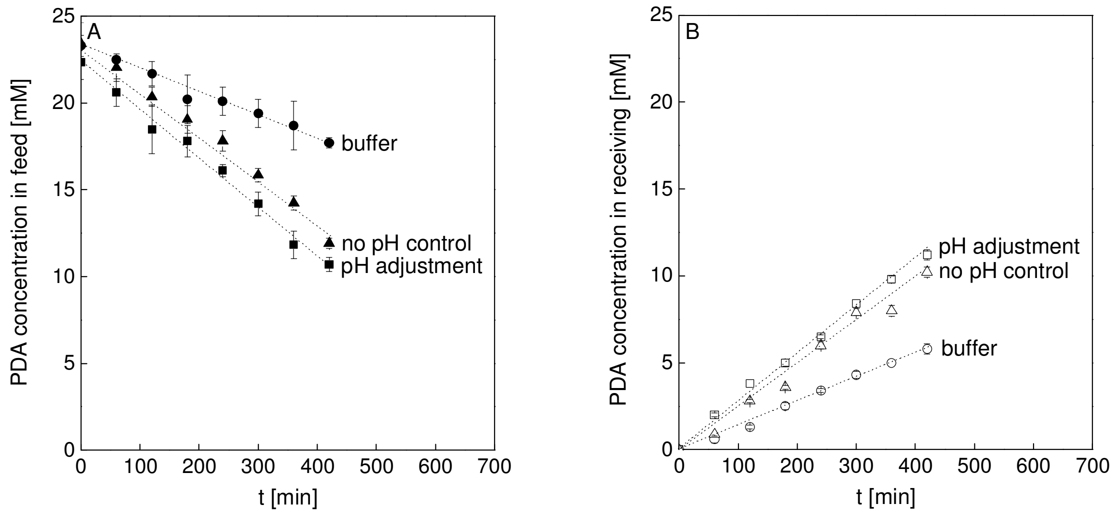

Figure 3.9. Concentration behavior of PDA in a) feed and b) receiving streams during the electrodialysis of $PDA^{+0.5}$ [25 mM] and Arg^{0} [25 mM] at pH = 10.9 and 25 ml/min without and with pH control (pH adjustment, buffer).

An increase of 25% in current efficiency and a decrease of 40% in energy consumption are obtained when adjusting the pH in time. This can be explained based on the average net charge of the amino acids present. At these pH conditions, PDA has an initial average net charge of +0.5. If the pH increases in time, the average net charge of PDA decreases. The current efficiency is directly related to the average net charge of the target amino acid [20]. When the pH is kept constant by adjusting it in time, the average net charge of PDA will remain constant throughout the experiment, leading to higher current efficiencies. At the same time, the higher (constant) average net charge of the PDA present in the feed represents a lower resistance throughout the experiment, as indicated by the lower voltage difference applied during the experiment. This has a direct positive effect on the values obtained for the energy consumption, as the energy consumption is directly proportional to the applied voltage difference [20]. The results are shown in Table 3.7.

Table 3.7. Process performance parameters for the recovery of $PDA^{+0.5}$ from Arg^{0} using ED with and without pH control (acid/base dosing, buffer). Initial feed pH = 10.9.

Amino acid system	$PDA^{+0.5}/Arg^{0}$ (pH = 10.9)		
	no pH control	acid/base dosing	buffer
Flux [10^{-5} mol/m^{2}s]	9.92 ± 0.86	11.43 ± 1.32	5.41 ± 0.24
Recovery [%]	50.34 ± 2.88	49.10 ± 1.56	26.18 ± 1.89
Current efficiency [%]	19.15 ± 0.17	22.06 ± 2.54	10.43 ± 0.45
Energy consumption [kWh/kg]	3.55 ± 0.02	2.36 ± 0.50	5.28 ± 0.41
Retention [%]	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00

The lower efficiency obtained for the process performance with a buffer can be explained based on the migration of the other ions present in the feed solution. The use of a buffer introduces other small ions in the solution, which can be transported as well under the influence of the electrical field. For practical reasons, we only analyzed the amino acid concentration. However, from the change in the conductivity of the receiving during the different experiments at pH = 10.9 (without pH control, with acid/base dosing and with buffer; Figure 3.10), migration of other ions from the feed towards the receiving compartment becomes obvious. The conductivity of the receiving solution during the three different experiments shows almost the same behavior. The concentration of the amino acids present in the receiving stream when using a buffer is only half the concentration of amino acids in the streams used in the experiments without pH control and with acid/base dosing. This suggests that other charged species have been transported to the receiving stream when a buffer is used instead of the amino acids only.

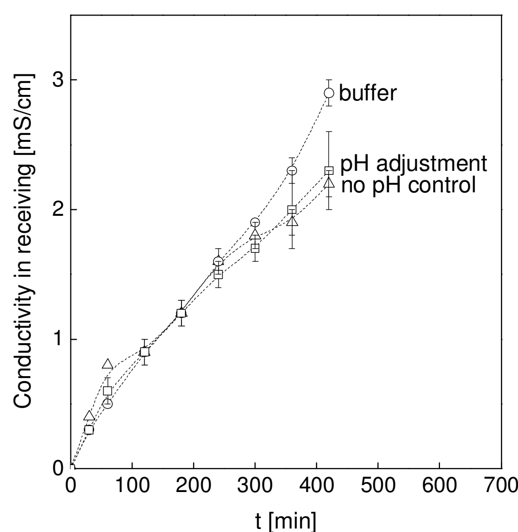

Figure 3.10. Conductivity of the receiving compartment during the electrodialysis of $PDA^{+0.5}$ [25 mM] and Arg^0 [25 mM] at pH = 10.9 and 25 ml/min without and with pH control (pH adjustment, buffer).

Effect of average net charge on product recovery: electrodialysis of $PDA^{+1.5}/Arg^0$ at pH = 10.0

A third possibility for the separation of PDA from Arg is the electrodialysis at pH = 10.0. At these pH conditions, PDA has an average net charge of +1.5, compared to an average net charge of +0.5 at pH = 10.9 and an average net charge of 0 at pH = 12.5. At pH = 10.0 the average net charge of the other amino acid in the mixture, Arg, is approximately +0.1, which is higher than its average net charge at pH = 10.9 (i.e. -0.2). It is expected that the separation will improve due to the higher average net charge of PDA (+1.5) but the purity of the receiving (product) stream

might decrease as transport of Arg towards the receiving stream might also occur due to its slightly positive average net charge (around +0.1). The behavior of the PDA concentration in time in the feed and the receiving compartment is shown in Figure 3.11.

The electrodialysis of $PDA^{+1.5}/Arg^{0}$ at pH = 10.0 results in high fluxes ($14 \cdot 10^{-5}$ mol/m^2s) and high amino acid recoveries (63%) when compared to previous experiments. High recoveries are obtained at very high current densities (83%) and low energy consumptions (3 kWh/kg). The explanation lies in the significantly higher average net charge of the amino acid to be transported, in this case PDA, which at pH = 10.0 has an average net charge of +1.5. The drawback of operating under these conditions is, as expected, the transport of the slightly positively charged Arg towards the receiving compartment as well. Arg retention obtained after 8 h of operation is nevertheless 96.5%.

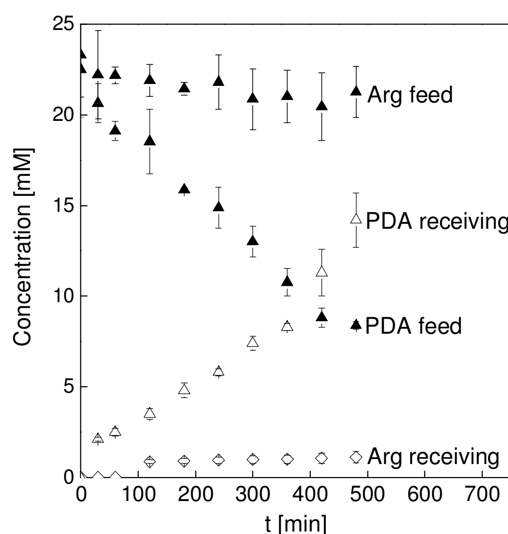

Figure 3.11. Concentration behavior of PDA and Arg in feed and receiving streams during the electrodialysis of $PDA^{+1.5}$ [25 mM] and Arg^{0} [25 mM] at pH = 10.0 and a flow rate of 25 ml/min (no pH control).

For these conditions, changes in pH (10.0 – 10.3) cause a decrease in the average net charge of PDA from 1.5 to 1.3 while the current remains constant. However, the variation in the limiting current density for $PDA^{+1.5}$ and $PDA^{+0.5}$ does not differ significantly, and still a significant concentration of charged amino acids are present, hence, water splitting does not set in as significant as for the case of $PDA^{+0.5}$ or $Arg^{-0.5}$ where the lack of ions caused by small pH changes significantly enhances water splitting. Therefore, higher recovery is possible in this case with no

pH control. However, as in the previous cases, longer operation leads to higher product recovery but compromises the purity.

It is important to mention that at the start of the experiment the current density corresponds to 80% of the determined limiting current density. However, with the depletion of target charged amino acids in the feed solution it is expected to reach the overlimiting region. This would result in significant pH changes due to water splitting limiting further amino acid recovery due to the barrier effect. This change in pH neutralizes the amino acids avoiding their further separation and further enhancing water splitting. Nevertheless, for the present case, the change in pH can be attributed to the transport of amino acids and other ions rather than to water splitting since it starts occurring from the beginning. Therefore, the focus is on finding a suitable method of pH control rather than on decreasing the occurrence of water splitting, for example, by decreasing the current in time.

To summarize the research, an overview of the process performance parameters of all experiments carried out is shown in Table 3.8. The results show that the separation is best performed at a pH = 10.0 (high flux, recovery, current efficiency and low energy consumption), having as trade-off a slight decrease in product purity only.

Table 3.8. Process performance parameters for the recovery of PDA and Arg using ED at different pH values.

Amino acid mixture	$PDA^0/Arg^{-0.5}$		$PDA^{+0.5}/Arg^0$		$PDA^{+1.5}/Arg^0$
pH	12.5		10.9		10.0
pH control mode	---	---	acid/base dosing	buffer	---
Flux [10^{-5} mol/m^{2}s]	6.12 ± 1.16	9.92 ± 0.86	11.43 ± 1.32	5.41 ± 0.24	14.04 ± 0.15
Recovery [%]	25.01 ± 3.64	50.34 ± 2.88	49.10 ± 1.56	26.18 ± 1.89	63.29 ± 0.73
Current efficiency [%]	11.81 ± 1.24	19.15 ± 0.17	22.06 ± 2.54	10.43 ± 0.45	82.79 ± 2.02
Energy consumption [kWh/kg]	3.51 ± 0.54	3.55 ± 0.02	2.36 ± 0.50	5.28 ± 0.41	2.77 ± 0.24
Retention [%]	99.86 ± 0.04	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	96.54 ± 2.85

3.4 Conclusions

The separation of basic amino acids combining electrodialysis and enzymatic conversion confirms that the proposed approach offers an interesting and promising route for the isolation of single amino acids with similar charge behavior for biorefinery applications.

Due to the sensitivity of the charge behavior of the amino acids with respect to pH changes during electrodialysis, different methods to control the pH were evaluated. Evaluation of process parameters, current efficiency and energy consumption, obtained when adjusting the pH in time for the electrodialysis at pH = 10.9 prove the importance of pH control and significantly improve the process performance.

The separation of PDA from Arg at pH = 10.0, when PDA has an average net charge of +1.5, resulted in the highest recovery (63%) at the highest current efficiency (83%) and significantly low energy consumption (3 kWh/kg). Depending on the conditions, pure streams of amino acids, either Arg (at pH = 12.5) or PDA (at pH = 10.9 and pH = 10.0) could be obtained. These results show the strength of the concept of enzymatic conversion combined with electrodialysis for the isolation of amino acids for biorefinery applications.

3.5 Acknowledgements

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4

Electrodialysis of neutral amino acids - Segmented bipolar membrane for internal pH control

This chapter has been submitted (in adapted form) to the Journal of Membrane Science as:
O.M. Kattan Readi, J.H. Kuenen, H.J. Zwijnenberg, K. Nijmeijer, Novel membrane concept for internal pH control in electrodialysis of amino acids using a segmented bipolar membrane (sBPM).

ABSTRACT

In electrodialysis of amino acids, pH-changes play an important role in terms of the efficiency of the process. Due to the zwitterionic character of amino acids, small pH changes may result in significant changes in the charge of the amino acids. This decreases either the recovery of the target ions or the product purity. An example of this is the separation of the modification product of serine (Ser) from ethanolamine (Etn) where the decrease in the pH in the feed compartment leads to a decrease in the product purity due to co-transport of alanine (Ala), that becomes charged at low pH. External pH control, like acid/base dosage or the use of a buffer, adds smaller ions that compete with the amino acids during the separation and decreases the process performance. Here we investigate the separation of Etn and Ala using electrodialysis with a segmented bipolar membrane (sBPM). The designed sBPM has both monopolar and bipolar areas. The transport of positively charged Etn at neutral pH is allowed through the monopolar areas without decreasing the separation performance. At the same time water splitting is enhanced at the bipolar areas keeping the pH constant during the experiment. This approach resulted in a recovery of 37 % of Etn, while Ala was completely retained in the feed stream. These results show the strength of the concept of using a segmented bipolar membrane to combine ionic transport and water splitting to control the pH simultaneously.

4.1 Introduction

With the aim of replacing the conventional process route that uses fossil feedstocks for the production of biobased chemicals, electrodialysis (ED) combined with enzymatic modification has been suggested and applied for the fractionation of amino acid mixtures [1-4]. Some amino acids, such as L-glutamic acid and L-aspartic acid (acidic amino acids), can be successfully separated from an amino acid mixture at neutral pH and pH changes occurring during the process do not affect significantly the performance [1]. Other systems, however, are more complex. For example, the isolation of the modification product of L-lysine (Lys), 1,5 – pentanediamine (PDA), from L-arginine (Arg) is more challenging since the charge of those amino acids is highly sensitive to pH changes occurring during the ED process [3]. External pH control such as acid/base dosing or the use of a buffer results in little or no improvement of the overall amino acid recovery due to the addition of smaller ions that compete with the target molecules [3]. Therefore, other ways of pH control are essential to allow this specific separation.

Bipolar membrane electrodialysis (BPM-ED) is a technology used for the production of protons and hydroxyl ions [5] widely studied and applied for a multitude of technical applications, amongst them the production of organic acids [6-16], in environmental applications such as waste water treatment [17-20] or carbon dioxide recovery [21] and in the food industry [22, 23]. A bipolar membrane consists of an anion exchange layer (positively charged) and a cation exchange layer (negatively charged). At the interface of both layers, water splitting takes place. H^+ that migrates through the cation exchange membrane towards the cathode and OH^- that migrates through the anion exchange membrane towards the anode are produced. Overall, at the anode side an alkaline solution is formed and an acidic solution appears at the cathode side [5]. At the same time due to the nature of the membrane, the transport of ions across the bipolar membrane is prevented. This concept can be used to control the pH in electrodialysis applications and is called BPM-ED.

Such is the case for example for the concentration and alkalization of monethanolamine salts [24, 25]. Similar to the production of organic acids, BPM-ED can be applied to produce an organic base, e.g. monoethanolamine, from its salt, in this case monoethanolamine salts produced by fermentation [25]. In these studies, besides achieving the concentration and alkalinization of ethanolamine salts with BPM-ED, the current efficiency of the process could be improved by using proton blocking anion-exchange membranes and a three compartment configuration [24,

25]. To convert the organic base salt into the organic base and the acid, water splitting and electroosmotic transport are beneficial.

In most of the cases where conventional electrodialysis is applied, pH changes during electrodialysis of amino acids occur as a product of water splitting. Anion exchange membranes are used to transport negatively charged target amino acids in the direction of the anode (feed solution containing deprotonated amino acids in the cathodic side of the membrane). When water splitting occurs at the membrane surface, the pH increases in the anode side while it decreases in the cathodic side of the AEM [26].

It was suggested that the water dissociation behavior is determined by the nature of the charged groups of the membrane [27] and that it hardly occurs at the surface of cation exchange membranes [28]. However, although less pronounced, pH changes are also observed when cation exchange membranes are used to transport cations [26]. Electric current efficiency for water splitting is found to be on average 2 – 3 orders of magnitude smaller for cation exchange membranes over anion exchange membranes [29]. Increased water splitting can take place when a cation exchange membrane is electrolyzed, for example, in $MgCl_2$ and $NiCl_2$ solutions due to the formation of $Mg(OH)_2$ and $Ni(OH)_2$ crystals in the boundary layer [29, 30] or in the presence of ionizable molecules such as amino acids [31-33]. In these cases, BPM-ED can also be applied to control the pH as shown in Figure 4.1 (Figure 4.1a for AEM, Figure 4.1b for CEM). The feed solution that consists of deprotonated amino acids is circulated in the cathodic side, i.e. the acidic side of the membrane and these negatively charged amino acids combine with either H^+ or OH^- that is produced by water splitting.

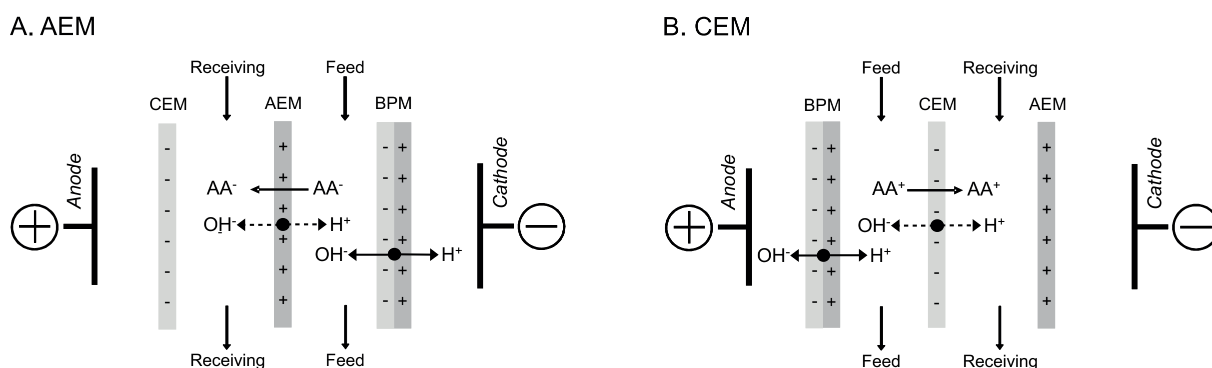


Figure 4.1. Schematic representation of pH control using bipolar membranes for a) AEM for the transport of negatively charged amino acids and b) CEM for the transport of positively charged amino acids.

As a result of this phenomena, reported as the barrier effect [1, 3, 34], further migration of the now differently charged ions is restricted, thus decreasing the yield. However, the pH can also change as a result of the transport of the ions itself. For example, consider a highly basic molecule that is transported in positive form in the direction of the cathode (Figure 4.1b) without water splitting taking place at the CEM surface. This is the case for the separation of ethanolamine (Etn) from neutral amino acids, for example alanine (Ala). Etn is the modification product of the decarboxylation of L-serine (Ser) using L-serine decarboxylase (SDC), a pyridoxal 5'-phosphate (PLP) dependent enzyme. Etn is an industrial product used as intermediate in many applications such as in the herbicide, textile, metal, detergent, plastics and personal care products industries [35]. Besides being an interesting industrial chemical intermediate, Etn exhibits a different charge behavior compared to the neutral amino acids, enabling its separation using ED. The charge behavior with pH of Etn compared to that of Ser and Ala is shown in Figure 4.2.

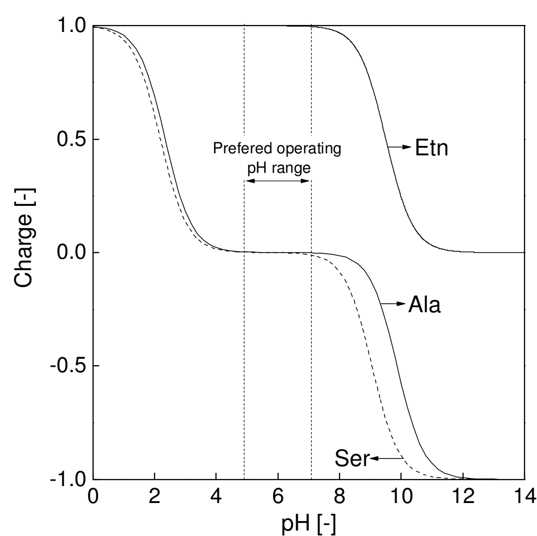


Figure 4.2. Charge behavior of ethanolamine (Etn), which is the modification product of L-serine (Ser) and alanine (Ala), a neutral amino acid, with pH.

The figure clearly shows the sensitivity of the charge of Etn, Ser and Ala for small variations in pH. Only small variations in pH in the range of >7 cause an immediate change in the charge of Etn from +1 to 0 while Ser and Ala go from 0 to -1. At lower pH the charge of Etn remains at a constant value of +1 but Ala and Ser become positively charged.

In standard electrodialysis (without the use of bipolar membranes) of Etn at neutral pH, the pH changes opposite to the effect induced by water splitting at the CEM used as transport membrane. The pH decreases in the anodic side of the membrane (feed side) while it increases in

the cathodic side of the membrane (receiving side). To maintain electroneutrality at the anodic side of the membrane (feed side) either a negative ion leaves the compartment or a positive ion comes in. In the case that a negative ion, i.e. OH^-, leaves the compartment or a positive ion, i.e. H^+, enters the compartment, the pH will decrease even further. In that case, the pH changes cannot be overcome using a conventional bipolar membrane as is used in BPM-ED (Figure 4.1b).

Water splitting at the surface of the transport membrane would overcome the pH changes caused by amino acid transport. This can be achieved by operating above the limiting current density. Nevertheless, water splitting is limited for CEMs (significantly lower when compared to AEM) and high current densities are needed [26]. Although the amino acid flux would increase and the pH can be controlled, increasing significantly the operational current density would result in a less efficient process in terms of current efficiency and energy consumption. Here we propose the integration of a different bipolar membrane concept to control the pH, while at the same time allowing the transport of amino acids. More specifically, we propose the use of a segmented bipolar membrane (sBPM) that contains defined monopolar areas for Etn transport and bipolar areas to enhance water splitting as shown in Figure 4.3.

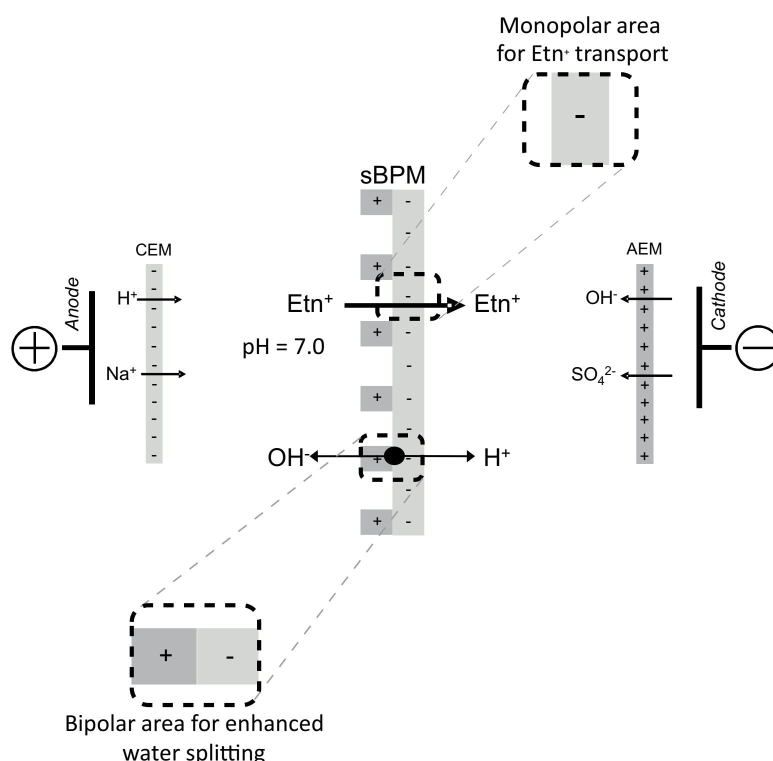
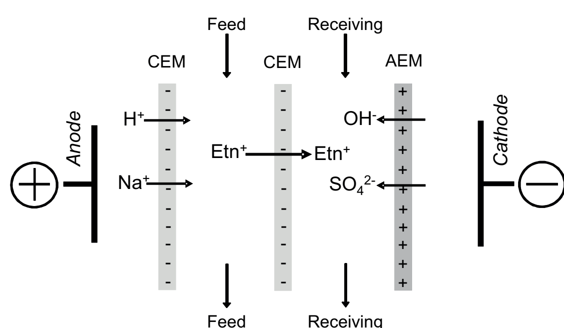


Figure 4.3. Schematic representation of the proposed use of a segmented bipolar membrane (sBPM) for pH control during electrodialysis of ethanolamine (Etn).

In this work we compare the process performance of conventional electrodialysis to that of a process with a segmented bipolar membrane for internal pH control and evaluate the effectiveness and limitations of this method for pH control for amino acid separation. Figure 4.4 shows a schematic representation of the ion transport during electrodialysis of ethanolamine with a standard cation exchange membrane (Figure 4.4a) compared to that of electrodialysis with the prepared sBPM (Figure 4.4b). As shown in Figure 4.4b, transport of the target ions, Etn in this case, can take place through the monopolar areas, while water splitting is expected to be enhanced at the bipolar areas.

A. Conventional electrodialysis



B. Electrodialysis with a sBPM

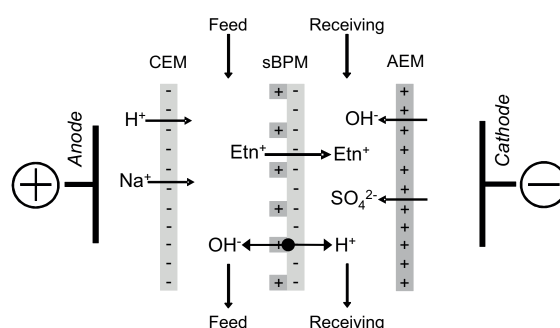


Figure 4.4. Schematic representation of ion transport during the electrodialysis of ethanolamine (Etn) with a) a standard CEM and b) a sBPM.

4.2 Experimental

4.2.1 Materials

Feed solutions used for the determination of the limiting current density, as well as for the electrodialysis experiments were prepared using Ala, Ser (solid state) and Etn (liquid) with a purity of 98% or higher obtained from Sigma-Aldrich. Sodium sulfate solutions (Merck Chemicals and Reagents) with a concentration twice as high as the total amino acid concentration in the feed solution were used as electrode rinsing solutions. Ion exchange membranes type Fumasep PEEK reinforced FKB (CEM) and Fumasep PEEK reinforced FAB (AEM) were purchased from FumaTech GmbH, Germany [1].

4.2.2 Methods

4.2.2.1 Preparation of the segmented bipolar membrane (sBPM)

The sBPM was prepared by perforating an AEM (FAB-PEEK) and gluing it onto a CEM (FKB-PEEK) using a sPEEK solution in methanol. In this case the CEM (FKB-PEEK) is the support membrane and serves also as transport membrane for positively charged Etn through the perforated areas from the AEM (FAB-PEEK). The non-perforated areas of the AEM (FAB-PEEK) glued together to the CEM (FKB-PEEK) serve as bipolar areas for enhanced water splitting.

4.2.2.2 Limiting current density

The limiting current density (LCD) of Etn was determined for different concentrations at neutral pH using a 4-compartment cell equipped with a platinized titanium electrode as the anode and a stainless steel cathode. The membrane under investigation was placed in the middle and the current was increased slowly via a power supply (Delta Elektronika), while the voltage over the membrane between two capillaries close to the membrane was measured with a voltage meter. The current density was plotted against the voltage over the membrane. A detailed description of the method used to determine the limiting current density is given elsewhere [26].

4.2.2.3 Electrodialysis

Electrodialysis experiments for the separation of Etn from Ala were carried out using a 4-compartment cell type ED-40 equipped with titanium/iridium plasma coated stainless steel electrodes (FumaTech GmbH) and an effective membrane area of 36 cm^2. Thick diamond structured welded mesh spacers with a thickness of 475 μm made of PVC/Polyester were placed between the ion exchange membranes in the feed and receiving compartments, while 900 μm thick diamond structured welded mesh spacers made of polyethylene were used for the electrode compartments [26]. The membrane configuration consisted of a CEM at the anode side (positive electrode), followed by another CEM in the middle and an AEM at the cathode side (negatively charged electrode) to avoid further migration of the positively charged amino acids towards the cathode (Figure 4.4a); for internal pH control, the sBPM was placed in the middle (Figure 4.4b).

1 L solution with a concentration of 25 mM of each amino acid and 1 L MilliQ water were used as feed and receiving solution, respectively. The electrode rinse solution consisted of 2 L, 0.1 M Na_2SO_4 and was pumped with two Masterflex pumps (Model 7521-25) at a flowrate of 50

ml/min. The experiments were carried out at constant current (at the previously determined i_{lim}) using a power supply from Delta Elektronika (0-30 V; 0-5 A). Every hour, pH and conductivity were monitored and a sample of each stream was collected and analyzed using U-HPLC [4].

4.2.2.4 Process evaluation

To assess the process, the amino acid flux, the recovery, the current efficiency, the power consumption and the amino acid retention were determined as explained in our previous work [1, 3]. All process parameters are calculated based on the real concentration of amino acids present in the receiving and the feed compartment and the overall current and voltage difference applied.

4.3 Results and discussion

4.3.1 Preparation of the segmented bipolar membrane (sBPM)

The prepared sBPM consisted of a perforated AEM (FAB-PEEK) and a standard CEM (FKB-PEEK) glued together. This resulted in a total monopolar area for ionic transport per membrane of 25.2 cm^2 (70%) and a total bipolar area for water splitting of 10.8 cm^2 (30%). Figure 4.5 shows a schematic representation of the prepared sBPM.

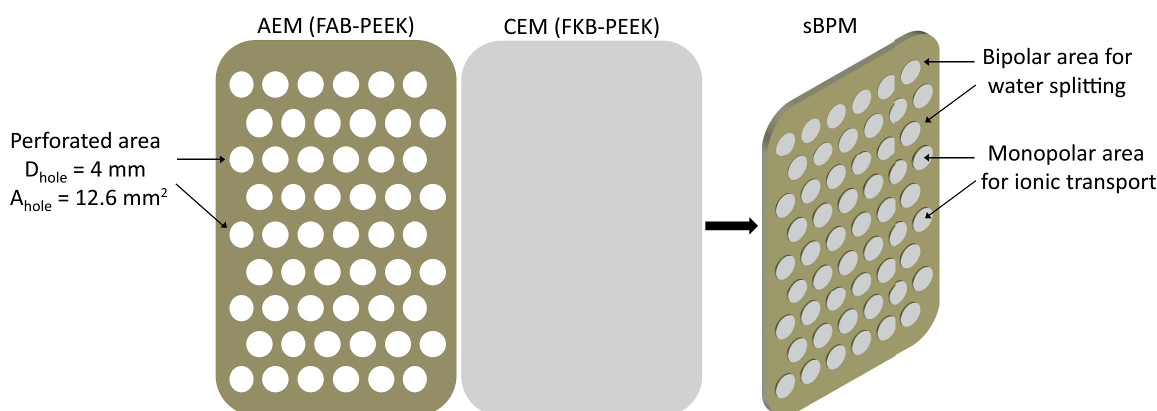


Figure 4.5. Preparation of the segmented bipolar membrane (sBPM).

4.3.2 Limiting current density

The LCD of Etn solutions with different concentrations (6, 12.5, 25 and 50 mM) was determined to allow the operation of ED at maximum current efficiency. The value obtained for 25 mM Etn is 2.0 mA/cm^2 and is in agreement with the LCD of other solutions of amino acids and modification products thereof [1, 3]. The results are summarized in Figure 4.6

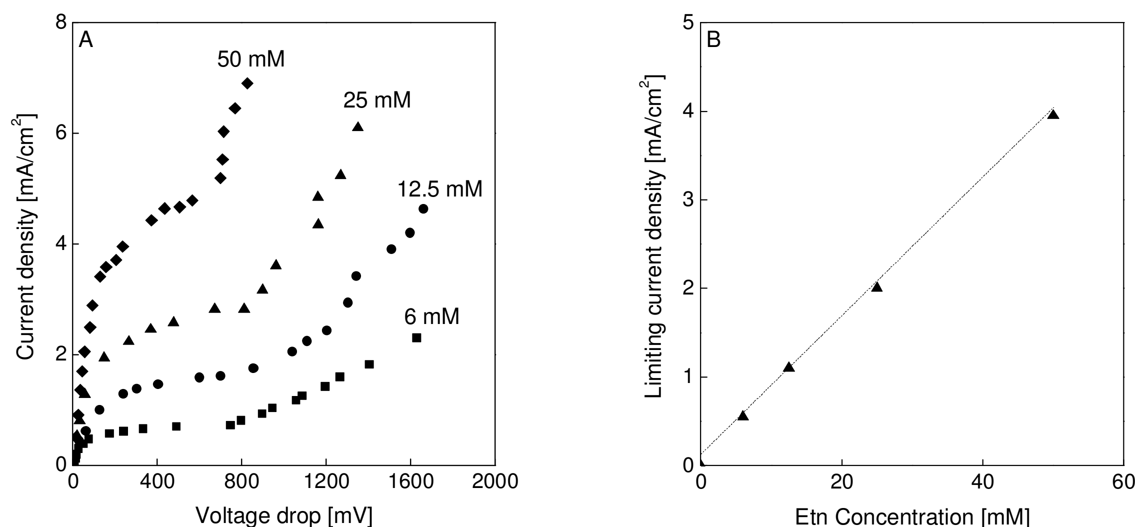


Figure 4.6. a) Current-voltage plot for ethanolamine (Etn) at different concentrations at pH = 7.0 for FKB-PEEK cation exchange membrane , b) linear relation between i_{lim} and ethanoleamine (Etn) concentration (for FKB-PEEK).

The LCD when using the sBPM was compared to that obtained for the standard commercially available FKB-PEEK membrane. The LCD of the sBPM was found to be between 2 and 3 mA/cm^2 which is comparable to the value of 2.0 mA/cm^2 obtained for the standard CEM membrane (FKB-PEEK). The LCD of the sBPM is somewhat more difficult to estimate as the onset of water splitting is very close to 1 V coinciding just with the LCD of the CEM part of the membrane. Above 1 V, part of the current will be transported through the bipolar regions of the membrane. As such a strict value of the current density of the membrane cannot be defined as the current distribution per region will vary as a function of voltage. The current increase at the plateau level, i.e. between 800 and 2000 mV, is a clear indication of an increased water splitting.

When water splitting occurs, OH^- and H^+ are produced. OH^- produced at the anodic side of the membrane can react with Etn^+ and deprotonate it. The result is a decrease in the concentration of the charged ions present and consequently an increase in resistance. H^+ produced at the cathodic side of the membrane cannot react further with Etn because it is already in its protonated form. If the current density would be increased further more water splitting is expected, producing a large excess in hydroxyl ions thereby decreasing the resistance again, as is normal for bipolar membranes. As a consequence the behavior of the sBPM, only as CEM or mostly as BPM, is tunable via the current density. In our ED experiments we chose to operate it in mixed mode, so above the water splitting voltage. Figure 4.7a shows a comparison between the current-voltage plot obtained for the standard CEM membrane (FKB-PEEK) and the sBPM.

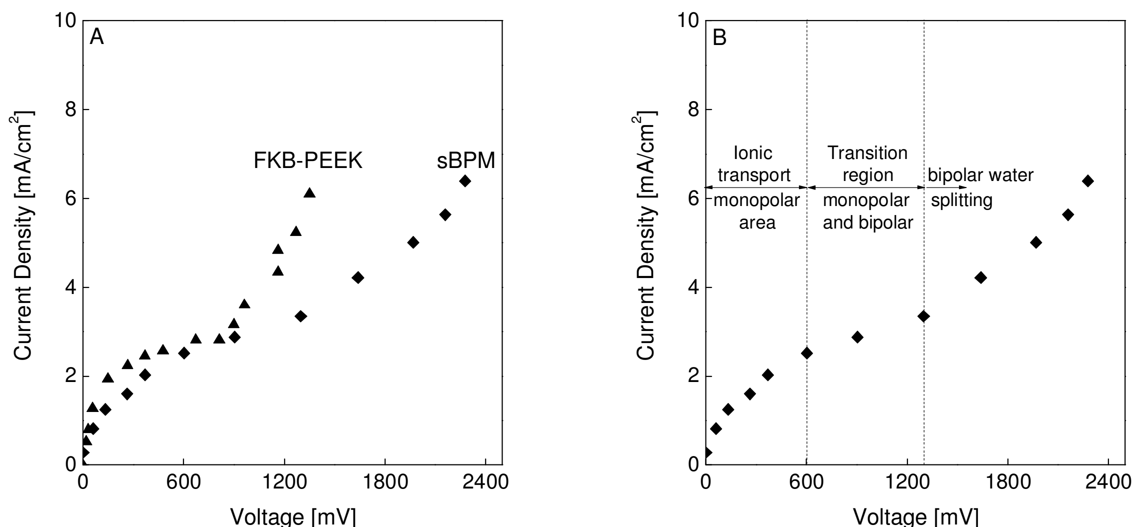


Figure 4.7. a) Current-voltage plot for ethanolamine at a concentration of 25 mM for FKB-PEEK and sBPM and b) Different regions identified for the LCD of the sBPM.

Figure 4.7b distinguishes three different regions for the LCD of ethanolamine at a concentration of 25 mM when a sBPM is used. Below 600 mV Etn transport occurs only through the cation exchange areas [36] since water splitting is not likely to occur below the limiting current density. The LCD of the sBPM has been calculated considering 0.7 of monopolar area. The plateau in the sBPM is not that easily recognizable due to the onset of water splitting. This area corresponds to a transition region where both Etn transport through the cation exchange layer as well as water splitting in the bipolar areas occur. The increase in the voltage drop observed for the sBPM is caused by the nature of the membrane, which consists of two monopolar layers. When protons and hydroxyl ions are generated at higher current densities, the resistance decreases [36]. At higher voltages water splitting occurs enhanced by the nature of the sBPM.

4.3.3 Electrodialysis

We assume that the decarboxylation reaction of Ser to Etn leads to sufficiently high conversions resulting in a mixture of Etn and other neutral amino acids prior to the separation process. As such, in this work only the electrodialytic separation of Etn and Ala is investigated in more detail. The feed solution consisted of 25 mM Etn and 25 mM Ala (model neutral amino acid). The concentrations were chosen arbitrarily with the only consideration of being far below the solubility limit of Etn and Ala. All electrodialysis experiments were performed with the membrane configurations shown in Figure 4.4.

First, conventional electrodialysis for the separation of Etn from Ala was applied, resulting in Etn recoveries of 39%. Etn migrates as cation from the feed to the receiving compartment. To maintain electroneutrality in the feed and due to the membrane configuration used, a positive ion, presumably H^+ replaces Etn^+. This causes the pH in the feed to decrease from 7.3 to 4.1 (Figure 4.8, ▲). The migration of Etn^+ to the receiving compartment is counterbalanced by the migration of either OH^- or SO_4^{2-} forming neutral Etn or ethanolamine sulfate. As a result, the pH in the receiving stream increases to values above 9 (Figure 4.8, Δ). At this pH, Etn acts as a buffer and therefore the pH in the receiving compartment does not change further. Ala present in the feed becomes slightly positive at a pH below 5.1 and is co-transported with Etn towards the receiving compartment. This results in an Ala retention of only 98% and consequently a decreased product purity.

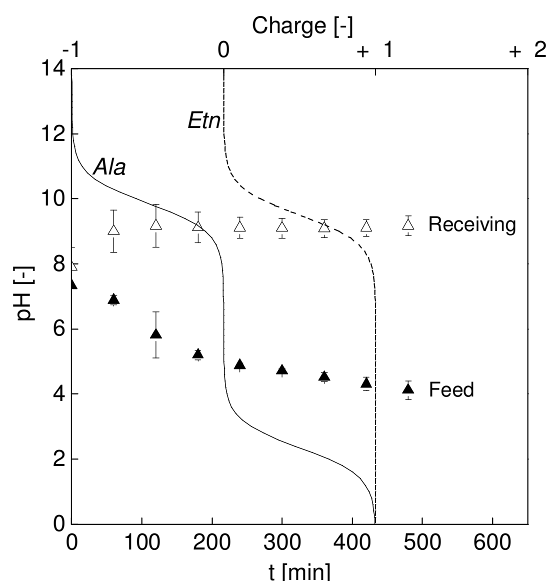


Figure 4.8. 1st x-axis: pH in the feed and the receiving streams plotted against time for ethanolamine/alanine Etn^{+1}/Ala^0 [25 mM/25 mM] during electrodialysis experiments with FKB-PEEK; 2nd x-axis: pH plotted against ethanolamine (Etn) and alanine (Ala) charge. Flow rate: 50 ml/min; Initial pH of the feed = 7.0.

To ensure product purity, strict pH control is needed. This limitation can be overcome when a sBPM is used in the electrodialysis stack (Figure 4.4b). Figure 4.9 clearly shows that the application of electrodialysis with the sBPM prevents pH changes and the pH in the feed throughout the experiment stays well above 7.0 (Figure 4.9a, ♦).

The constant pH in the feed obtained when using the sBPM prevents a change in charge behavior of Ala and as such limits the migration of Ala towards the receiving compartment increasing the product purity from 98% to 100%. Similar Etn recoveries of 39% and 37% were

obtained when electrodialysis with a standard CEM or with a sBPM are applied, respectively. Figure 4.10 shows the concentration of Etn and Ala in the receiving compartments for both concepts (ED with standard CEM (FKB-PEEK; Δ,▲) and ED with sBPM (◊,♦).

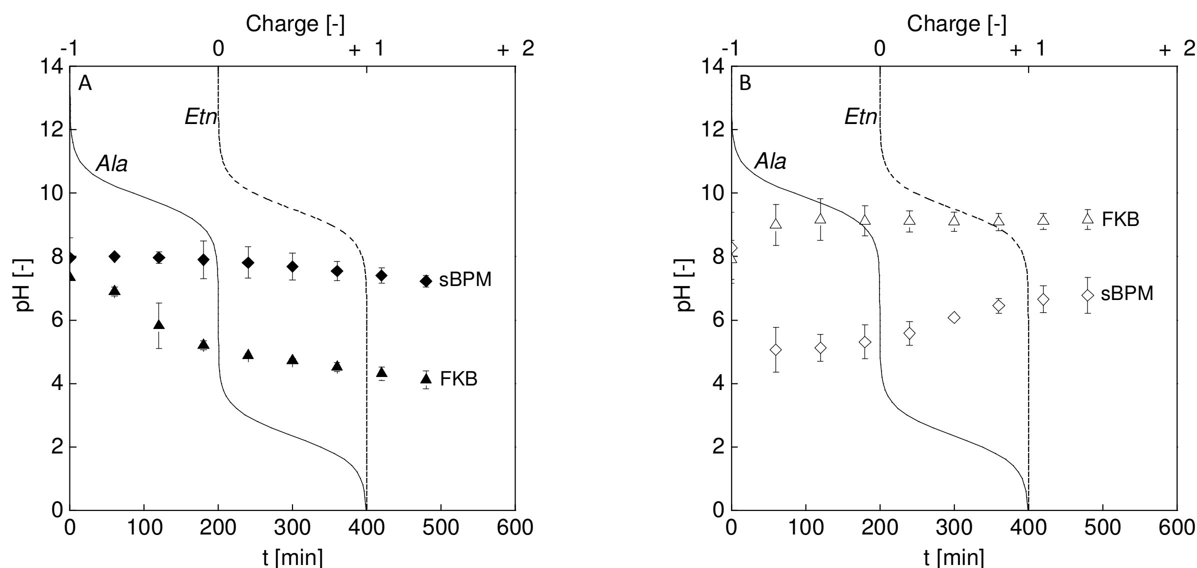


Figure 4.9. 1st x-axis: pH against time for ethanolamine/alanine [25 mM/25 mM] during electrodialysis experiments with FKB-PEEK for a) feed stream and b) receiving stream; 2nd x-axis: pH plotted against ethanolamine and alanine charge. Flow rate: 50 ml/min; Initial pH of the feed = 7.0.

For electrodialysis with a standard CEM, as well as for electrodialysis with a sBPM, the pH in the electrode compartments is balanced as the same vessel is used to feed both compartments. Based on pH changes in the outlet of both electrode rinsing streams it can be concluded that most of the produced H^+ is transported from the anode to the feed compartment. The same H^+ transport from the electrode compartment to the feed compartment is obtained for both process configurations. In the receiving compartment, SO_4^{2-} is transported preferably over OH^-. When electrodialysis with a sBPM is applied, water splitting is assumed to occur from the start of the experiment on. In the feed, OH^- is generated while Etn^+ migrates to the receiving compartment. Similar Etn^+ recoveries are obtained for both membrane configurations investigated. This suggests that the OH^- produced by water splitting is counterbalanced by the migration of Na^+ from the anode to the feed stream keeping the pH constant in the feed compartment.

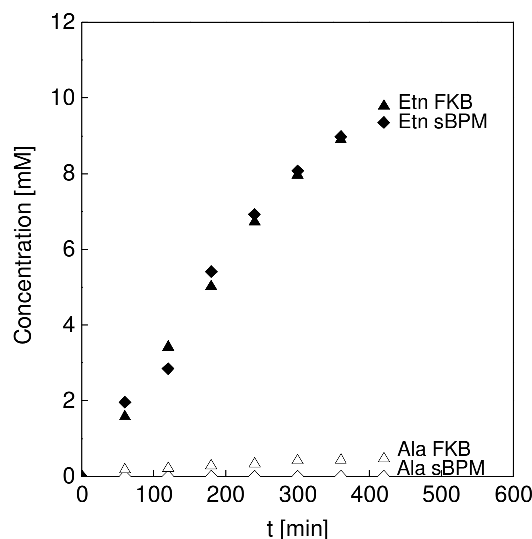


Figure 4.10. Concentration behavior of ethanolamine (Etn) and alanine (Ala) in the receiving stream during the electrodialysis of ethanolamine [25 mM] and alanine [25 mM] with FKB-PEEK and sBPM at pH = 7.0 and a flow rate of 50 ml/min.

When water splitting occurs at the interface of the sBPM also H^+ is produced and migrates to the receiving compartment. Together with the Etn^+ migration from the feed to the receiving compartment they are counterbalanced by the migration of SO_4^{2-} and OH^- from the cathode to the receiving stream. At the start, mainly water splitting occurs at the sBPM due to the high voltage drop over the compartment and the produced H^+ combines with SO_4^{2-} and causes a decrease in pH (Figure 4.9b, ◊). Later in the experiment the conductivity increases and water splitting is reduced allowing for an increased flux of Etn^+ that combines with OH^- and leads to an increase in pH (Figure 4.9b, ◊).

Both processes are evaluated with respect to their amino acid recovery, current efficiency, energy consumption, amino acid flux and retention (product purity). Electrodialysis with the sBPM shows similar performance in terms of flux, current efficiency, energy consumption and recoveries while at the same time a pure product stream is obtained. Both recovery of Etn and current efficiency using a sBPM are about 2% lower (relative to initial concentration in the feed) than that obtained during electrodialysis with a standard CEM (FKB-PEEK). This difference in performance is most probably due to water splitting and recombination of the produced H^+ and OH^- with those ions coming from the electrode compartments.

Considering the overall pH change in the feed during electrodialysis with a standard CEM (FKB-PEEK) and during electrodialysis with a sBPM, it can be estimated that 0.08 mM of OH^-, and

consequently 0.08 mM H^+, are produced, suggesting a current efficiency for OH^- and H^+ transport through the AEM and CEM layer of the sBPM of less than 2%. When calculated, the current efficiency of OH^- and H^+ through the AEM and CEM layer of the sBPM is 0.4%. The current efficiency of OH^- transport (0.4%) should be lower than the difference in current efficiency of Etn^+ between the two membrane configurations (2%). This is the case and it indicates that the pH changes obtained are realistic based on the OH^- concentration in the feed and supports the strength of this novel membrane concept for internal pH control.
The results are summarized in Table 4.1.

Table 4.1: Process parameters of the electrodialysis of Etn^{+1} from Ala at pH = 7.0 with a standard CEM (FKB-PEEK) and with a sBPM to control the pH of the feed after 420 min of operation. The given fluxes are normalized on available membrane area.

Transport membrane	FKB-PEEK	sBPM
Flux [mol/m²s]	$1.1 \cdot 10^{-6}$	$1.5 \cdot 10^{-6}$
Recovery [%]	39.2	37.4
Current efficiency [%]	46.9	44.7
Energy consumption [kWh/kg]	4.48	3.93
Ala Retention [%]	98.1	100.0

In the above shown process the influence of water splitting at the electrodes could be successfully counterbalanced by using a sBPM. In normal ED separation the influence of the electrodes can generally be neglected beyond the first two-three repeat units. However, as is known from amino acid separation, water splitting directly at a CEM and more strongly at the AEM can be severely disturbing for the process operation [1, 34]. It is therefore expected that using sBPM in the industrial ED stack configurations stabilizes process operation and product purity significantly.

4.4 Conclusions

The separation of the modification product of Ser, Etn, from neutral amino acids using electrodialysis is promising for the production of biochemicals in the biobased economy. However changes in the pH due to charge transport in ED introduce changes in the charge behavior of the amino acids. Consequently the product purity decreases.

The separation of Etn from Ala at neutral pH with a commercially available CEM (FKB-PEEK) results in high recovery (39%), satisfactory current efficiency (47%) and low energy consumption

(4.5 kWh/kg) but the product purity is compromised. Internal pH control using a segmented bipolar membrane similar process performance is obtained in terms of current efficiency (45%) and energy consumption (3.9 kWh/kg) while leading to similar Etn recovery (37%) and a pure product stream. These results show the strength of the concept of using a segmented bipolar membrane to integrate ionic transport and water splitting to control the pH. Optimization of the sBPM ratio and structure is required to further understand and improve the process.

4.5 Acknowledgements

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5

Electrodialysis of complex amino acid mixtures - Overcoming the poisonous effect of arginine

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ABSTRACT

The potential of electrodialysis (ED) for the separation of amino acids (zwitterionic molecules that exhibit a specific charge behavior dependent on pH) has been demonstrated in the past. However, even though successful for the separation of specific amino acids, ED is not applicable for the separation of mixtures containing the positively charged amino acid arginine (Arg) due to its poisonous effect on commercially available cation exchange membranes (CEMs), while Arg is one of the major components in biobased feeds and an important precursor for the production of chemicals. The present study confirms that this inhibiting effect is directly related to the water content of the cation exchange membrane during the ED experiments. To overcome this limitation, ED with self-prepared cation exchange membranes with a high swelling degree (SPEEK) and ED with ultrafiltration membranes (EDUF) was applied. The results clearly proof that the separation of lysine (Lys) and Arg is possible with the two proposed alternative ED configurations. Separation of more complex biobased amino acid mixtures containing Arg showed the simultaneous separation of the acidic amino acids, glutamic acid (Glu) and aspartic acid (Asp), and the basic amino acids, Lys and Arg, from neutral alanine (Ala) validating the potential of ED for biorefinery applications.

5.1 Introduction

As an alternative to fossil feedstocks, chemicals can be produced from amino acids present in biomass, for example, in side streams of biofuel production. As amino acids are usually present as a mixture, appropriate separation technologies are a prerequisite towards a biobased society. Electrodialysis (ED), an electro-membrane process that uses an electrical potential difference over the membrane as driving force, is an attractive approach for the isolation of amino acids due to their zwitterionic character.

Studies on the applicability of electro-membrane processes for the separation of amino acids (aa's) with different charge behavior have been published previously [1-5]. Isolation of a single amino acid from another one with almost identical charge behavior is more complex. The approach of combining enzymatic modification of amino acids to obtain new molecules with pronounced differences in their iso-electric point (pI) and consequently in their charge behavior, thus enhancing the successful fractionation of one of the two aa's, has been successfully demonstrated for the acidic amino acids, Glu and Asp (Glu converted into γ-amminobutyric acid, GABA) [6,7], and for the basic amino acids, Lys and Arg (Lys converted into 5-pentanediamine, PDA) [8,9]. Sandeaux et al. showed however that the presence of the amino acid Arg causes some unexpected poisonous effects and significantly reduces the separation efficiency [10]. They studied the performance of a cation exchange membrane (CMV Selemion) in the presence of positively charged Arg and reported a humongous increase in the membrane resistance from 2 $\Omega\cdot cm^2$ to 80 $\Omega\cdot cm^2$ when the concentration of Arg^+ (ArgCl) in the solution increased from 0 M to 0.1 M (Arg concentration in the membrane increased from 0 mmol/g to 1.5 mmol/g). At the same time it decreased from 2317 $\Omega\cdot cm^2$ to 79 $\Omega\cdot cm^2$ when the concentration of Na^+ (NaCl) was increased with the same amount [10]. Simultaneously, the water content in the membrane decreased from 33% to 28.6% with increasing Arg^+ concentration, suggesting that the increase in the membrane resistance might be related to the decrease in water content in the membrane in the presence of Arg. A second part of this study was carried out by Fares et al., who determined the individual fluxes of sodium and Arg counter ions and chloride co-ions [11]. They concluded that the presence of Arg also decreased the membrane permselectivity, thus, adversely affecting the process economics [11].

A clear problem arises: Due to the counteracting effect of Arg on CEMs, the separation possibilities for complex mixtures of aa's also containing Arg using ED are limited. To the best

of our knowledge, no solution has been proposed to make the separation of such mixtures containing Arg with ED suitable, while Arg is one of the major components in biobased feeds and an important precursor for the production of chemicals such as 1,4-butanediamine (for the plastics industry) and urea (for the fertilizers industry). As such a suitable separation process for amino acids is essential for the transition towards a biobased society. To overcome this and to extend the use of ED towards the separation of complex mixtures, we carried out a systematic study where we propose two different approaches.

First we identify the problem and confirm the hypothesis published elsewhere [10,11]. As the poisonous effect of Arg is assumed to be related to the water content of the membrane, we determine the swelling degree (SD) of the membrane in the presence of Arg. Additionally we evaluate the ED process performance for the separation of aa's using a commercial membrane. Next, we consider the use of a tailor made CEM with a significantly higher SD and show that this results in significant improvement and allows the separation of complex mixtures of aa's. The results confirm the hypothesis that the water content of the membrane to a large extent governs the aa transport. Based on earlier studies on the application of electro-membrane filtration (EF) for the separation of proteins, peptides and amino acids [12, 13, 14], an alternative approach that combines electrodialysis (ED) and ultrafiltration (UF) in one single process (EDUF) is investigated for the separation of aa's. EDUF, besides successful for the isolation of biomolecules with a molecular weight higher than 500 Da [15], might also have benefits for the separation of smaller molecules such as amino acids [16], especially for the ones with relatively high molecular weight such as Arg due to the significantly higher molecular weight cut off (MWCO) of the membrane with respect to the target molecules [17] where the transport rate is expected to be higher since no retardation effects such as friction between the molecule and the pore walls of the membrane occur [17].

Finally, we apply ED using tailor made CEMs for the separation of complex mixtures of aa's representative for industrially relevant separations. More specifically we evaluate the simultaneous separation of the acidic (negatively charged) amino acids, Glu and Asp, from the basic (positively charged) amino acids, Lys and Arg, and the uncharged amino acid Ala (used as model neutral amino acid) to validate the potential of ED for the isolation of amino acids using the charge behavior of the aa's to perform the separation.

5.2 Experimental

5.2.1 Materials

Model amino acid solutions were prepared using lysine monohydrochloride (LysHCl), arginine monohydrochloride (ArgHCl) and Ala, Glu and Asp in solid state with a purity of 98% or higher obtained from Sigma-Aldrich. The iso-electric point of the different amino acids used is reported elsewhere [6]. Sodium sulfate solutions (Merck Chemicals and Reagents) with a concentration twice as high as the total amino acid concentration in the feed solution were used as electrode rinsing solutions.

Ion exchange membranes type Fumasep PEEK reinforced FKB (CEM) and Fumasep PEEK reinforced FAB (AEM) were purchased from FumaTech GmbH, Germany [8,15]. As ultrafiltration (UF) membrane PES5K (Milipore), a polyethersulfone (PES) based membrane with a MWCO of 5 kD was used. Tailor made cation exchange membranes were produced from sulfonated (poly ether ether ketone) (SPEEK) with a sulfonation degree (DS) of 67%. SPEEK (DS 67%) was obtained from FumaTech GmbH and the membrane prepared thereof showed an average thickness of 80 μm, a permselectivity of 88% and a swelling degree (in MilliQ water) of 50%.

5.2.2 Methods

5.2.2.1 Swelling degree (SD)

The swelling degree (SD) of FKB membranes was determined similar to the method described by Dlugolecki et al. [19]. Different solutions containing 25 mM Lys or Arg in different concentrations (2 mM, 4 mM, 8 mM, 16 mM, 25 mM) were used. As a comparison, the SD of FKB membranes in MilliQ Water was also determined. The membrane samples were immersed in MilliQ water or in the amino acid solutions at room temperature. After 24 hours the samples were removed from the solution, the surface water was removed with blotting paper and the weight of the membrane samples was recorded subsequently. Afterwards, the membrane samples were placed in a vacuum oven at 30°C. After 24 h the membranes were taken out from the oven, the dry weight was recorded and the SD was calculated as follows [19]:

$$SD = \frac{m_{wet} - m_{dry}}{m_{dry}} * 100\% \qquad \text{Eq. 5.1}$$

5.2.2.2 Electrodialysis

Electrodialysis experiments for the separation of either Lys or Lys and Arg (varying concentration of Arg: 0 mM, 2 mM, 4 mM, 8 mM, 16 mM, 25 mM) from neutral Ala were carried out using a 2-compartment (in addition to the two electrode compartments) cell type FT-ED-40-2 equipped with titanium/iridium plasma coated stainless steel electrodes (FumaTech GmbH). The height of the compartments corresponded to 9 cm with a width of 4 cm resulting in an effective membrane area of 36 cm^2. Thick diamond structured welded mesh spacers with a thickness of 475 μm made of PVC/Polyester were placed between the ion exchange membranes in the feed and receiving compartments (intermembrane distance of 475 μm). For the electrode compartments 900 μm thick diamond structured welded mesh spacers made of polyethylene were used.

For the separation of positively charged amino acids, the membrane stack consisted of a CEM at the anode side (positive electrode) to avoid migration of chlorine ions (Cl^-) to the electrode, followed by another CEM in the middle and an AEM at the cathode side (negatively charged electrode) to avoid further migration of the positively charged amino acids towards the cathode (Figure 5.1a). As CEM, FumaTech FKB or the tailor made SPEEK membrane was used. For the EDUF experiments, the middle CEM membrane was replaced by the PES5K ultrafiltration membrane (UF, Figure 5.1b).

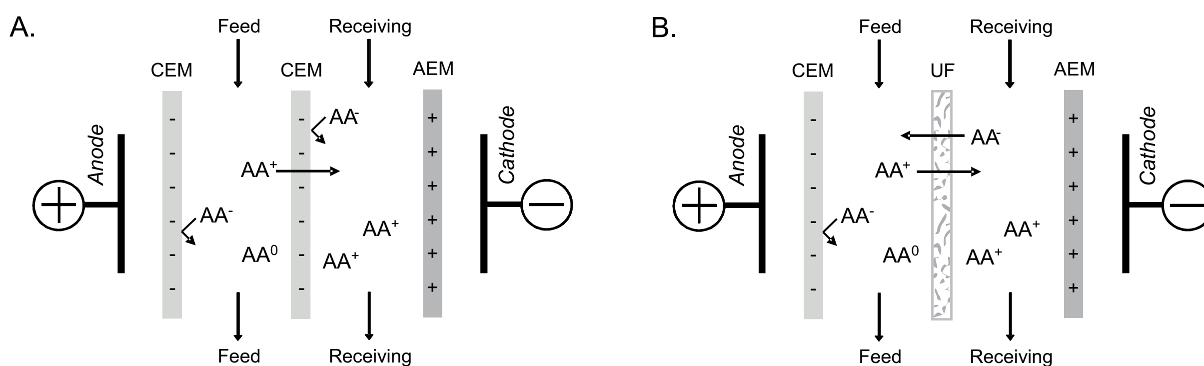

Figure 5.1. Schematic representation of the membrane configuration used in electrodialysis experiments for the separation of positively charged amino acids. a. ED; CEM: FKB or SPEEK; b. EDUF; UF membrane: PES5K.

Furthermore, ED experiments using a complex mixture of 5 aa's where positively charged (basic) amino acids, Lys and Arg, were separated simultaneously from the negatively charged (acidic) amino acids, Glu and Asp and from uncharged Ala were performed. These experiments were carried out using a 4-compartment (in addition to the 2 electrode compartments) cell type FT-

ED-100-4 equipped with stainless steel electrodes. The height of the cell corresponded to 10 cm with a width of 10 cm resulting in an effective membrane area of 100 cm^2. Thick diamond structured welded mesh spacers (similar to the previous experiments) with a thickness of 475 μm (intermembrane distance) made of PVC/Polyester were placed between the ion exchange membranes in the feed and both receiving compartments. 900 μm thick diamond structured welded mesh spacers made of polyethylene with a silicon frame were used for the electrode compartments. The membrane configuration is shown in Figure 5.2.

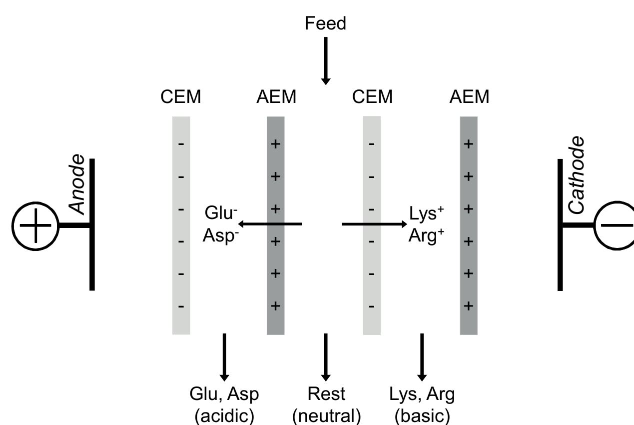

Figure 5.2. Schematic representation of electrodialysis for the separation of basic and acidic amino acids from the neutral ones. CEM: SPEEK, AEM: FAB.

For all electrodialysis experiments the current was applied via a power supply ES 030 – 5 (Delta Elektronika, Voltage range: 0 – 30 V, Current range: 0 – 5 A). pH meters (InLab Routine, Metler Toledo) and conductivity sensors (TetraCon DU, WTW) were used to monitor pH and conductivity, respectively, of all streams throughout the experiments. Every hour a sample of each stream was collected and analyzed using U-HPLC [9]. 1 L solution with a concentration of 25 mM of each amino acid present and 1 L of MilliQ water were used as feed and receiving streams, respectively. The electrode rinse solution consisted of 2 L Na_2SO_4 with a concentration twice as high as the total amino acid concentration present in the feed stream. Feed and receiving streams were circulated through each corresponding compartment and the electrode rinse solution was circulated through both electrode compartments and collected again in one vessel as described in previous work using Masterflex pumps (Cole Parmer Instruments & Co) [6]. The experiments were carried out at an initial pH of the feed of 6.0 with no pH control. The feed and receiving flow rates were 50 ml/min resulting in a linear velocity of 263 cm/min for the FT-ED-40-2 (width = 4 cm, intermembrane area = 0.0475 cm) and 105 cm/min for the FT-ED-100-4 (width = 10 cm, intermembrane area = 0.0475 cm).

To be consistent with previous work, the experiments were performed at the limiting current density (LCD) for all amino acids studied up to date [6, 8, 15], where the LCD was studied for individual components but also for mixtures such as Glu and Asp together under constant feed and receiving volume and concentration of the target ions. This value was also used for the EDUF experiments. In the experiments performed in this work, small changes in the volume in the receiving compartment occur for the different membranes investigated and the feed concentration changes in time due to the transport of the target compounds, what means that at some point of the experiment, operation in the over limiting region might occur and needs to be considered. However, this was not taken into account and the current density was not adapted during the experiments. A summary of all ED experiments is given in Table 5.1.

Table 5.1. Summary of electrodialysis experiments.

Transport membrane	Current density [mA/cm^2]	C_{Ala} [mM]	C_{Lys} [mM]	C_{Arg} [mM]	C_{Glu} [mM]	C_{Asp} [mM]
FKB	2.5	25	25	0 2 4 8 16 25	0	0
SPEEK	2.5 3.6	25 25	25 25	0 25	0	0
PES5K	2.5 3.6	25 25	25 25	0 25	0	0
SPEEK/FAB*	3.6	25	25	25	25	25

*SPEEK: used for the transport of positively charged amino acids, Lys and Arg; FAB used for the transport of negatively charged amino acids, Glu and Asp.

5.2.2.3 Process evaluation

To assess the process, the amino acid flux, the recovery, the current efficiency and the power consumption were determined as explained in previous work [6]. In addition, the retention of uncharged Ala in the feed stream is calculated as explained elsewhere [20].

This process parameter indicates the purity of the final product. All process parameters are calculated based on the real concentration of amino acids present in the receiving and the feed and the overall current and voltage difference applied.

5.4 Results and discussion

5.4.1 Poisonous effect of Arg

The negative effect of the presence of Arg on the process performance of ED using CMV membranes has been reported previously [10,11] and it is hypothesized that this is related to the water content of the membrane. Figure 5.3 obviously visualizes this poisonous effect of the Arg concentration on the Lys and Arg recovery using ED with FKB membranes after 5 h of operation.

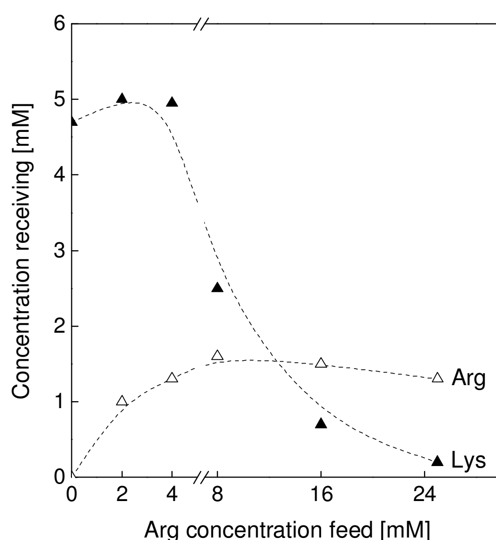

Figure 5.3. Concentration of Arg and Lys in receiving stream during electrodialysis of Lys (25 mM) with increasing Arg concentration (0 mM, 2 mM, 4 mM, 8 mM, 16 mM, 25 mM). Flow rate: 50 ml/min; Initial pH of the feed = 6.0 (no pH control); Current density: 2.5 mA/cm^2. Transport membrane: FKB.

Figure 5.3 shows that the concentration of Lys in the receiving stream after 5 h of operation decreases significantly with increasing Arg concentration. Competition between Arg and Lys is excluded since the Arg concentration in the receiving stream after 5 h is more or less the same for all experiments (~1.0 - 1.5 mM) when the Arg concentration is increased from 0 – 25 mM. Since the same current density was applied for all experiments, the same transport of the target amino acids is expected provided that the current efficiency does not change significantly due to arginine concentration. This indicates that the difference (decrease) in transport of charged molecules is actually caused by the presence of Arg in the feed solution what was the aim of the experiments carried out. These results confirm the observations reported by Sandeaux et al. [10] and Fares et al. [11] on the extraordinary deteriorating effect of positively charged Arg on the ED

process performance. Quantitatively this is summarized in Table 5.2, which shows the process performance based on the recovery of Lys (R_{Lys}), the recovery of Arg (R_{Arg}) and the overall recovery (R_{Total}).

Table 5.2. Process performance of ED with commercial FKB membrane based on the recovery of Lys, Arg and the overall recovery. Lys concentration is in all cases 25 mM.

Concentration Arg [mM]	R_{Lys} [%]*	R_{Arg} [%]	R_{Total} [%]**
0	22.7	n.a.	22.7
2	22.2	39.6	24.8
4	22.0	49.6	24.7
8	11.5	24.6	14.5
16	4.0	14.4	8.0
25	0.9	7.2	4.1

*R_{Lys} during ED experiments related to the increase in Arg concentration in the feed.
**R_{Total} during ED experiments determined based on the overall amount of amino acids transported divided by the total amount of charged amino acid present.

It can be clearly seen in Table 5.2 that the Lys recovery R_{Lys} and the total recovery R_{Total} decrease with increasing Arg concentration. These results confirm that the poisonous effect of positively charged Arg on the ED process performance when using commercial CMV membranes [10,11] also applies to commercial FKB membranes. Next to this, also the Arg recovery, R_{Arg}, decreases with increasing Arg concentration. However, the decrease in R_{Arg} refers in all cases to a similar Arg concentration in the receiving compartment of between 1.0 mM – 1.5 mM. Since the Arg concentration in the feed increases, the same Arg concentration in the receiving stream corresponds to a lower R_{Arg}.

In general, ion exchange membranes need to have specific properties, such as a high permselectivity (the membrane should be highly permeable to counter ions but impermeable to co-ions), low electrical resistance (high permeability of the ion exchange membrane for counter ions under the electrical potential gradient), a good mechanical stability (low swelling degree) and a high chemical stability (over the whole pH range). However, these properties are interrelated. For example, a high concentration of fixed charges in the membrane matrix means low electrical resistance but leads to a high swelling degree, resulting in low mechanical stability. On the other hand, also the type and concentration of the fixed ionic charges has an influence on permselectivity and electrical resistance [21]. For instance, the preferred ionic groups should be completely dissociated over the entire pH range, otherwise the resistance of the membrane will

increase and the permselectivity will decrease as a result of the neutralization of the charges [18]. Sandeaux et al. [10] and Fares et al. [11] did observe an increase in resistance and a decrease in permselectivity. At the same time they observed a decrease in the swelling degree of the membrane investigated (CMV) [10,11].

These statement and the results of Sandeaux et al. [10] and Fares et al. [11] suggest that the decrease in Lys recovery with increasing Arg concentration in the feed might be related to the water content of the membrane. Consequently we determined the swelling degree of FKB membranes in Arg solutions with different concentrations, obtaining a decrease in the SD with increasing Arg concentration, which coincides with a significant decrease in the total recovery, R_{total}. The results are summarized in Figure 5.4.

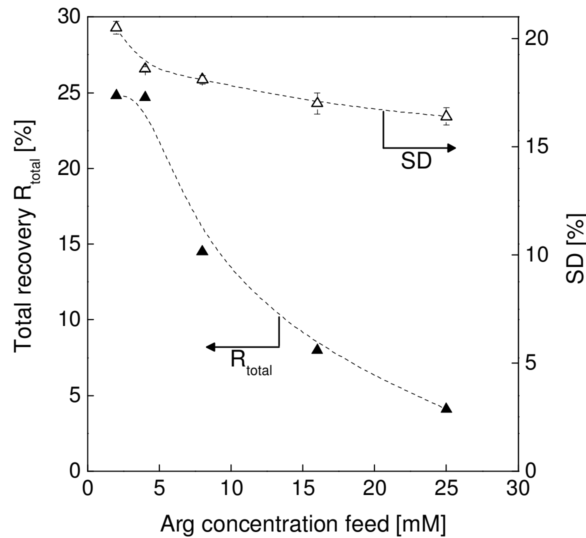

Figure 5.4. Relation between the swelling degree (SD) of FKB membranes in Arg solutions with increasing concentration and the total recovery (R_{total}) of charged amino acids during the ED experiments of Arg and Lys with constant Lys concentration [25mM] and increasing Arg concentration.

As hypothesized, the trend shown in Figure 5.4 for the swelling degree of FKB membranes for different amino acid solutions and the total recovery of amino acids during ED with FKB membranes clearly confirms the poisonous effect of Arg on the performance of the FKB membranes due to the decrease in the swelling degree with increasing Arg concentration. What is surprising from the results summarized in Figure 5.4 is that the small decrease in the swelling degree (Δ) has such a big negative impact on the process performance of ED with FKB membranes based on total recovery of amino acids (R_{total}, ▲).

Assuming that a decrease in the swelling degree of the FKB membranes is the main cause of a decrease in transport of both Arg and Lys, a membrane that in the presence of Arg shows a significantly higher SD might solve the problem. Therefore, and based on its accessibility and easiness of handling, we selected a highly hydrophilic tailor made membrane made of sulfonated poly(ether ether ketone) (SPEEK). SPEEK is a well-known polymer used for the fabrication of ion exchange membranes in electro membrane processes. The ion exchange group is sulfonic acid and it can be produced with different sulfonation degrees (67% for the present study, thickness: 80μm). The swelling degree of this tailor made membrane in Arg solutions with different concentrations was also determined. As a reference we determined the swelling degree of the membranes in water and in a 25 mM Lys solution as well. The results are summarized in Table 5.3.

Table 5.3. Swelling degree of a commercial FKB membrane and a tailor made SPEEK membrane in amino acid solutions with different concentrations.

Amino acid	Concentration [mM]	SD_{FKB} [%]	SD_{SPEEK} [%]
MilliQ Water	n.a.	22.3 ± 0.2	50.3 ± 0.9
Lys	25	18.0 ± 0.3	34.3 ± 0.7
Arg	2	20.5 ± 0.3	36.9 ± 1.9
	4	18.6 ± 0.1	29.9 ± 0.7
	8	18.1 ± 0.2	23.1 ± 5.0
	16	17.0 ± 0.5	26.5 ± 1.1
	25	16.4 ± 0.4	27.1 ± 0.1

As can be clearly seen from Table 5.3, the swelling degree shows its highest value for water for both membranes investigated and it decreases with increasing Arg concentration. This is in agreement with the decrease in R_{Lys}, R_{Arg} and in the total amino acid recover R_{Total} as shown before (Table 5.2). The decrease in Arg recovery, R_{Arg} in Table 5.2, corresponds to similar Arg concentrations in the receiving compartment for all cases, ranging between 1.0 and 1.5 mM (Figure 5.3). It can be argued that the decrease in swelling is a result of an increase in electrolyte concentration in the feed what would lead to higher friction between solute and membrane reducing the transport of the target compounds [22]. However, even though an increase in Lys concentration also leads to a decrease in swelling, a concentration of 25 mM of Lys does not have such a negative effect on the performance of the cation exchange membrane investigated as the presence of Arg. Because of this, the negative effect of Arg on the performance of the cation exchange membranes investigated (Table 5.2) can be considered as specific for the case of Arg

and is referred to as a poisonous effect as referred to in previous works [10, 11]. Even though the swelling degree also decreases with increasing Arg concentration for the tailor made SPEEK membranes, the values are still significantly higher than the ones obtained for the FKB membranes for all tested Arg concentrations, which suggests that the separation of complex amino acid mixtures in ED with tailor made SPEEK membranes might be possible even at higher Arg concentrations. This will be presented in the next paragraph.

5.4.2 Effect of membrane swelling on separation performance

The differences in the swelling degree of FKB and the tailor made SPEEK membranes suggest the latter would not exhibit the poisonous effect of Arg in ED. However, a fair comparison of the original process performance of both membranes can only be established in the absence of Arg due to its inhibiting effect on the FKB membrane. Therefore, ED experiments for the separation of Lys from Ala using either a FKB membrane or a tailor made SPEEK membrane (Figure 5.1a) were compared first to establish that no unexpected phenomena other than the poisonous effect of Arg occurs during the experiments. In a later stage, the performance in the presence of Arg is evaluated. As first process parameter we discuss the behavior of the voltage. The voltage represents the resistance of the membrane and indicates how easy the ions are transported. The results are summarized in Figure 5.5.

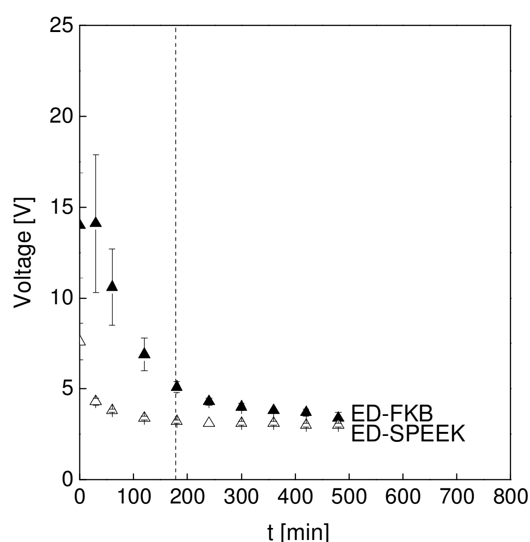

Figure 5.5. Comparison of the behavior of voltage in time during ED experiments with FKB or SPEEK membranes for the separation of Lys and Ala. Flow rate: 50 ml/min; Initial pH of the feed = 6.0 (no pH control); Current density: 2.5 mA/cm².

Not of less importance is the behavior of pH. The pH of the feed compartment is a crucial parameter in the separation of amino acids with electrodialysis, since its value determines the charge of the amino acids present and consequently the separation performance. On the other hand, the pH in the receiving compartment also helps to understand the process performance and might also complement the explanation of the phenomena taking place regarding pH changes in the feed. Figure 5.6 summarizes the pH behavior in the feed and receiving compartments throughout the experiments.

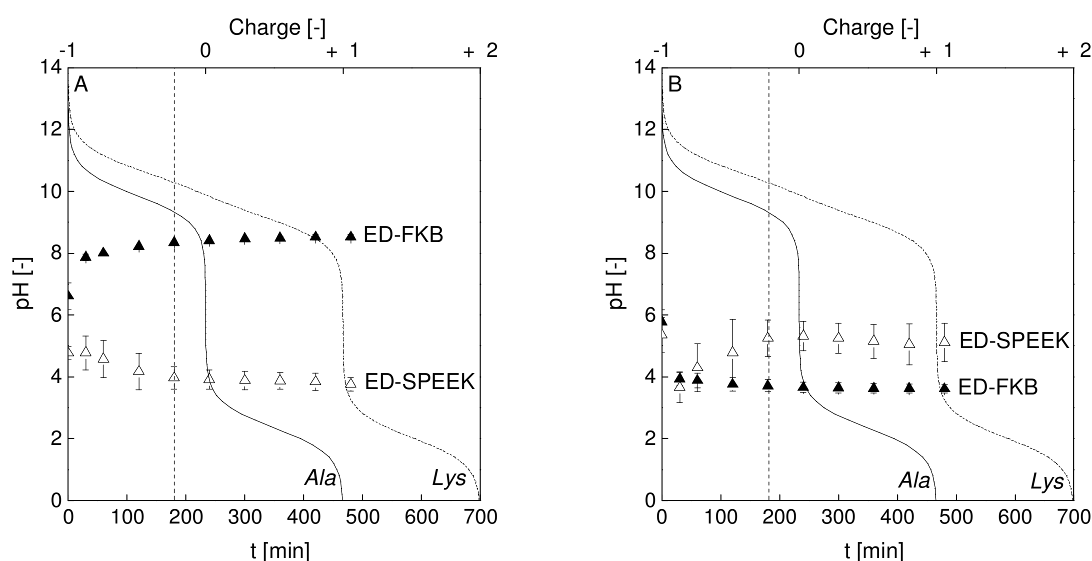

Figure 5.6. ED experiments with FKB or SPEEK membranes for the separation of Lys and Ala. 1st x-axis: Feed pH plotted against time for the separation of $Ala^{0}/Lys^{+1.0}$ [25 mM/25 mM] using a FKB or a SPEEK membrane. 2nd x-axis: pH plotted against average net charge of Ala and Lys for a) Feed compartment and b) Receiving compartment. Flow rate: 50 ml/min; Initial pH of the feed = 6.0 (no pH control); Current density: 2.5 mA/cm^2.

Figure 5.6a shows a clear trend. For the FKB membrane and the SPEEK membrane, the pH increases and decreases respectively during the first three hours of the experiment. This behavior can be explained based on two phenomena. On one hand, the change in pH is related to the behavior of the voltage (Figure 5.5). The increase in the voltage means an increase in the resistance of the membrane as a result of a limitation in the amino acid transport. Consequently, water splitting occurs at the surface of the FKB to provide enough ions, OH^- and H^+, to transport the imposed current. Based on the membrane configuration used (Figure 5.1), the OH^- ions produced remain in the feed compartment, causing the pH of the feed stream to increase (Figure 5.5a). The decrease in pH in the receiving compartment for the FKB membrane supports this explanation, where H^+ ions, also products of water splitting, end up in the receiving compartment. On the other hand, no significant increase in the voltage is observed for the

SPEEK membrane in the first three hours of the experiment. For this case the pH decrease (from 5 to 4) is not attributed to water splitting but to the maintenance of electroneutrality. When an amino acid ion migrates from the feed to the receiving stream, a proton migrates from the anode to the feed compartment leading to a decrease in pH in the feed stream. The decrease in pH in the receiving compartment for the SPEEK membranes can be attributed to the transport of H^+ ions coming from the electrolyte compartment and also to a less extent to the dissociation of $LysH^+$ to Lys and H^+ in the receiving compartment. These results are probably also related to the difference in the swelling degree of both membranes, where a higher swelling degree corresponding to the SPEEK membranes facilitates amino acid transport through the membrane.

The behavior of pH is also attributed to the permselectivity (or lack thereof) of the different membranes. SO_4^{-2} ions can migrate from the cathode to the receiving compartment through the AEM. When the FKB membrane is applied (middle membrane, Figure 5.1a), the SO_4^{-2} ions cannot migrate any further towards the feed stream since they are blocked by the almost completely selective FKB membrane (permselectivity >98%). On the other hand, SO_4^{-2} ions do migrate to the feed stream when SPEEK membranes are used (permselectivity = 88%). The tailor made SPEEK membrane is still selective, but its value is significantly lower than the corresponding value of the FKB membrane. Hence, a decrease in pH can be observed in the feed compartment when SPEEK membranes are applied.

Even though it is interesting to understand the nature of the pH changes, no significant effect on the separation of the amino acids is to be expected at this stage, since over the whole interval of the experimental pH the charge of the amino acids remains constant for all cases (Figure 5.6a), leading to a similar process performance of both investigated membranes. This might however become relevant when other amino acids are selected that have a smaller pH operating range [8].

Next, to elucidate the reason for the poisonous effect of Arg, the separation of Arg, Lys and Ala using SPEEK membranes has been performed. Also during these experiments, the pH in the feed compartment increases when using FKB membranes and decreases when applying the tailor made SPEEK membranes (results not shown). The voltage when the FKB membrane is used exhibits an enormous increase directly at the start of the experiment while the voltage during the experiments with the SPEEK membranes (Figure 5.7a) remains consistently low. These results already clearly show the effect of the water contents of the membrane on the separation efficiency. As a consequence, no Arg nor Lys is transported to the receiving compartment

through the FKB membrane. Therefore, only the concentration in the receiving stream for the separation of Lys and Arg from Ala with SPEEK membranes for the three amino acids present is shown in Figure 5.7b.

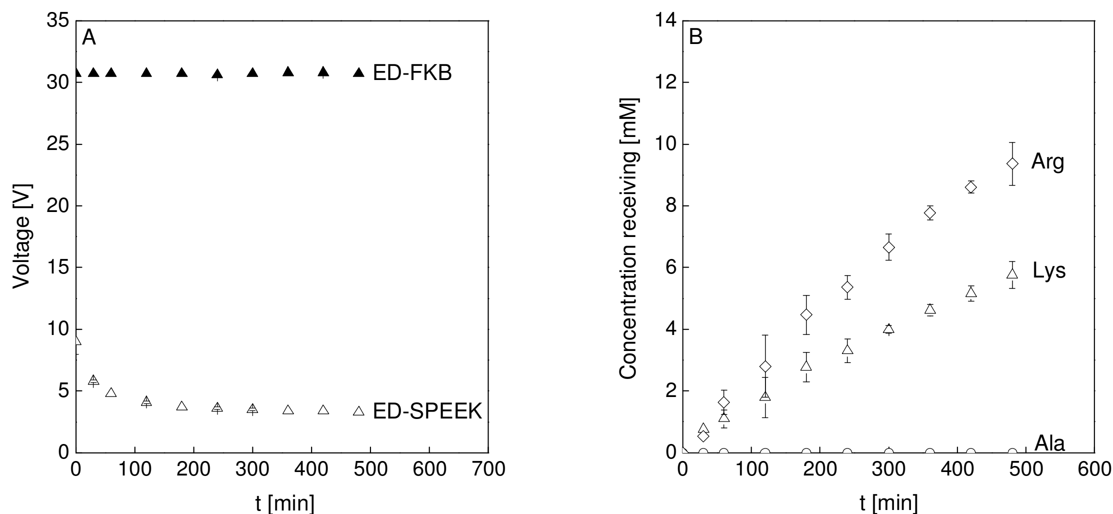

Figure 5.7. Separation of Lys and Arg from Ala with ED. a) Voltage behavior in time for FKB and SPEEK membranes b) Concentration in the receiving stream for SPEEK membranes. Flow rate: 50 ml/min; Initial pH of the feed = 6.0 (no pH control); Current density: 3.6 mA/cm^2.

The concentration of Arg in the receiving stream is higher than the concentration of Lys, suggesting the selectivity of SPEEK for Arg over Lys (Figure 5.7b). This can also be deduced from Figure 5.3, where Arg is transported preferably over Lys and at the same time poisons the membrane decreasing the overall process performance. This is shown in the decrease in Lys concentration and a constant Arg concentration in the receiving stream with increasing Arg concentration in the feed. The results shown in Figure 5.7b make this hypothesis extensive also to the tailor made SPEEK membrane investigated, where Arg is transported preferably over Lys but no poisonous effect is observed.

Based on these results, we conclude that no significant difference in the process performance is observed between the FKB and the SPEEK membranes for the separation of Lys from Ala, allowing us to directly relate the poisonous effect of Arg to the low water content of the CEM.

5.4.3 ED versus EDUF

An alternative for the separation of Lys and Arg from neutral amino acids is the combination of ED and UF membranes, a process known as EDUF [12-17]. Since the swelling degree does not play a role in the transport mechanism of UF membranes, any poisonous effect of Arg is not expected. However, UF membranes do not exhibit any ion selectivity, what might lead to a decrease in the purity of the product due to the co-transport of unwanted ions to the receiving stream. As before, for a fair comparison, ED experiments for the separation of Lys from Ala using PES5K, an ultrafiltration membrane, are carried out first (Figure 5.1b). Next, the performance in the presence of Arg is evaluated. Figure 5.8 voltage behavior throughout the ED experiment with the FKB or the SPEEK membrane and during the EDUF experiments for the separation of Lys from Ala.

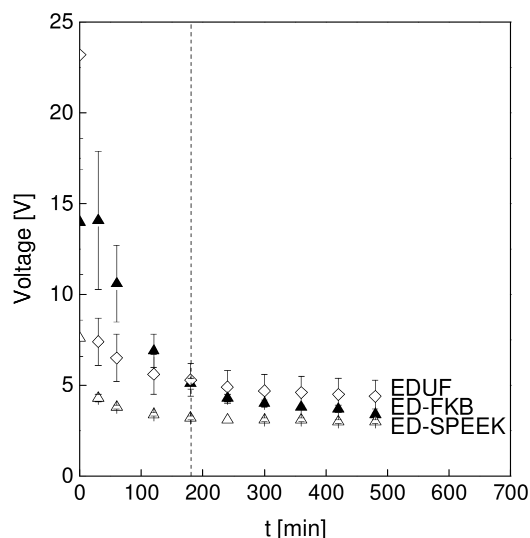

Figure 5.8. Voltage behavior in time for the separation of Lys from Ala with ED using FKB or SPEEK membranes and EDUF. Flow rate: 50 ml/min; Initial pH of the feed = 6.0 (no pH control); Current density: 2.5 mA/cm^2.

Compared to ED, an exceptionally high voltage at the start of the EDUF experiment is observed. This is a direct consequence of the lack of pretreatment of this membrane. Generally speaking, porous membranes need to be pressurized prior to the experiment to open their pores. This was no possible in the present case due to the large membrane area used. Already after 30 min of operation the voltage decreases and EDUF performs similar to ED (with respect to voltage).

Next to the voltage, the behavior of pH during the EDUF process is also worth attention. For the same reasons mentioned before for the SPEEK membrane, the pH in the feed stream decreases in EDUF as well. The results are summarized in Figures 5.9a and 5.9b for the feed and receiving streams, respectively.

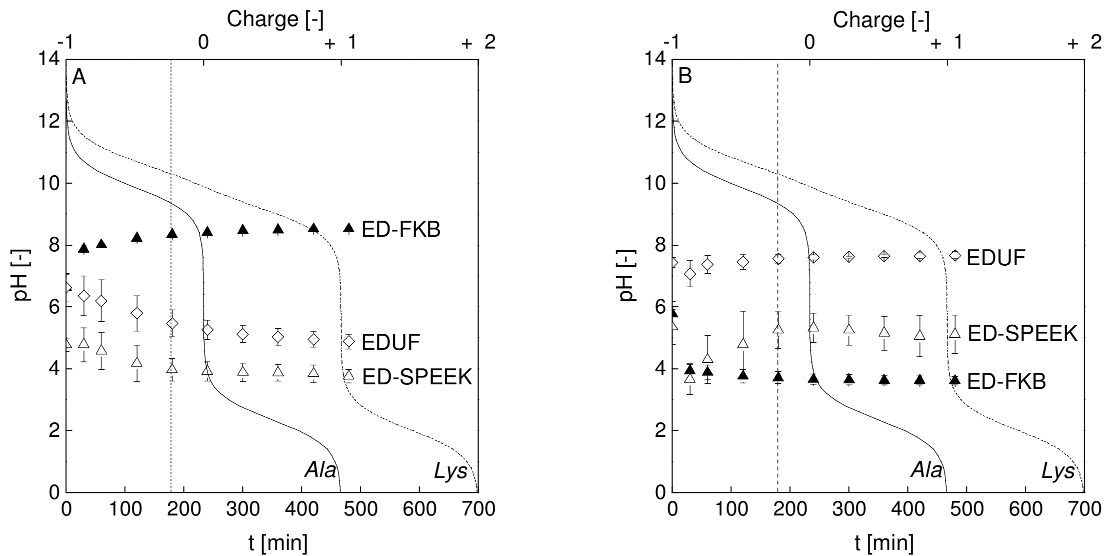

Figure 5.9. Separation of Lys from Ala with ED using FKB or SPEEK membranes and EDUF. a) pH behavior in time. Flow rate: 50 ml/min; Initial pH of the feed = 6.0 (no pH control); Current density: 2.5 mA/cm^2.

When assessing the separation of Lys and Ala, no significant differences can be observed for the transport of Lys from the feed towards the receiving compartment for the different membranes investigated (results not shown). Since there is no significant difference in the voltage, the current, flow rate, volume of the streams and the membrane area is the same for all experiments, similar process performance can be obtained for all configurations. However, due to the use of the porous UF membrane, a significant amount of Ala can be found in the receiving stream for the EDUF experiments, decreasing the purity of the product. The transport of Ala is most probably a result of both electroosmotic transport and diffusion due to the difference in concentration in the feed and the receiving stream. Alanine convection due to pressure differences is unlikely to take place since the same flow rate is used for both feed and receiving compartment, avoiding a pressure difference between feed and receiving compartment. Electroosmotic transport might be reduced by lowering the operational current density, which may also decrease the recovery of the target products. Diffusive transport is more difficult to control since it is related to the concentration in the feed and receiving compartment. It is important to mention that a relatively open membrane (PES5K, MWCO = 5kDa) has been used

for the EDUF experiments. Probably when using a nanofiltration membrane or an ultrafiltration membrane with a smaller molecular weight cut-off, e.g. 1kDa, a decrease in the transport of Ala towards the receiving compartment would have been observed while Arg and Lys transport would have remained the same (or higher). Even though optimization of the EDUF process for the separation investigated in the present work might be possible, it is not within the scope of this research.

To complete this part of the investigation, EDUF is also applied for the separation of Lys and Arg from Ala and compared to the process performance of ED with FKB and SPEEK membranes. Figure 5.10 shows the voltage in time for the three membrane configurations investigated (Figure 5.10a) and the concentration in the receiving compartment in time of the three amino acids present in the feed mixture (Figure 5.10b).

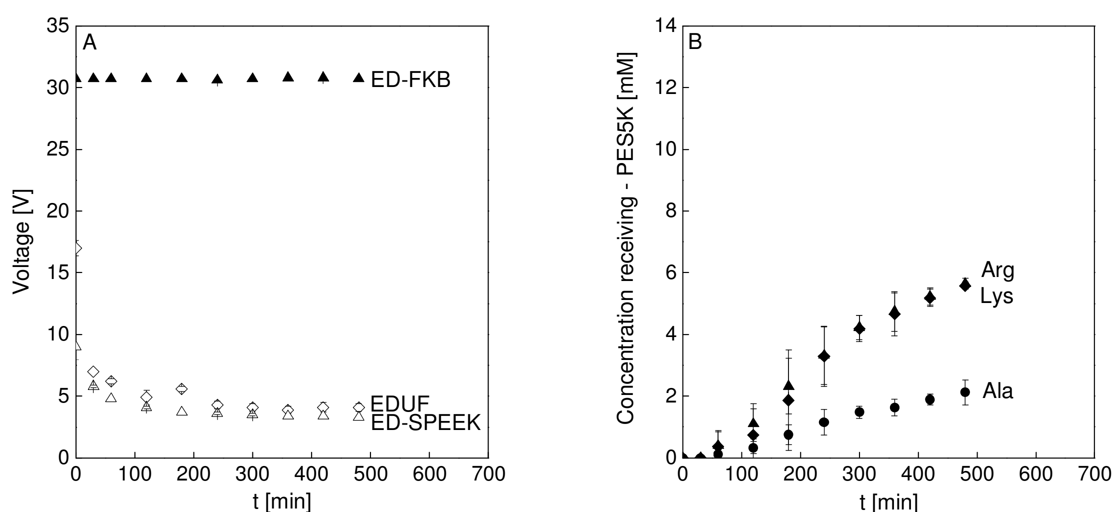

Figure 5.10. Separation of Lys and Arg from Ala with EDUF. a. Comparison of the voltage behavior in time of ED using FKB and SPEEK membranes and EDUF. b. Concentration in the receiving stream of Lys, Arg and Ala when applying EDUF. Flow rate: 50 ml/min; Initial pH of the feed = 6.0 (no pH control); Current density: 3.6 mA/cm^2.

As can be seen in Figure 5.10a, the voltage during the EDUF of Lys, Arg and Ala is similar to the one observed for the ED experiments with SPEEK membranes. Furthermore, the behavior of the voltage in time during the separation of Arg and Lys from Ala with SPEEK or EDUF is similar to the one obtained for a mixture of Lys and Ala only (Figure 5.8) suggesting that the presence of Arg does not have any effect on the process performance for the two alternative concepts (ED with SPEEK and EDUF).

Figure 5.10b shows that separation of Lys and Arg from Ala was successful when applying EDUF. Similar Lys and Arg recoveries are obtained when applying EDUF, contrary to the values obtained for ED with SPEEK membranes (Figure 5.7b), suggesting that the ultrafiltration membrane investigated has no selectivity of Arg over Lys. As previously discussed, migration of Ala through the porous UF membrane occurs as well, resulting in a 89.7% Ala retention, thus decreasing the product purity.

For all the experiments performed, attention was paid to the volume changes in feed and receiving compartment in the end of the experiment. For EDUF and ED-SPEEK an increase in the volume in the receiving compartment of 20 ml – 50 ml was observed. All calculations were corrected for the volume increase. Furthermore, a quick assessment on the osmotic pressure (osmotic flow) has been carried out, suggesting that water transport is a result of mainly electroconvection. Water transport numbers have been calculated as described by de Groot et al. [23], resulting in water transport numbers of 12 (corresponding water transport of 0.016 $mol/m^2{\cdot}s$) and 8 (corresponding water transport of 0.011 $mol/m^2{\cdot}s$) for EDUF and ED-SPEEK, respectively.

Process performance parameters such as recovery, current efficiency, energy consumption, flux and Ala retention were determined for the three membrane configurations investigated: ED with FKB membranes, ED with SPEEK membranes and EDUF (PES5K) for the separation of Lys from Ala and Lys and Arg from Ala. The results are summarized in Table 5.4, where a clear improved performance is obtained when using SPEEK as transport membrane, showing a higher recovery at higher current efficiency and lower energy consumption while no Ala migrates towards the receiving stream. All calculations, based on three replicates performed for each process condition, show an error <8% mainly attributed to errors in the analysis.

Table 5.4. Comparison of EDUF with the process performance of the separation of Lys from Ala and Lys and Arg from Ala using ED with FKB and SPEEK membranes.

Process parameters	ED of Lys from Ala			ED of Lys and Arg from Ala			
	FKB	SPEEK	EDUF	SPEEK		EDUF	
		Lys		Lys	Arg	Lys	Arg
Recovery [%]	38.7	46.0	36.2	27.0	46.3	24.1	24.8
Current efficiency [%]	33.7	36.9	31.3	14.8	24.1	24.1	24.8
Energy consumption [kWh/kg]	2.5	1.1	2.2	4.1	2.4	5.4	5.1
Flux [10^{-5} mol/m^2s]	8.7	9.6	8.1	5.6	9.0	5.5	5.4
Ala retention [%]	100.0	100.0	83.2	100.0		89.7	

Figure 5.11 is a graphical summary of the superior performance of ED with SPEEK membranes and EDUF for different Arg concentrations in the feed stream. No poisonous effect of Arg could be detected when SPEEK membranes with a high swelling degree are used or when ED is combined with the use of porous UF membranes. As mentioned before, a clear selectivity of Arg over Lys is exhibited by the FKB and the SPEEK membranes, whereas the transport of Lys and Arg is equal for the UF membranes.

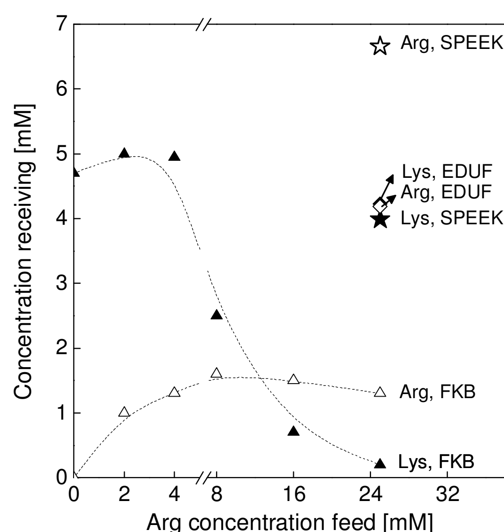

Figure 5.11. Concentration of Arg and Lys in receiving stream during the separation of Lys (25 mM) and increasing Arg concentration (0 mM, 2 mM, 4 mM, 8 mM, 16 mM, 25 mM) using ED with FKB membranes and SPEEK membranes and with EDUF. Flow rate: 50 ml/min; Initial pH of the feed = 6.0 (no pH control); Current density: 2.5 mA/cm², 3.6 mA/cm² (Table 5.1).

Based on the superior process performance of the ED with SPEEK membranes over EDUF (PES5K) and ED with FKB membranes, ED with SPEEK membranes is applied further for for the separation of the basic amino acids from the acidic group and neutral Ala (Section 5.4.4).

5.4.4 Separation of complex biobased mixtures

As a final confirmation and to prove the strength of ED for the separation of complex biobased mixtures of aa's containing Arg, ED with tailor made SPEEK membranes is applied to fractionate a mixture of five amino acids.

Figure 5.12a shows the voltage behavior during the separation of a complex mixture containing basic, acidic and neutral amino acids. The initial value of the voltage shows in general a variation of around 10 – 20% but reaches a stable value between 4 V and 5 V after 100 minutes of operation. Figure 5.12b indicates the pH in the three streams, receiving 1, receiving 2 and in the feed. As for previous experiments significant changes in the pH in receiving 1 and receiving 2 can be identified at the start of the experiment. This might be due to non-conductive parts in the membrane that cause a higher transport of target ions in other (same overall transport is achieved), while water splitting occurs in the non-conductive spaces. Water splitting might occur immediately after turning on the power supply mainly on the surface of the AEM. OH^- ions migrate towards the receiving 1 compartment, causing an increase in the pH while H^+ ions migrate towards the feed and immediately towards the receiving 2 compartment, causing a decrease in pH in this stream. Already after 60 min the pH reaches a constant value meaning that the transport of amino acids towards both compartments overtakes the current transport and water splitting is suppressed. As can be seen, the pH in the feed shows no significant variations staying stable at around 5.5. This means that as long as basic and acidic amino acids are present in the feed they will be either positively or negatively charged, respectively, and an increase in the voltage is unlikely to happen, while the neutral amino acids present will show no net charge.

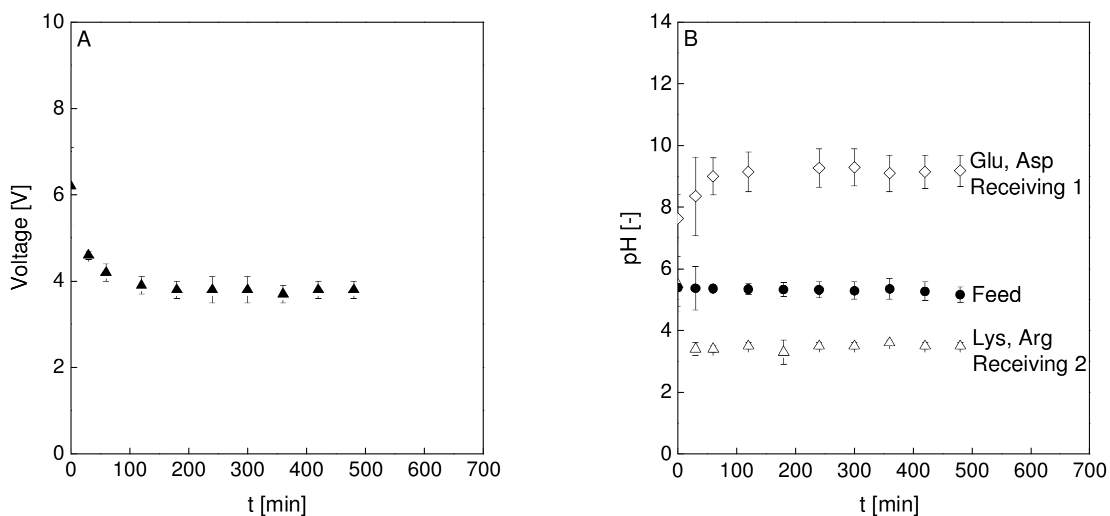

Figure 5.12. ED with SPEEK membranes of Lys, Arg, Glu, Asp and Ala. a. Behavior of voltage with time; b. behavior of pH with time. Flow rate: 50 ml/min; Initial pH of the feed = 6.0 (no pH control); Current density: 3.6 mA/cm^2. Membrane configuration: Figure 5.2.

The concentration behavior for the amino acids in the feed stream and both receiving compartments is shown in Figure 5.13. It is divided in two separate parts: the concentration

behavior of the acidic amino acids, Glu and Asp in the feed and the corresponding receiving stream (receiving 1, Figure 5.13a) and the concentration of the basic amino acids, Lys and Arg, in the feed and the corresponding receiving stream (receiving 2, Figure 5.13b). The Ala concentration is not shown since due to its neutral character this amino acid is not transported and completely retained in the feed compartment.

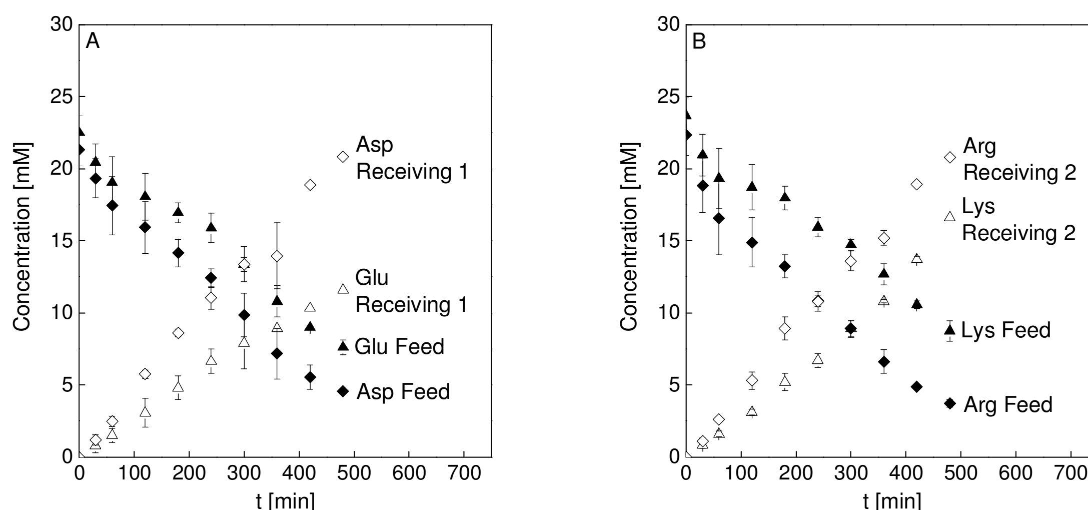

Figure 5.13. Concentration behavior in time during the separation of Lys, Arg, Glu, Asp and Ala with ED-SPEEK. a. Migration of acidic amino acids, Glu and Asp to the receiving 1 compartment. b. Migration of basic amino acids, Lys and Arg, to the receiving 2 compartment. Flow rate: 50 ml/min; Initial pH of the feed = 6.0 (no pH control); Current density: 3.6 mA/cm². Membrane configuration: Figure 5.2.

To the best of our knowledge, here we show, for the first time, the simultaneous separation of negatively (Glu, Asp) and positively (Lys, Arg) charged amino acids from neutral ones from a complex biobased mixture. The process performance of this separation is quantitatively summarized in Table 5.5. Three replicates of each process condition were performed leading to an error of <8% attributed to analysis errors.

Table 5.5. Process performance of ED with SPEEK membranes of complex mixture containing five amino acids.

Process performance parameter	Amino acid				
	Glu	Asp	Lys	Arg	Ala
Recovery [%]	72.1	97.8	70.3	93.2	---
Current efficiency [%]	41.9	53.7	42.9	53.7	----
Energy consumption [kWh/kg]	1.7	1.3	1.9	1.1	----
Flux [10^{-5} mol/m²s]	15.6	20.1	16.1	20.1	----
Ala retention [%]	----	----	----	----	100.00

5.5 Conclusions

The application of conventional electrodialysis for the separation of feed mixtures containing Arg is limited mainly due to a decrease in the SD of the membrane in the presence of Arg. This poisonous effect of Arg to cation exchange membranes was overcome with two alternative approaches: the use of a tailor made cation exchange membrane with a significantly higher SD and the combination of ED with ultrafiltration (EDUF, PES5K). The results clearly proof that the separation of Lys and Arg is possible with the two proposed alternative ED configurations. The poisonous effect of Arg towards the SPEEK membrane is absent, which confirms the hypothesis that the poisonous effect of Arg is directly related to a reduction in the water content of the membrane. By applying this approach (ED with SPEEK membranes) to more complex mixtures of aa's, we successfully achieved the simultaneous separation of the acidic amino acids, Glu and Asp, and the basic amino acids, Lys and Arg, from neutral Ala and validated the potential of ED for biorefinery applications.

5.6 Acknowledgements

This work is a GSPT project performed in close collaboration with Wageningen University (NL), which investigates the specific modification of the amino acids. Susan Witte is especially acknowledged for the amino acid analysis.

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6

Mixed matrix membranes for process intensification

This chapter has been submitted (in adapted form) to the Journal of Chemical Technology and Biotechnology as:

O.M. Kattan Readi, E. Rolevink, K. Nijmeijer, Mixed matrix membranes for process intensification in electrodialysis of amino acids.

ABSTRACT

Amino acids are valuable intermediates in the biobased economy for the production of biobased chemicals. Electro-membrane processes combined with enzymatic modification have been investigated as an alternative technology for the fractionation of a mixture of amino acids with almost identical charge behavior. Up to now, the modification and subsequent separation were performed in two separate reactors. Integration of both unit operations into one single device would lead to further process intensification and optimization. In this concept, mixed matrix membranes containing Relizyme EP403/S as enzyme carrier and the enzyme glutamic acid decarboxylase (GAD) are prepared and characterized. Their activity is evaluated for L-glutamic acid conversion into γ-aminobutyric acid, an interesting intermediate for the production of industrial chemicals, and the subsequent separation is evaluated. The prepared mixed matrix membranes, though showing a decrease in enzyme activity compared to the functionalized particles, proof to be effective for L-glutamic acid conversion. Together with the high mechanical stability obtained, this opens the route towards process intensification, combining enzymatic conversion and separation with electrodialysis in one integrated process for the successful isolation of amino acids for biorefinery applications.

6.1 Introduction

It is well known that green alternatives for the production of energy, fuels and chemicals are needed to overcome the depletion of fossil fuels, the increasing oil prices and the rise in the emissions of CO_2. The potential of different biomass sources for different applications has been studied intensively in the last years, e.g. microalgae to biofuel [1, 2], the application of the waste-to-energy technologies where waste streams can be converted into valuable biofuels [3] and the potential of bioplants such as *Jatropha Curcas* [4-6] or the use of lignocellulosic biomass for the production of biofuels and commodity chemicals [7-9].

While energy and fuels can be obtained from renewable resources such as wind and sun, chemicals need the carbon source present in biomass. A wide range of biomass sources has an attractive concentration of amino acids [10]. The major advantage of amino acids is that they already contain the required functionalities (i. e. –N and –O) for the production of biobased chemicals [11]. Alternative technologies for the production of new and different products to those derived from fossil resources have been investigated, developed and commercialized in the past, however often without considering the use of biomass. One of the difficulties of biomass is the complexity of the mixture. As usually for specific applications, only individual components can be used and advanced separation methods are a prerequisite for the transition towards a biobased economy.

Electro-membrane processes use an electrical field as driving force for the separation of charged molecules. Such processes have been successfully applied for the isolation of amino acids with different charge behavior with respect to pH [12-27]. To fractionate a mixture of amino acids with almost identical charge behavior represents a challenge. The combination of electrodialysis and enzymatic modification to modify the charge behavior of the amino acids was proven to be successful for their isolation [22, 23, 28, 29].

Integration of enzymatic conversion and subsequent separation into one single process would lead to further intensification of the process. An attractive approach is the use of mixed matrix membranes as platform for enzymatic reactions [30]. Mixed matrix membranes are characterized as the incorporation of a solid (dispersed) phase into a continuous polymer matrix [31]. They find their application e.g. in gas separation [32], as alternative for traditional chromatographic columns [33, 34], as adsorbers for protein recovery [35-37] and in medical applications such as blood

purification [38]. Selection and tailoring of the proper mixed matrix membrane offers the possibility to use this concept for process intensification purposes for the selective modification and separation of amino acids.

We present a systematic study on the development and application of such mixed matrix membranes for the selective modification and subsequent separation of amino acids using electrodialysis. Relizyme EP403 is used as enzyme carrier and glutamic acid decarboxylase (GAD) is immobilized on that. GAD initiates the modification of the amino acid Glu into γ-aminobutyric acid (GABA), an interesting intermediate for the production of industrial chemicals. This mixed matrix membrane is integrated in an electrodialysis stack and the enzymatic conversion of one of the amino acids by the immobilized enzyme is followed by the subsequent fractionation of the amino acid mixture using an electrical field.

The objectives of the present study are (1) to prepare mechanically stable MMMs containing Relizyme EP403 for enzyme immobilization; (2) to study the performance of the prepared MMMs as platform for the enzymatic decarboxylation of Glu; 3) to investigate the potential of MMMs for process intensification in electrodialysis for the selective conversion and further separation of amino acids.

6.2 Materials and methods

6.2.1 Materials

Amino acid solutions were prepared using L-glutamic acid and L-aspartic acid (Sigma-Aldrich) in solid state with a purity of 98% or higher. The enzyme glutamic acid decarboxylase (GAD, type I, 0.73 Units/mg at pH = 5.0 and 37 °C) was purchased from Sigma-Aldrich. Pyridoxal 5'phosphate monohydrate ($PLP \cdot H_2O$, Sigma-Aldrich) with a purity of 97% or higher was added as enzyme cofactor. Relizyme EP403/S (Biokal) with a particle size range of 100 – 300 μm and a pore size range of 40 – 60 nm was used as enzyme carrier.

Sodium phosphate buffer (pH = 7.0) used for enzyme immobilization was prepared by mixing 65.5 g/L Na_2HPO_4 anhydrous (dibasic, Acros Organics) with 40.5 g/L NaH_2PO_4 anhydrous (monobasic, Acros Organics) and adjusting the pH with pure NaOH (Merck). A washing solution of 0.60 g/L glacial acetic acid (100%, HAc, Merck) with 0.40 g/L NaOH (Merck) was

prepared and the pH was adjusted to 4.6 with pure NaOH. All solutions were prepared with MiliQ water.

For the membrane preparation, polyethersulfon (PES, Ultrason E6020P, Amoco) and polyethylene glycol (PEG) with a molecular weight of 400 g/mol (PEG MW400, Merck) were used as polymeric matrix, while N-methylpyrrolidone (99% NMP extra pure, Acros Organics) and MiliQ water were used as solvents.

For the electrodialysis experiments amino acid solutions containing Glu and Asp were used and the pH was adjusted with pure NaOH (Merck). A sodium sulfate solution (Merck Chemicals and Reagents) with a concentration of 0.1 M was used as electrode rinsing solution. Ion exchange membranes type Fumasep PEEK reinforced FKB (CEM) and Fumasep PEEK reinforced FAB (AEM) were purchased from FumaTech GmbH, Germany [22].

6.2.2 Enzyme carrier conditioning

The properties of Relizyme EP403/S used as enzyme carrier, obtained in wet state, are summarized in Table 6.1.

Table 6.1. Properties of Relizyme EP403/S.

Relizyme EP403/S	
Particle size range [μm]	100 - 300
Average pore diameter [nm]	40 - 60
Functional group	Epoxy (oxirane)

For the preparation of the mixed matrix membranes, dried material is required to guarantee the complete dispersion of the resin in the polymeric mixture. Therefore, as first step, the Relizyme EP403/S was completely dehydrated at room temperature under a dry nitrogen stream until a constant weight was obtained.

Dried Relizyme EP403/S was then milled using a Fritsch Pulverisette type P-0150 with a steel ball. The milled material was sieved using a Retsch AS200 sieving machine. The different fractions (Table 6.2) were used for enzyme immobilization and for mixed matrix membrane preparation.

Table 6.2. Fractions of unmilled and milled Relizyme EP403/S.

Fraction	Particle size [μm]
R_1	Unmilled (100 - 300)
R_2	32 - 60
R_3	20 – 32

6.2.3 Membrane preparation

Mixed matrix membranes were prepared using the different fractions (Table 6.2) of milled and unmilled dried Relizyme EP403/S. The casting solution with a particle loading of 50 wt% was prepared by adding Relizyme particles to a base solution consisting of PES, PEG MW400, NMP and miliQ water. After that a mixture of NMP/PEG/miliQ water was added to obtain the desired concentration. For comparison, a microfiltration membrane without particles was also prepared (R_0). The composition of the base solution as well as that of the final casting solution is summarized in Table 6.3.

Table 6.3. Composition of base and final casting solution for membrane preparation.

Component	Base solution [wt%]	MMM [wt%]	Base membrane [wt%]
Particle fraction[1]	---	12.00	---
PES	16.00	12.00	12.00
PEG MW400	38.55	34.75	40.30
NMP	38.55	34.75	40.30
miliQ water	6.90	6.50	7.40

[1]Particle fractions used for MMM preparation: Table 6.2.

The mixtures were homogenized on a roller bank during 3 days. Prior to casting the solution was kept standing without mixing for 2 hours to allow degassing. To obtain MMMs as thin as possible, the thinnest applicable casting knife was used for the different fractions. A 1000 μm casting knife was used for the membranes with particle fractions R_1 and R_2. Membranes with particle fraction R_3 and the base membrane for comparison were casted using an 800 μm casting knife.

6.2.4 Characterization techniques

6.2.4.1 SEM

The structure and morphology of the particles and membranes were analyzed using a JEOL JSM-5600LV scanning electron microscope (JEOL, Japan). The samples were dried at room

temperature under nitrogen. For cross section analysis, the membranes were cryogenically broken in liquid nitrogen. All samples were coated with a BalzersUnion SCD 040 sputter coater (Oerlikon Balzers, Belgium).

6.2.4.2 Swelling degree

The swelling degree (SD) of the prepared membranes in miliQ water was determined similar to the method described by Dlugolecki et al. [40]. The membrane samples were immersed in miliQ water at room temperature. After 24 hours the samples were removed from the solution, the remaining surface water was removed with blotting paper and the weight of the membrane samples was recorded subsequently. Afterwards, the membrane samples were placed in a vacuum oven at 30°C. After 24 h the membranes were taken out from the oven, the dry weight was recorded and the SD was calculated as follows [40]:

$$SD = \frac{m_{wet} - m_{dry}}{m_{dry}} * 100\% \qquad \text{Eq. 6.1}$$

6.2.4.3 Clean water flux

To determine the clean water permeance (Lp) of the membranes, a nitrogen pressurized dead-end "Amicon type" cell was used. The experiments were performed at room temperature and with miliQ water. Flat membranes with an active surface area of 3.46 cm^2 were pre-pressurized for 10 min at 1.00 bar (highest applicable pressure). The clean-water flux was determined at 0.25, 0.50, 0.75 and 1.00 bar. The flux was plotted against the transmembrane pressure and the slope of the linear area gave the clean water permeance.

6.2.4.5 Zeta-potential

The zeta-potential of the prepared membranes was determined using a SurPASS Electrokinetic Analyzer (Anton Paar GmbH, Austria) by measuring the streaming potential or the streaming current. Two pieces of the flat sheet membranes were placed in the cell while 1 mM KCl, used as electrolyte solution, was circulated through the measurement cell. 0.1 M nitric acid and 0.1 M NaOH were used for automatic pH adjustment. The zeta-potential of the surface of the membrane was determined assuming that the electrolyte solution flows in one direction and does not enter the porous structure.

6.2.4.6 Oxirane density

The oxirane density of dried unmilled Relizyme EP403/S was determined as described elsewhere [41]. 300 mg of particles were suspended in deionized water and the pH was adjusted to 7.0 and 15 ml of 1.3 M $Na_2S_2O_3$ solution was added and shaken for two hours. The reaction produced OH^- ions that were back titrated with 0.01 M HCl solution giving the oxirane density.

FT-IR analysis was performed on the MMMs to determine the relative oxirane density [42-44]. Samples were analyzed without further conditioning using an ALPHA FT-IR Spectrometer (Bruker Optics) with a spectral range of 375 – 7500 cm^{-1}. The peak at a wavelength of 915 cm^{-1} characteristic of epoxy groups [44] was integrated and compared relative to the oxirane density exhibited by unmilled Relizyme EP403/S particles (R_0).

6.2.5 Enzyme immobilization and activity assay

6.2.5.1 Enzyme immobilization

GAD immobilization onto Relizyme EP403/S was carried out similar to the procedure described elsewhere [28]. Dry Relizyme EP403/S beads (500 mg or equivalent) were suspended in sodium phosphate buffer (5ml or equivalent, 1 M at pH 7.0) with a concentration of 0.1 mg/ml GAD. The suspension was mixed overnight at room temperature. Afterwards, the suspension was filtered. To remove all non-covalently bound enzyme, the beads were washed two times with sodium acetate (0.01 M, pH 4.6). To determine the enzyme immobilization efficiency, the washing solution (supernatant) containing the non-covalently bonded enzyme was analyzed for its protein content by determining the absorbance of the samples at 595 nm using a Cary 300 Scan UV-visible spectrophotometer (Varian).

6.2.5.2 Activity assay

For the determination of the enzyme activity, 500 mg of beads or equivalent were contacted with 10 ml substrate solution containing 8 mM L-glutamic acid, 8 mM L-aspartic acid and 0.5 mM PLP at pH 4.6 (adjusted with NaOH). The reaction was performed at room temperature, without pH control or a buffer. Samples were taken every hour and analyzed via U-HPLC as described in the following section. The initial enzyme activity was calculated at 5% of substrate conversion and expressed as U/mg of enzyme. Relative activities are reported in % of base activity when using R_1 particle fractions in wet or dried state or the mixed matrix membranes prepared with the R_1 fraction (indicated for each specific case).

The amino acid concentration in the different samples was analyzed via Ultra High Pressure Liquid Chromatography, U-HPLC using a Dionex U-HPLC instrument (Dionex Corporation, Sunnyvale, CA, USA). The instrument was equipped with an Ultimate 3000 RS (rapid separation) pump, an Ultimate 3000 Autosampler, an Ultimate 3000 column compartment with temperature control and an Ultimate 3000 variable wavelength detector. Dionex Chromeleon™ 6.8 Software is used to run the system and analyze the data. For the separation, an Acquity UPLC® BEH C18 reversed phase column (1.7 μm particle size, 2.1 x 50 mm) and an Acclaim® 120 C18 guard column (5 μm, 2.0 x 10 mm) were selected. The sample preparation and the analysis method for the amino acid concentration is explained elsewhere [29].

6.2.6 Electrodialysis with integrated MMM

As a final step the integration of the prepared MMMs and electrodialysis for simultaneous conversion of Glu into GABA and its further separation from Asp was investigated. For that, we used an electrodialysis stack with next to the anion and cation exchange membranes the MMM combined with an anion exchange membrane (Figure 6.1). As such this membrane consisted of two individual layers, place together: the MMM to induce enzymatic conversion and the AEM to allow the selective transport of negatively charged amino acids.

At first the separation of Glu and Asp from Ala at pH = 4.6 (both negatively charged) through an anion exchange membrane (FAB-PEEK, FumaTech GmbH) was investigated (Figure 6.1a). Secondly, the conversion of Glu to GABA was considered. In that case the receiving stream 1 consisted of unconverted Glu, Asp and GABA. Lastly, unconverted Glu and Asp were separated further to the receiving stream 2 through an AEM (FAB-PEEK, FumaTech GmbH). For all experiments performed, amino acids (Glu and Asp) were already added to all streams (feed, receiving 1 and receiving 2) to decrease the overall resistance of the stack. Two different experiments were performed: 1) Receiving stream 1 consisted of Glu, Asp, native GAD and PLP as cofactor. The enzymatic reaction took place in receiving stream 1 using native GAD (Figure 6.1a) and 2) Receiving stream consisted of Glu, Asp and PLP as cofactor. The enzymatic conversion of Glu to GABA took place when the amino acid migrated from the feed through the AEM coupled with the prepared MMM towards the receiving stream 1 (Figure 6.1b). The schematic representation of the different configurations used for the electrodialysis experiments is shown in Figure 6.1.

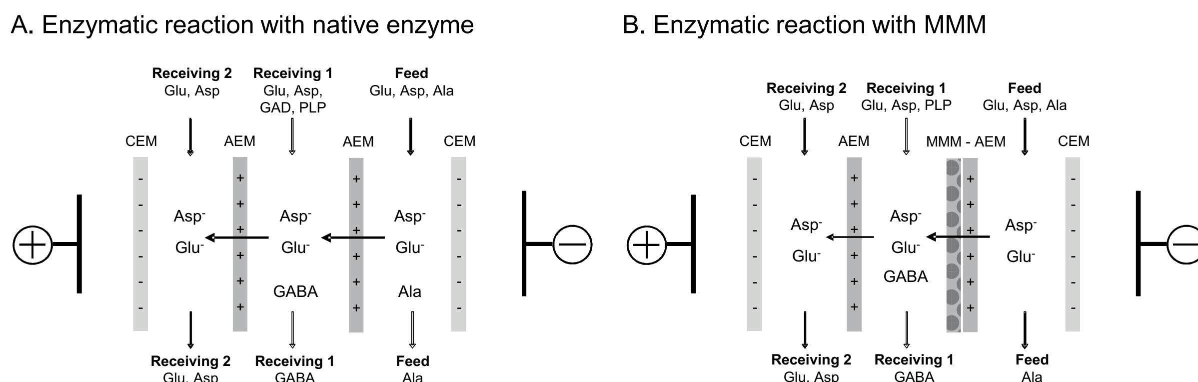

Figure 6.1. Schematic representation of electrodialysis for the integration of reaction and separation. a) Enzymatic reaction using native GAD and PLP as cofactor and b) Enzymatic reaction using a MMM coupled with an AEM.

A 5-compartment ED stack equipped with a platinized titanium electrode at the anode side and a stainless steel electrode at the cathode side was used for all electrodialysis experiments. The current was applied via a power supply (Delta Elektronika). Every hour, samples from both streams were taken and analyzed with U-HPLC as described in the following section. For all experiments cation exchange membranes (FKB-PEEK, FumaTech GmbH) were placed at both anode and cathode side to prevent the further migration of negatively charged Asp and Glu. For all solutions, the pH was adjusted to 4.6 with NaOH.

The experiments were performed at a current density of 0.9 mA/cm^2 which corresponds to 80% of the previously determined limiting current density (1.16 mA/cm^2) [22]. The effective membrane area used was 44.2 cm^2. The process was assessed based on amino acid flux, current efficiency, energy consumption and GABA retention [22, 23]. Additionally, the conversion of Glu to GABA relative to the amount of Glu transported from feed to receiving 1 stream was determined. The different compositions of feed and both receiving streams are summarized in Table 6.4.

Table 6.4. Composition of feed, receiving 1 and receiving 2 streams for the integration of enzymatic conversion and further separation with electrodialysis.

Experiment	Feed	Receiving 1	Receiving 2
1	8 mM Asp, 8 mM Glu, 8 mM Ala	8 mM Asp, 8 mM Glu, 0.5 mM PLP, 0.1 mg/L GAD	4 mM Asp, 4 mM Glu
2		8 mM Asp, 8 mM Glu, 0.5 mM PLP	

6.3. Results and discussion

6.3.1 Enzyme carrier conditioning

The morphology of the unmilled Relizyme EP403/S particles was analyzed with SEM and shows smooth spherical particles (Figure 6.2a) with a very porous structure (Figure 6.2b).

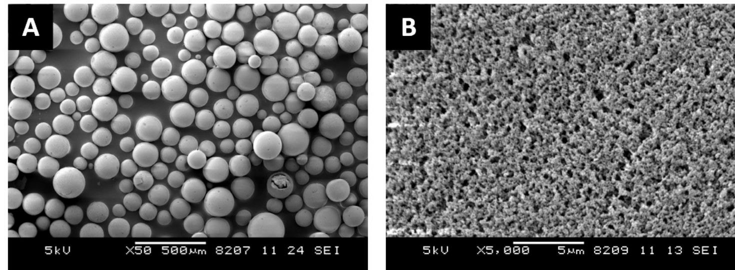

Figure 6.2. Morphology of unmilled Relizyme EP403/S (R_1): a) Particles (magnification: 50x), b) Porous surface (magnification: 5000x).

To guarantee complete dispersion of the resin in the polymeric mixture, Relizyme EP403/S was completely dehydrated at room temperature under a dry nitrogen stream until a constant weight was obtained (48 h). A water content of 66.5 wt% was found. After drying, Relizyme EP403/S was milled as explained in Section 6.2.2. Figure 6.3 shows the SEM analysis of the morphology of the different fractions obtained.

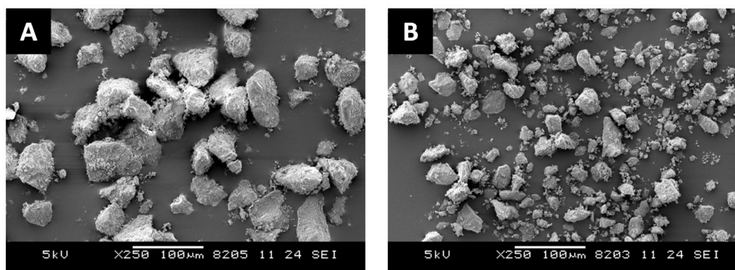

Figure 6.3. Morphology of milled Relizyme EP403/S fractions (magnification: 250x). a) R_2: 32 - 60 μm, b) R_3: 20 – 32 μm.

As a result of the milling, the smooth spherical structure was destroyed for all Relizyme EP403/S fractions prepared. The non-spherical shape of the particles might have an influence on the morphology of the mixed matrix membranes and the creation of e.g. voids around the particles may occur. However, this will depend on the adhesion between the particles and the membrane material.

6.3.2 Membrane preparation

The different Relizyme EP403/S fractions obtained after drying, milling and sieving were used for the preparation of the mixed matrix membranes (Table 6.2) as described in the experimental part. For comparison, a microfiltration membrane without particles was also prepared (R_0).

6.3.3 Characterization

6.3.3.1 SEM

Mixed matrix membranes were prepared using different fractions of dried Relizyme EP403/S. The morphology of the membranes was analyzed using Scanning Electron Microscopy (SEM). Figure 6.4 shows the cross section of the mixed matrix membranes containing dried unmilled Relizyme particles (Figure 6.4a), particle fraction R_2 (Figure 6.4b) and particle fraction R_3 (Figure 6.4c).

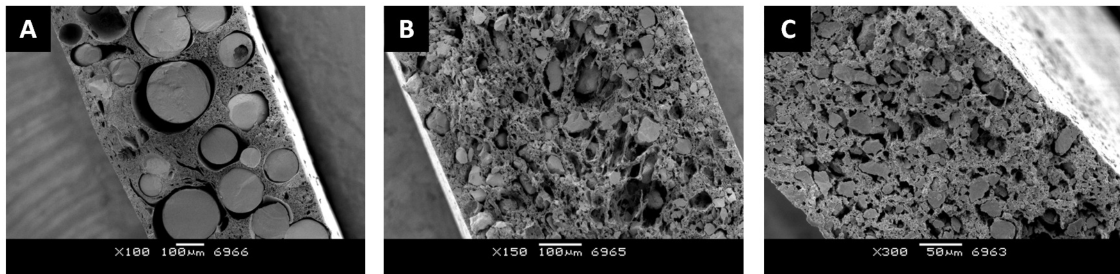

Figure 6.4. Morphology of the cross section of the prepared membranes with different Relizyme EP403/S fractions (no enzyme immobilization yet): a) R_1: 100 – 300 μm (magnification: 100x), b) R_2: 32 – 60 μm (magnification: 150x), c) R_3: 20 – 32 μm (magnification: 300x).

The use of unmilled particles resulted in low mechanical stability of the prepared membranes. Voids were created around the particles for all mixed matrix membranes prepared (Figure 6.4). This most probably coincides with an increase in porosity and leads to an increase in clean water permeance of the mixed matrix membranes. This effect would not be beneficial for the integration of the MMM in an electrodialysis stack as this will give rise to high water fluxes from one compartment to the other.

For comparison, a membrane without particles was prepared as well (Table 6.3). Figure 6.5 shows the cross section (Figure 6.5a) and the bottom surface (Figure 6.5b) of the prepared microfiltration membrane without particles.

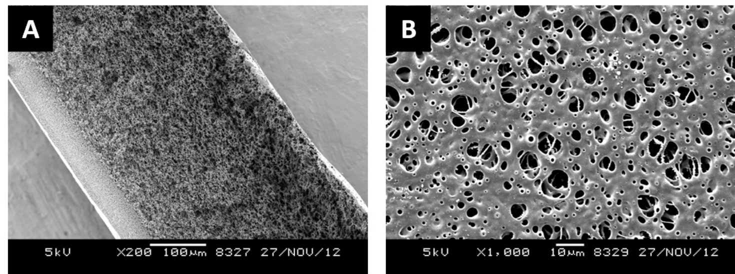

Figure 6.5. Morphology of the prepared microfiltration membrane R_0: a) Cross section (magnification: 200x), b) Bottom surface (magnification: 1000x).

The membrane obtained is a macro void free microfiltration membrane with a highly porous top surface with surface pores up to 6 μm.

6.3.3.2 Swelling

The swelling degree of all prepared membranes was determined based on the dry weight of the membranes. A swelling degree of 139% was obtained for the base membrane without particles (R_0). For all MMMs tested, swelling degrees higher than 150% were obtained regardless of the particle size of the resin. The values obtained were 179%, 173% and 176% for the membranes containing the fractions R_1, R_2 and R_3, respectively. As the uptake of water predominantly occurs in the pores of the membrane rather than resulting in swelling of the polymer itself, this indicates that a higher porosity was obtained for the MMMs relative to the base membrane without particles (R_0). The obtained swelling degrees are characteristic for micro-, ultra- and nanofiltration membranes and ultra- and nanofiltration membranes have been already successfully applied in electrodiaysis [19, 21, 27].

6.3.3.3 Clean water flux

The clean water flux provides information about the clean water permeance of the membranes and is an important measure to quantify the cross transport of water from one compartment to another in the ED stack. Clean water permeances for the base membrane without particles (R_0) and for the prepared membranes with particle fractions R_1, R_2 and R_3 were determined. After stabilization of the membranes for 10 min at the highest applied pressure (1 bar), the clean water flux was measured for different pressures. The slope of the curve of the flux against each corresponding pressure indicates the clean water permeance of the MMMs. The results of the different membranes are summarized in Figure 6.6.

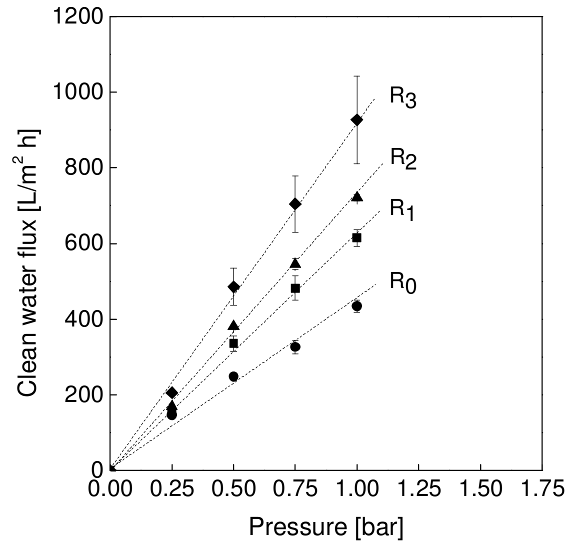

Figure 6.6. Average clean water flux against the corresponding transmembrane pressure of the native membrane and the mixed matrix membranes (without enzyme immobilization, R_0) with different particle fractions R_1, R_2 and R_3.

The clean water permeance of the MMMs increases with decreasing particle size. The values obtained for the native membrane without particles and the different MMMs investigated are summarized in Table 6.5.

Table 6.5. Clean water permeance of the prepared native membrane and MMMs.

Particle fraction	Clean water permeance [L/m² · h · bar]	Thickness [μm]
R_0 (native membrane, no particles)	487 ± 3.0	600
R_1	679 ± 14.0	630
R_2	806 ± 13.5	530
R_3	949 ± 9.0	300

The membrane without particles (R_0) and the MMM prepared with particle fraction R_1 have similar thicknesses and can be compared. Comparison of the clean water permeances of these two membranes reveals an increased clean water permeance for the MMM suggesting an increase in porosity due to the creation of voids around the particles (Figure 6.4). The obtained swelling degrees corresponding to these two membranes (SD_{R0} = 139%, SD_{R1} = 179%) supports this assumption. For the MMMs with particle fractions R_2 and R_3 the permeance increases further. However, these membranes have considerably lower thicknesses also resulting in an increase in permeance. As such the higher water permeances cannot be attributed to the decrease in particle

size only. The similar values obtained for the SD (SD_{R2} = 173%, SD_{R3} = 176%) suggest that the increase in permeance is not related to porosity but rather to membrane thickness and tortuosity. Conventionally used membranes for electrodialysis are dense ion exchange membranes and water transport between compartments is usually avoided. Porous membranes with permeances comparable to those of microfiltration membranes [38, 45] might induce significant water transport. Ultrafiltration and nanofiltration membranes on the other hand have been successfully applied in electrodialysis processes [19, 21, 27].

3.3.4 Zeta-potential

To evaluate the surface charge of the developed mixed matrix membranes, their zeta potential was determined. The results obtained for the native membrane without particles and the MMMs investigated are summarized in Figure 6.7.

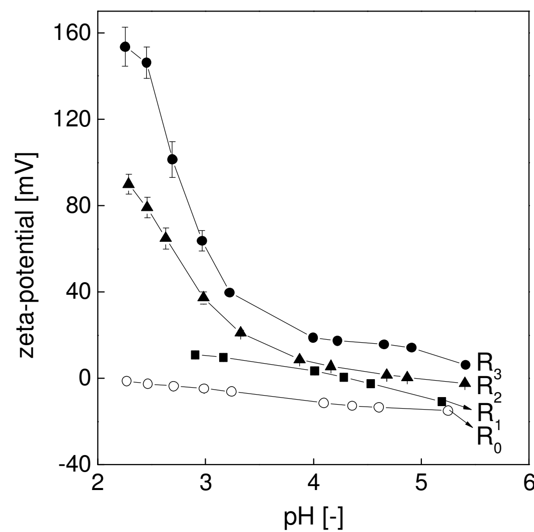

Figure 6.7. Zeta-potential of the prepared native membrane and the mixed matrix membranes measured as a function of pH. pH range: 2 – 6. Operational pH in ED experiments: 4.6

In the surroundings of the operating pH (pH = 4.6) the MMMs show either a slightly positive (R_2, R_3) or no net charge (R_1) while the native membrane without particles shows a slightly negative charge. The charge of the prepared MMMs increases with decreasing particle size what suggests that the particles have a positive charge..

6.3.3.5 Oxirane density

FT-IR spectra of the prepared MMMs of particle fraction R_1, R_2 and R_3 were also recorded (Figure 6.8a, continuous lines) and compared with the FT-IR spectra of the single particles of fraction R_1 (Figure 6.8a, dotted line). The results show an increase in the oxirane density (peak obtained at a wavelength of approximately 910 cm^{-1}) with decreasing particle size. Previously the functional group density of unmilled Relizyme EP403/S was determined in its wet and dry state. This resulted in an oxirane density of 119 ± 15 μmol/g and 108 ± 12 μmol/g, respectively. The results of the oxirane density for dry and wet Relizyme suggest that no change in functional group density took place as consequence of the drying process. The functional group density of the MMMs prepared was determined relative to functional group density of these dried unmilled particles (fraction $R_{1,}$ 119 ± 15 μmol/g) (Figure 6.8b).

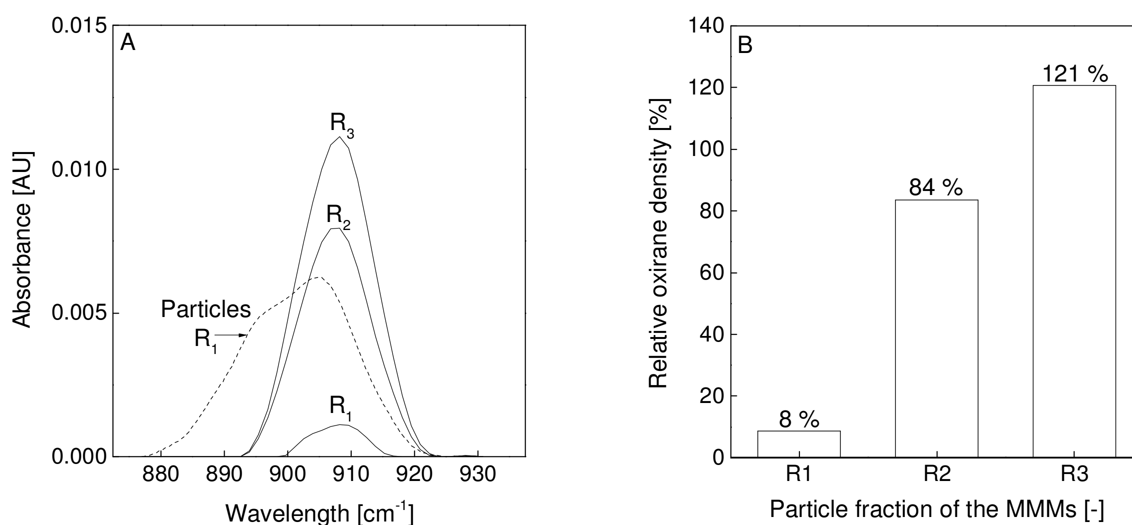

Figure 6.8. a) FT-IR analysis of the prepared MMMs with different Relizyme EP403/S particle fractions at λ = 870 - 940 cm^{-1} and b) Oxirane density of the different Relizyme EP403/S fractions relative to unmilled dried Relizyme EP403/S fraction R_1. No enzyme immobilization on the particles yet.

Figure 6.8 shows that the oxirane density increases with decreasing particle size. This might result in a decrease in enzyme activity. Conventionally speaking, an increase in the density of the functional groups would give an increased capacity or activity. However, in most of the cases, an optimal density exist. Too low densities of the epoxy groups results in low immobilization capacities, but, on the other hand, too high densities can result in a loss of the number of active sites of the enzyme available for Glu conversion due to immobilization of the enzyme at multiple active sites simultaneously thus decreasing its activity.

Table 6.6 summarizes the results discussed above regarding the characterization of the prepared membranes.

Table 6.6. Characterization of the prepared membranes.

Fraction	R_0	R_1	R_2	R_3
Particle size [μm]	No particles	100 - 300	32 - 60	20 - 32
Thickness [μm]	600	630	530	300
Swelling degree [%]	139 ± 7.8	179 ± 12.8	173 ± 1.9	176 ± 3.5
Permeance [$l/m^2 \cdot h \cdot bar$]	487 ± 3.0	679 ± 14.0	806 ± 13.5	949 ± 9.0
Relative oxirane density[1] [%]	n.a.	9	84	121
Charge at pH = 4.6 [-]	negative	neutral	positive	positive

[1]Relative to particle fraction R_1.

6.3.4 Enzyme immobilization and activity assay

The same procedure for enzyme immobilization as was used for the single particles was also applied for the immobilization of GAD onto the prepared MMMs. For all cases, an immobilization efficiency of around 25% was obtained.

Static activity assays of the prepared mixed matrix membranes were performed as explained in the experimental part and the results are summarized in Figure 6.9 relative to the activity of the unmilled particles or the membranes with the unmilled particles. The results show a slight decrease in the activity of the enzyme immobilized on MMMs with decreasing particle size.

Relatively higher oxirane densities are obtained for the MMMs containing dried milled particles from fractions R_2 and R_3 compared to that of the MMMs containing the dried unmilled particles from fraction R_1. On the other hand, the oxirane density of the MMMs containing particle fractions R_2 and R_3 is similar to the oxirane density found for the pure dried unmilled particles of fraction R_1. If we assume that the pure unmilled Relizyme particles of fraction R_1 already have the optimal functional group density, the maximum enzymatic activity will be obtained for that oxirane density. Then it appears logical that the enzymatic activity obtained for the MMMs of particle fractions R_2 and R_3 is comparable to that obtained for the pure dried unmilled Relizyme particles.

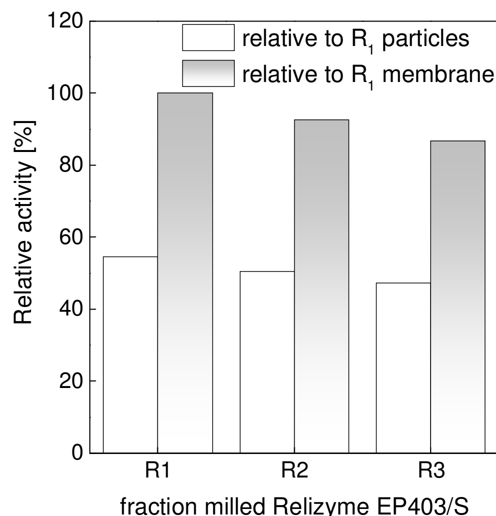

Figure 6.9. Relative activity of the mixed matrix membranes with different fractions of dried Relizyme EP403/S particles. Values relative to those of dried unmilled Relizyme EP403/S particles (fraction R_1) and to those of the mixed matrix membrane produced with particle fraction R_1.

Surprisingly, the MMMs containing the unmilled fraction R_1 show higher activity while showing a lower relative oxirane density (8%) when compared to pure dried unmilled R_1 particles. The reason might be the mechanical instabilities of the membrane. Due to the large particle size, some of the particles are removed from the membrane while stirring during the enzymatic activity test, but these were still present in the reaction vial. To determine the functional group density, FT-IR measurements were performed to three different dried membrane samples. It cannot be excluded that before the measurement unmilled Relizyme particles had detached from the membrane. Then the oxirane density measured is an apparent value and lower than the real functional group density.

6.3.5 Electrodialysis with integrated MMM

As a final step, integration of enzymatic modification and separation in one single process has been evaluated. Electrodialysis with an integrated MMM was carried out to proof the principle of the simultaneous conversion of Glu into GABA and its further isolation from Asp. The MMM of particle fraction R_2 and R_3 showed both high mechanical stability. MMM of particle fraction R_2 were used for the integration experiments due to their lower water permeance compared to those of particle fraction R_3.

For comparison, ED experiments without the MMM but with native GAD added to the receiving 1 compartment for enzymatic conversion of Glu to GABA were carried out as well. In that case the enzyme was not immobilized on the MMM and the electrodialysis stack did not contain an MMM.

Preliminary experiments (results not shown) resulted in a high water flux between feed and receiving 1 compartment when only the MMM was used as both transport membrane and platform for enzymatic conversion. To avoid the transport of water, the MMM was than combined with a standard anion exchange membrane (FAB-PEEK), resulting in one membrane consisting of two layers. The two different membrane configurations used in the ED experiments are shown in Figure 6.1. Table 6.7 shows an overview of the process performance parameters obtained for both membrane configurations used.

Table 6.7. Process performance parameters for an integrated system combining enzymatic conversion and separation for the fractionation of Glu and Asp. Comparison of a system with native enzyme and a system with enzyme immobilized onto an MMM.

No.[1]	Membrane	Amino acid	Glu to GABA conversion[2] [%]	Location	Flux $\cdot 10^{-7}$ [mol/m^2 s]	Current efficiency [%]	Energy consumption [kWh/kg]
1	FAB-PEEK (1)	Glu	100	Receiving compartment 1	2.7	31.4	1.9
		Asp			2.9	28.2	1.8
	FAB-PEEK (2)	Glu		Receiving compartment 2	2.8	30.7	2.0
		Asp			2.9	30.2	2.0
2	MMM-FAB-PEEK (1)	Glu	33	Receiving compartment 1	3.7	39.6	2.8
		Asp			4.1	43.5	2.6
	FAB-PEEK (2)	Glu		Receiving compartment 2	4.0	42.2	2.9
		Asp			4.4	47.5	2.6

[1]Membrane configuration: see Figure 6.1. Composition of feed and receiving solutions: see Table 6.1.

[2]Relative to amount of Glu transported from feed to receiving compartment 1.

For the experiment using native GAD (not immobilized) a single amino acid flux (Glu or Asp) of around $3 \cdot 10^{-7}$ mol/m$^2 \cdot$ s was found, while the system with the integrated MMM showed a higher single amino acid flux ($4 \cdot 10^{-7}$ mol/m$^2 \cdot$ s). The higher amino acid flux for the system with the MMM is related to the lower Glu to GABA conversion achieved. This conversion takes place in receiving compartment 1. When a molecule of Glu is converted into GABA, OH^- is released. This OH^- competes with Glu^- and Asp^- and is because of its high mobility transported preferably towards receiving compartment 2. At equal conditions, an increased conversion of Glu thus results in a decrease in Glu or Asp amino acid transport towards receiving compartment 2 due to

an increase in the transport of OH^-. When native GAD is used, 100% conversion of Glu into GABA was reached in receiving compartment 1, while only 33% conversion was obtained for the experiment with the integrated MMM while for both configurations the same amount of GAD was available. Consequently, a lower single amino acid (Glu or Asp) flux for receiving compartment 2 was obtained for the system with native GAD and no MMM. The same effect is visible when comparing the current efficiencies of both systems. Current efficiencies of around 30% and 40% for the experiment with native GAD and the integrated MMM, respectively, were obtained.

For both experiments, a decrease in pH in the feed compartment, a more or less constant pH in receiving compartment 1 and an increase in pH in receiving compartment 2 were observed (results not shown). This indicates the occurrence of water splitting. Current is not only used for amino acid transport, but part of it is used for water splitting to produce the necessary ions for charge transport.

When the experiment with native GAD is compared to that when using electrodialysis with an integrated MMM, a two times higher stack resistance for the system with the MMM is obtained. The potential difference across the electrodialysis stack for the electrodialysis experiment with native GAD and with an integrated MMM were 15 V and 30.8 V, respectively. This obvious result is not predominantly related to the nature of the MMM but to the extra membrane layer added to the system. Comparable electrodialysis systems, using bipolar membranes that consist of an anionic and a cationic membrane layer, also show an increased resistance compared to a system with a single ion exchange membrane, even though the nature of the membranes is equal in that case (both have ion exchange properties) [46]. For the case of bipolar membranes, the stack resistance decreases once water splitting sets in due to the production of H^+ and OH^- [46] and overall it contributes to the improvement of the process economics (Chapter 5). Enhanced water splitting does not take place for an integrated MMM (positive surface charge, same charge as the AEM layer in the combined membrane). Consequently the voltage remains high during the experiment, resulting in higher energy consumptions and less favorable process economics than those obtained for the system without the integrated MMM.

Here we reported for the first time the use of an mixed matrix membrane as a way to integrate enzymatic conversion and subsequent separation using electrodialysis for the fractionation of amino acids for the biobased economy. Although these are initial results, these satisfactory proof

the principle of the integration of reaction and separation using MMMs and set the crucial areas for further optimization. Improvement of MMMs towards an increase in activity and a decrease in water transport to prevent the use of an AEM will result in an efficiently integrated process for simultaneous enzymatic conversion and amino acid isolation.

6.4 Conclusions

This work presents a systematic study of mixed matrix membrane fabrication for process intensification in the isolation of amino acids with electrodialysis. Mixed matrix membranes were produced using Relizyme EP403/S as enzyme carrier and the enzyme GAD as model enzyme. The mixed matrix membranes obtained showed activity towards L-glutamic acid conversion into GABA and a good mechanical stability.

The principle of the integration of enzymatic conversion and further separation with an electrodialysis stack was possible. ED with commercially available anion exchange membranes and a mixed matrix membrane for the transport of negatively charged L-aspartic acid and L-glutamic acid and simultaneous enzymatic decarboxylation of L-glutamic acid to GABA was performed. The further separation from L-aspartic acid and the unconverted L-glutamic acid was proven to be successful. The results show the applicability of this approach and opens a broad area for further investigation towards process optimization in biorefinery processes.

6.5 Acknowledgements

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7

Preliminary process design and economic evaluation

This chapter is in preparation for publication as:

O.M. Kattan Readi, Y. Wu, A.G.J. van der Ham, K. Nijmeijer, Process design and economic evaluation of electrodialysis for the separation of amino acids.

ABSTRACT

As alternative for conventional refineries, electrodialysis combined with enzymatic modification can be applied for the successful fractionation of amino acid mixtures from a biobased feedstocks. Once isolated, amino acids can be used for the production of industrial chemicals. The objective of this work was to evaluate the economic feasibility of the suggested alternative route for the production of biochemicals. One of the most interesting chemical intermediates is γ–aminobutyric acid (GABA), the modification product of the decarboxylation of glutamic acid (Glu) because of its high price. Glu is a non-essential amino acid as it does not compete with food or feed applications. Additionally, the separation of Glu and Asp from an amino acid mixture and further separation of Asp from GABA does not need strict pH control. After evaluation based on price and glutamic acid content, DDGS was selected as raw material for the isolation of amino acids with electrodialysis. Literature data based on experimental results on amino acid recovery, conversion and amino acid flux were used for the cost evaluation. Amino acid flux and the cost of the ion exchange membranes were found to be the limiting parameters for the economic feasibility of the process. An increase in amino acid flux by a factor of 10^2 is needed to guarantee a profitable process without a decrease in the membrane cost and with a payback period of one year. Furthermore, process intensification results in a decrease in effective membrane area, hence a decrease in both capital and operating costs of 25 %.

7.1 Introduction

The use of renewable resources such as wind and sun has been exploited for the generation of energy and fuels. However, alternative routes for the production of chemicals conventionally obtained from fossil feedstocks, require carbon sources for their production, which are e.g. present in biomass. Biomass sources have an attractive concentration of amino acids [1], which already contain the necessary functionalities (i. e. –N and –O) for the production of biobased chemicals [2]. Separation and conversion are an integral part of such biobased facilities for the production of chemicals. Electro-membrane processes are successful for the isolation of amino acids with differences in charge behavior with respect to pH [3-18]. The technical feasibility of the combination of electrodialysis with enzymatic modification is successful for the isolation of specific amino acids with almost identical charge behavior [13, 14, 19, 20].

Process and cost evaluation of the enzymatic enzymatic decarboxylation of Glu to GABA [19] suggest that the cost of the enzyme (2 – 3€ per ton product, same range as amylase used for the hydrolysis of starch) and co-factor (PLP for the enzyme GAD) are not expected to contribute considerably. Lammens et al. [19] consider the high cost of Glu, now produced by fermentative processes, to be the main obstacle of the application of the process and suggest the isolation of Glu from protein streams as essential to make the process cost-effective. Electrodialysis is a successful alternative for Glu and Asp separation and further isolation of GABA from Asp. In electrodialysis processes it is very difficult to perform an accurate economic analysis. Its complexity lies on the influence of several factors such as location, individual application, membrane price, membrane properties, feed conditions, product quality requirements, operation parameters [21]. In most of the work performed on the application of electrodialysis for the separation of biomolecules it does not go any further than the calculation of the energy consumption as an indication for the economic feasibility of the process [9, 13, 14, 18, 22-24].

In this work we perform a preliminary process and economic evaluation to address some of the major issues regarding the economic feasibility of such an electrodialysis process for the isolation of amino acids from biobased feedstocks. We evaluate and select the most suitable biobased raw material for the isolation of amino acids and perform an initial process design and cost evaluation based on available literature data. The results indicate the limiting factors for the economic feasibility of the suggested alternative process route and set future directives to further improve the development of the process.

7.2 Raw material selection

In the last years, the potential of different biomass sources has been studied for different applications. For example, microalgae [25, 26] and the application of the waste-to-energy technologies [27] have been investigated for the production of biofuels. Also *Jatropha Curcas* has been considered for the application as bioplants [28-30] and lignocellulosic biomass has been studied for the production of biofuels and commodity chemicals [31-33].

Different biomass sources are available in substantial amounts and exhibit a high amino acids content [1]. Several amino acids are interesting for the production of chemical intermediates. For example ethanolamine (Etn), the modification product of the decarboxylation of L-serine (Ser) using L-serine decarboxylase (SDC), is an industrial product used as intermediate in many applications such as in the herbicide, textile, metal, detergent, plastics and personal care products industries [2]. Furthermore, L - lysine (Lys) can be converted into 1,5 – pentanediamine, an aliphatic α,ω – diamine used for the production of nylon [2, 20]. One of the most interesting amino acids is L - glutamic acid (Glu), which can be decarboxylated producing γ – aminobutyric acid (GABA). GABA is a high value product used as intermediate for the synthesis of e.g. 2 – pyrrolidone, the most important precursor of N - vinylpyrrolidone (NVP) [19]. Electrodialysis of Glu and Asp and further separation of GABA from Asp results in pure product streams with high recoveries at high current efficiency and low energy consumption [13]. pH changes that occur during the electrodialysis of Asp and Glu and the further separation of GABA from Asp do limit the maximum amino acid recovery. Nevertheless, the influence of pH changes on the overall process performance is not a significant drawback because the charge of the acidic amino acids and GABA remains constant over a wide range of pH (pH = 4 – 8) [13].

The technical feasibility combined with the high value of GABA as chemical intermediate makes this separation highly interesting for industrial applications. Furthermore, Glu is a non–essential amino acid that does not compete with food or feed as is the case for e.g. Lys. Combined with its relatively high price, selection of the raw material is based, next to protein content, more specifically on the amount of glutamic acid present in the raw material. The most attractive biomass resources are summarized in Table 7.1.

Table 7.1. Percentage of major amino acid in the different biomass resources (Adapted from [1]) .

Amino acid	Wheat DDGS[1]	S. microalgae	Poultry feather	Sugarbeet vinasse
Glutamic [%]	12.79	9.42	10.09	19.85
Aspartic [%]	2.13	6.48	5.05	3.03
Arginine [%]	1.24	4.12	6.73	0.67
Lysine [%]	1.07	2.94	2.10	2.36
Phenylalanine [%]	1.78	2.94	3.79	0.34
Serine [%]	1.78	2.94	8.83	0.67
Total protein content in biomass [%]	38.00	63.00	90.00	36.00

[1]DDGS: Dried distillers' grains and solubles.

DDGS and sugarbeet vinasse have higher glutamic acid content than spirulina microalgae and poultry feather. However, the total protein content in spirulina microalgae and poultry feather is very high. Also, the raw material price and its availability play an important role. The price of the raw materials and major products can be found in ICIS chemical business [34] and the availability is reported elsewhere [1].

Table 7.2. Price and availability of different biomass sources. (Source: ICIS chemical business [34], [1]).

Raw material	Price [€/ton]	Availability [kton/year]
DDGS	170	282
s. microalgae	250	382
Poultry feather	487	357
Sugarbeet vinasse	170	181

A first rough overall economic evaluation of the process based on the cost of the raw material, availability and the revenues of the main product GABA gave the highest positive result for DDGS and sugarbeet vinasse. Because of its higher total protein content, DDGS is selected for further calculations.

7.3 Conceptual design

Based on data of a chemical plant located in Rotterdam, The Netherlands [1, 35], the yearly production of DDGS is about 360 kton. Based on the data of Table 7.1, we assume that Glu occupies approximately 13% of the DDGS raw material.

A simplified functional process diagram of the proposed approach is presented in Figure 7.1. As a first step, protein is extracted from the raw material and further hydrolyzed [36] to obtain a complex mixture containing multiple amino acids. Next, the amino acid mixture enters the first separation unit (S_1) where the acidic amino acids Glu and Asp are separated with electrodialysis with a recovery of 98% [13]. Subsequently, the Glu and Asp mixture is fed into an enzymatic reactor (R_1) where Glu is converted into GABA with an overall conversion of 80% [19]. Afterwards, GABA, Asp and unconverted Glu enter the second separation unit (S_2) where GABA is separated from Asp and Glu (recovery of 98%, [13]). Based on these data, we assume an annual production of 25 kton of GABA.

The stream containing Asp and unconverted Glu is partially recycled to the first separation unit, S_1 (purge = 10%). During the process, three water streams enter the process in order to keep the amino acids dissolved and avoid precipitation as these have fairly low solubilities (Glu = 8.6 g/L, Asp = 4.5 g/L, GABA = 51.6 g/L, [37]). Figure 7.1 shows the functional process scheme of the suggested route.

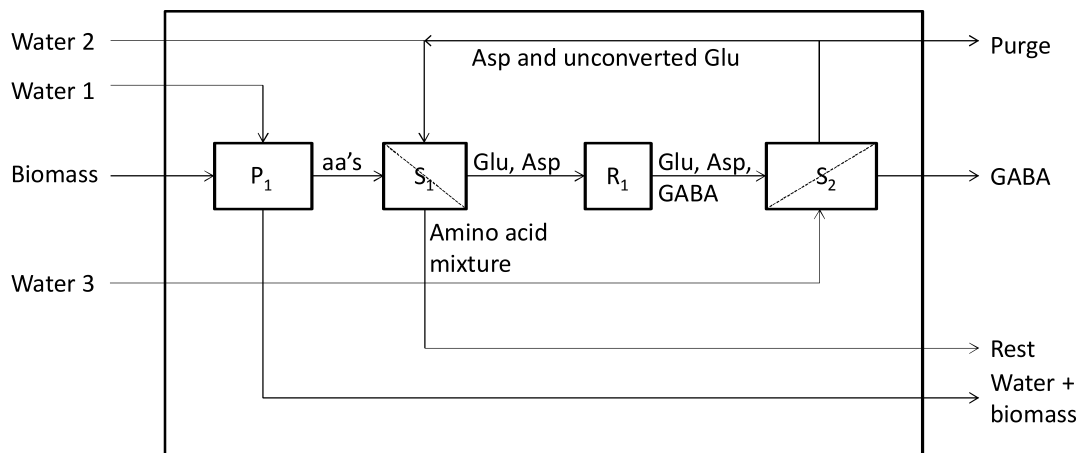

Figure 7.1. Functional process scheme.

7.4 Methods

The economic evaluation is based on capital cost and the payback period (PP). The capital costs refer to the membrane module costs and the peripheral costs [21, 38] that are estimated based on the required membrane area and the price of 500 €/m^2 of the commercially available ion exchange membranes FKB-PEEK and FAB-PEEK (FumaTech GmbH) used in previous work [13, 14, 18]. The process parameter that gives the effective membrane area needed for the specific separation, and therefore determines the total membrane cost, is the amino acid flux. The lifetime

of the ion exchange membranes is assumed to be 5 years. The amino acid flux used for the calculations is 10^{-5} mol/m^2 · s [13, 14, 18]. The membrane module costs and the peripheral costs are calculated as follows:

$$Membrane\ module\ cost = 1.5\ \cdot membrane\ cost \qquad \text{Eq. 7.1}$$

$$Peripheral\ costs = 1.5\ \cdot membrane\ module\ cost \qquad \text{Eq. 7.2}$$

Additionally, the payback period (PP) is calculated to determine the cost effectiveness of the process. It relates the capital costs and the annual cash inflows. The annual cash inflows are estimated subtracting the raw material cost and the operating costs, including maintenance costs, utility costs, labor costs and depreciation costs, estimated as 50% of the membrane module cost [38] from the product market price. The PP is calculated as follows:

$$PP = \frac{investment\ cost}{annual\ cash\ inflows} \qquad \text{Eq. 7.3}$$

7.5 Membrane module design, cost evaluation and sensitivity analysis

Preliminary process simulations with the commercial flowsheet program UniSim® and first rough economic calculations indicate that pretreatment, enzymatic reaction and downstream processing do not contribute significantly to the total costs compared to the membrane modules and peripheral costs. The total membrane costs are very significant as huge membrane areas are required due to the relatively low fluxes of amino acids through the membrane (despite the high selectivities). Consequently, the preliminary cost evaluation and sensitivity analysis were performed considering the membrane separation units.

First, the effective membrane areas needed for the separation of Glu (S_1) and GABA (S_2) were calculated based on a production of 25 kton/year of GABA. The determining factors were found to be amino acid fluxes that are generally in the order of 10^{-5} mol/m^2 · s [13, 14, 18] and the cost of the ion exchange membranes. The latter was estimated based on the commercially available cation exchange membranes FKB-PEEK (FumaTech GmbH) and anion exchange membranes FAB-PEEK (FumaTech GmbH) used in previous works (500 €/m^2) [13, 14, 18].

A sensitivity analysis was performed to evaluate the sensitivity of the overall process costs for the membrane costs. Decreases of 10%, 20%, 30% and 40% were evaluated. The results indicate that such variations in the membrane cost would not improve the process economics unless accompanied by a significant increase in the amino acid flux through the membrane. An increase in amino acid flux by a factor of 10^1, 10^2, 10^3 and 10^4 was considered. Figure 7.2a and 7.2b show the increase in cash inflow and PP, respectively, with respect to the amino acid flux increase and the decrease in membrane price. The results show that an increase in the amino acid flux of at least 10^2 is needed to make the process cost effective. Without a decrease in the membrane price, an increase in the amino acid flux by a factor of 10^2 results in a PP of 1 year.

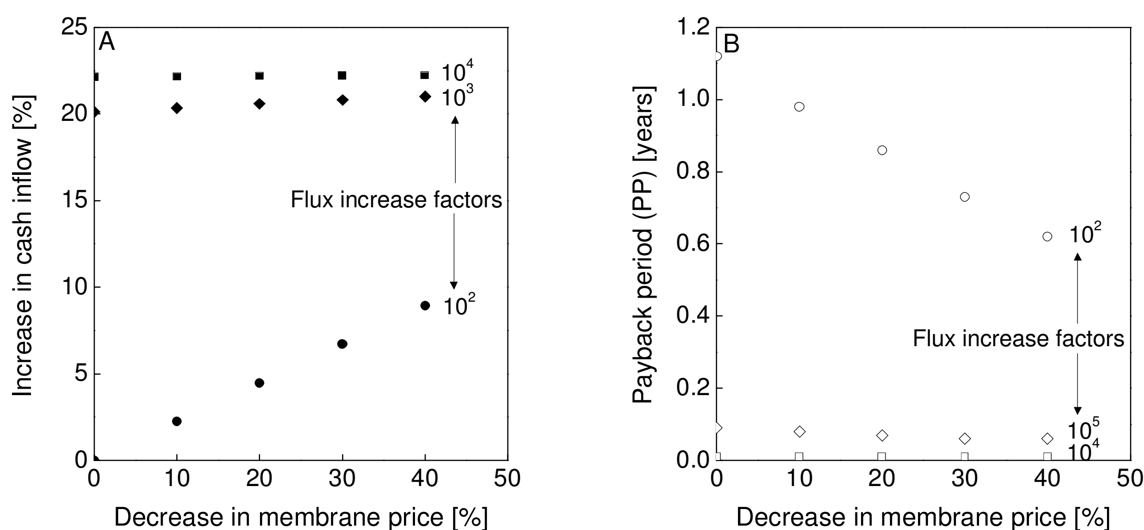

Figure 7.2. a) Cash inflow increase in % and b) payback period (PP) with respect to the amino acid flux and the decrease in membrane price.

Figure 7.2a shows that the decrease in the membrane price has a significant positive influence on the cash inflow if the amino acid flux is increased by a factor of 10^2. When the amino acid flux is increased by factors of 10^3 and 10^4 a decrease in the membrane price has no significant influence. A similar behavior can be observed for the payback period (Figure 7.2b).

Process intensification based on the integration of enzymatic conversion of the amino acid and subsequent separation using mixed matrix membranes (MMMs) as a platform for enzymatic conversion was experimentally considered. We also included that in the economic evaluation (Figure 7.3). For the calculations, the MMMs price is assumed to be in the same order of magnitude as the commercially available ion exchange membranes (e.g. FAB-PEEK) and the same recoveries, conversions and recycle as the standard process (Figure 7.1) are considered.

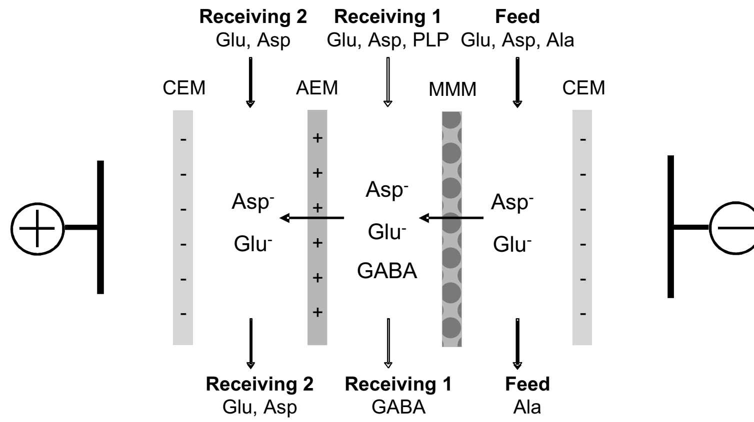

Figure 7.3. Schematic representation of the integration of reaction and separation using mixed matrix membranes as a platform for enzymatic conversion.

Figure 7.4a and 7.4b show the increase in cash inflow and PP, respectively, with respect to the amino acid flux increase and the decrease in membrane price for the integrated process (Figure 7.3). For the integrated process, a 40% decrease in the membrane cost combined with an increase in the amino acid flux by a factor 10^1 results in a cost-effective process with a PP of 3 years (results not shown). It can be clearly seen in Figure 7.4 that the increase in the flux has a bigger influence especially when it is increased by factors higher than 10^2 where a further decrease in the membrane price does not significantly influence the cash inflow nor the PP.

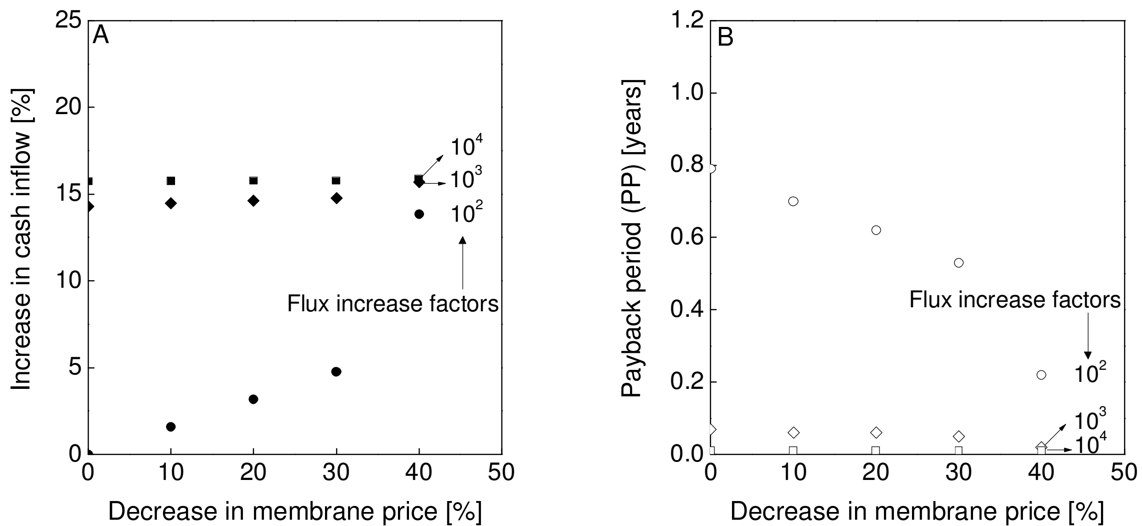

Figure 7.4. a) Cash inflow increase in % and b) payback period (PP) with respect to the amino acid flux and the decrease in membrane price for the integrated process (Figure 7.3).

7.6 Evaluation

Two different process configurations have been evaluated. One considering a first electrodialysis stage followed by the enzymatic conversion of Glu to GABA and the subsequent separation of GABA from Asp. The second approach refers to an integrated process where reaction and separation occur in a single step.

If the standard process (Figure 7.1) is compared with the integrated process (Figure 7.3), the main difference lies in the payback period. For the standard process, at an amino acid flux increase by a factor of 10^1 and a decrease in the membrane cost of 50%, 60% and 70% result in payback periods of 50, 15 and 6 years, respectively. For the standard process, a PP of 3 years is obtained at an amino acid flux increase by a factor of 10^1 and if the membrane cost is decreased at least by 80%. For the integrated process a PP of 3 years is already obtained at an amino acid flux increase of 10^1 and a decrease in the membrane cost of 40%. Furthermore, integration represents a 25% decrease in both capital and operating costs.

For both cases, the influence of two parameters, amino acid flux and membrane cost, on the profitability of the suggested approach was evaluated. The membrane cost by itself cannot help the process to reach cost effectiveness but has a positive influence when combined with an increase in the amino acid flux. However, the increase in the amino acid flux by itself has an important influence on the process performance. These results suggest that the focus of further research should be on the improvement of the process performance of the separation of amino acids in terms of amino acid flux.

7.7 Conclusions

The objective of this work was to explore the economic feasibility of the suggested alternative route for the production of chemical intermediates from biobased feedstocks. More specifically, the focus of this evaluation was on the production of γ-aminobutyric acid (GABA) from glutamic acid (Glu) by combining electrodialysis and enzymatic modification. An assessment on the possible amino acid sources was performed based on raw material price, protein and glutamic acid content and availability. Dried distillers' grains and solubles (DDGS) was selected for further calculations. Preliminary results suggested that the membrane module and membrane cost were the limiting factors for the cost evaluation. Therefore, focus was specifically on this aspect

starting from experimentally obtained amino acid fluxes, which gave the membrane area needed for the specific separations. A sensitivity analysis was performed regarding membrane costs and amino acid fluxes.

The process design study demonstrated that a decrease in the membrane costs by itself, though being a limiting factor, could not guarantee the economic feasibility of the process. The amino acid flux, on the other hand, if improved (by a factor of 10^2) makes the process profitable. In addition, the integration of reaction and separation using mixed matrix membranes in an electrodialysis stack would result in savings of around 25% in membrane area needed, hence in both, fixed and operational costs. Therefore, future research should focus on the increase of amino acid flux through ion exchange membranes together with the production of cheaper membranes and the improvement of an integrated process for the simultaneous enzymatic conversion and further separation of amino acids. This approach could also be expanded towards the modification and separation of other amino acids such as Lys and Ser, which can be enzymatically decarboxylated to PDA and Etn, respectively. Nervertheless, due to the low price of PDA and Etn when compared to GABA, the process needs to be optimized further before it reaches economical attractiveness to be applied to the fractionation of more complex mixtures.

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8
Closing remarks and outlook

ABSTRACT

This research aimed to show the potential of membranes in the biobased economy. More specifically, it dealt with the combination of enzymatic modification and electrodialysis for the fractionation of amino acid mixtures towards the production of biobased chemicals. The concept was applied experimentally to the separation of acidic, basic and neutral amino acids. pH changes during the process could dramatically influence the charge behaviour of the amino acids, thus affecting the process performance. Methods to control the pH, such as acid/base dosing and the use of a buffer, were evaluated. Additionally a novel membrane concept using a structured bipolar membrane (sBPM) for internal pH control was successfully developed. Experimental results with the amino acid Arg present in the feed showed that Arg has a poisonous effect and decreases the process performance significantly. Two approaches to overcome this effect, i.e. the use of ultrafiltration membranes or tailor made cation exchange membranes were successfully applied. As a first step towards process intensification, both specific modification of amino acids and separation using electrodialysis have been integrated. Finally, the preliminary process design and economic evaluation of the suggested route is carried out. The results obtained show the potential of an integrated membrane process in the biobased economy and suggest directions for further research: 1) improvement of ion exchange membranes for amino acid separation in terms of limiting current density increase to be able to apply higher currents and obtain higher amino acid fluxes without enhancing water splitting, 2) improvement of the developed MMMs in terms of mechanical stability, electrical resistance and enzyme activity, 3) application to other enzymatic conversions for process intensification combining the three selected enzymatic modifications and further separation with electrodialysis and 4) application and further optimization of the concept to real feed mixtures.

8.1 Summary

8.1.1 Introduction

In this work the potential of the combination of enzymatic modification with electrodialysis for the separation of amino acids from biomass was presented. The systematic study consisted of the application of the concept to three different systems. The concept was validated considering the successful fractionation of a complex amino acid mixture into the three groups, acidic, basic and neutral amino acids. As first step towards the integration of enzymatic conversion and further separation with electrodialysis, mixed matrix membranes (MMMs) with enzyme activity were prepared, characterized and applied in an electrodialysis stack. Furthermore the economic feasibility of the suggested approach was evaluated to determine the sensitive parameters to make the process interesting for industrial applications. In the following paragraphs, the main results of each chapter are presented and the chapter is completed with a discussion on limitations, advantages and future directions of the research.

8.1.2 Proof of principle

Chapter 2 presents for the first time the application electrodialysis combined with enzymatic modification for the isolation of target amino acids. The proof of principle was shown for the acidic amino acids. As a first step Glu and Asp were separated together, followed by the enzymatic decarboxylation of Glu to GABA with the enzyme GAD. The produced GABA was further separated from Asp at neutral pH based on their differences in charge behavior with respect to pH. Outstanding process performance parameters were obtained for both separations investigated.

The maximum recovery of amino acids (~ 90% for all cases) was strongly influenced by the pH during the electrodialysis process. However, though limiting, the pH changes during the separation of acidic amino acids did not have a significant negative effect on the process performance, as for Glu and Asp, as well as for GABA, the average net charge of the amino acids remained constant in a pH range of 4 – 8 approximately.

8.1.3 pH changes during electrodialysis: a challenge for the basic amino acids

The separation of basic amino acids, Lys and Arg, was achieved by considering the enzymatic decarboxylation of Lys to PDA with the enzyme LDC. Unfortunately, for this system, at the operational pH, small changes in pH cause significant changes in the charge of the amino acids.

The separation of PDA from Arg is, in theory, possible at pH 12.5 (Arg^{-1}, PDA^{0}), 10.9 (Arg^{0}, $PDA^{+0.5}$) and 10.0 ($Arg^{+0.1}$, $PDA^{+1.5}$). For the separation of PDA from Arg, pH changes occurring during the process dramatically change the charge of the target amino acids. In **Chapter 3** the use of a buffer and acid/base dosing for pH control were investigated for the separation at pH = 10.9. Due to the presence of additional small ions, the process performance was decreased when a buffer was used. The addition of acid/base did not have a negative influence on the process economics (current efficiency and energy consumption) due to the controlled addition of only the necessary amount of ions to compensate for the amino acids present. Nevertheless, the recovery was not improved.

The best performance was achieved at a pH of 10.0, where the recovery of PDA was 64% while the current efficiency reached 83%. However, due to the slight positive charge of Arg, the product purity was compromised, leaving still room for improvement.

8.1.4 A novel membrane concept for internal pH control

In **Chapter 4** a novel segmented bipolar membrane (sBPM) containing monopolar areas to allow ion transport and bipolar areas to enhance water splitting was prepared. During electrodialysis with a standard cation exchange membrane for the separation of ethanolamine (Etn) and alanine (Ala) at neutral pH, the pH decreased below 4, causing Ala to gain a slightly positive charge. The product purity was decreased due to the co-transport of Ala together with Etn towards the receiving stream.

The application of the prepared sBPM in electrodialysis maintained the pH in the feed above 7 and avoided the transport of Ala to the receiving compartment. This resulted in an increase in product purity from 98% to 100% while the same recovery was obtained.

8.1.5 Validation of the concept

Chapter 2, 3 and 4 proofed that pure product streams consisting of single amino acid or the modification product thereof could be successfully obtained by combining enzymatic modification and electrodialysis. Subsequently, the fractionation of an amino acid mixture containing Arg is more complex: When Arg is positively charged (neutral pH) it has a poisonous effect on commercially available cation exchange membranes.

In **Chapter 5** the effect of the presence of positively charged Arg on the separation of Lys and Arg from an amino acid mixture at neutral pH was investigated. An increasing Arg concentration caused a decrease in the swelling degree of the membrane and decreased the overall amino acid recovery. A concentration of 25 mM of Arg leads to almost no recovery. Two approaches were considered to overcome this problem. On one hand, the combination of electrodialysis with ultrafiltration membranes was applied. This lead to high Arg and Lys recoveries but at the expense of product purity due to the use of a non-selective (porous) ultrafiltration membrane. Alternatively, the use of a high swelling tailor made membrane was studied. A slight decrease in swelling degree with increasing Arg concentration was still observed but the membrane remained sufficiently swollen to overcome the poisonous effect of Arg.

Separation of Arg and Lys with electrodialysis using the tailor made membrane lead to sufficiently high recoveries and an Ala retention of 100%. Next, electrodialysis with the tailor made membrane was applied for the fractionation of a complex amino acid mixture containing acidic, basic and neutral amino acids. It resulted in recoveries of Glu, Asp, Lys and Arg of 72%, 98%, 70% and 93%, respectively, while uncharged Ala was completely retained in the feed, showing the strength of the concept for the separation of complex biobased mixtures.

8.1.6 Towards process intensification

In **Chapter 6** mixed matrix membranes were prepared and studied with the aim to integrate enzymatic conversion and further separation with electrodialysis using mixed matrix membranes (MMMs) with enzymatic activity in a single process.

Mixed matrix membranes were prepared and characterized in terms of thickness, swelling degree, zeta-potential, relative oxirane (functional group) density and L-glutamic acid conversion into γ-aminobutyric acid (GABA). The use of unmilled resin lead to a mechanically unstable MMM not

suitable for integration of reaction and separation. Though an increase in oxirane density was observed, a decrease in activity was obtained when the resin particle size was decreased. This suggested that the unmilled Relizyme EP403/S particles show already the optimum oxirane density needed for GAD immobilization. Even though decreased, still 50% of enzyme activity remained.

The prepared mixed matrix membranes were applied in an electrodialysis stack for simultaneous reaction and separation. Glu and Asp contained in the feed were separated at pH = 5.0 through an AEM coupled with the prepared MMM for enzymatic conversion. The coupling of the MMM with the AEM was needed due to the high water flux through the MMM, resulting in a high volume change in the compartments during the electrodialysis experiment. The enzymatic conversion using MMMs was 33% of the transported Asp and Glu. Unconverted Asp and Glu were further separated, while GABA was retained in the middle compartment.

A first limitations of the prepared membranes was a decrease in the activity of the membranes due to an increase in the oxirane density of the carrier above the optimum. Most probably, the enzyme was immobilized at so many active sites that the activity towards substrate conversion was limited. Secondly, a too high water flux was obtained. Membranes with smaller pores might restrict the use of an extra AEM. Lastly, even with the smallest particle size fraction of the enzyme carrier, the resulting membranes were too thick. Thinner membranes are necessary to decrease their resistance in the electrodialysis stack. If the resistance is too high, the energy consumption increases, but most important, the enzyme activity might be affected, or the enzyme might be completely destroyed due to the high potential difference.

8.1.7 Resource availability and preliminary economic evaluation

Chapter 7 presents the economic analysis of the suggested approach based on the separation of acidic amino acids from biomass. Among the acidic group, the enzymatic decarboxylation of L-glutamic acid (Glu) into γ-aminobutyric acid (GABA) was considered [1]. The process design was evaluated using dried distiller's grains solubles (DDGS) as starting material.

Two main drawbacks were identified: the relatively low amino acid flux that leads to large membrane area needed for the specific separations combined with the high price of the

commercial ion exchange membranes. Preliminary results show that an increase in the amino acid flux by a factor of at least 10^2 is needed to make the process cost-effective.

Integration of enzymatic conversion and further separation with electrodialysis using MMMs significantly improves the process economics. If integrated, a decrease of 40% in membrane cost with an increase by a factor of 10^1 in the amino acid flux results in a payback period of 3 years. Additionally, the integration results in savings of around 25% in both, fixed and operational costs.

The results set directives for further research, which should focus on the increase of amino acid flux through ion exchange membranes together with the production of cheaper membranes and the improvement of an integrated process for the simultaneous enzymatic conversion and further separation of amino acids. Once optimized, this approach could also be applied to the fractionation of more complex mixtures, e.g. for the modification and separation of other amino acids such as Lys and Ser, which can be enzymatically decarboxylated to PDA and Etn, respectively.

8.2 Outlook

Starting from biomass, isolating amino acids with membrane technology based on their charge behavior with respect to pH and producing industrial chemicals: The concept sounds relatively easy and in practice seems not to be that complex. Nevertheless, the way is still long, broad and undefined.

Further research should focus on the improvement of the ion exchange membranes. The low flux obtained for amino acid separation results in high effective membrane areas needed for the separation if a competitive market production is considered (i.e. 25 kton/year of GABA for NMP production). With the existing commercial ion exchange membranes, limiting current densities (LCDs) of around 2.5 mA/cm^2 are obtained for all amino acids and modification products investigated. Operation at the LCD results in amino acid fluxes in the order of 10^{-4} $mol/m^2 \cdot h$ while operation above the LCD enhances water splitting and changes the pH. Consequently, ion exchange membranes with improved LCD need to be developed so that higher amino acid fluxes can be obtained.

In this research a novel membrane concept was presented: The use of a segmented bipolar membrane (sBPM) for internal pH control during the separation of Etn from Ala. However, it would be interesting to determine the influence of different process parameters and membrane characteristics on the efficiency of the membrane to control the pH such as the ratio between bipolar and monopolar area and the current efficiency.

Attention must be also paid to the poisonous effect of Arg to commercially available membranes. Although this problem was solved by the use of electrodialysis with high swelling tailor made membranes, a fundamental understanding of the effect would enhance further development of ion exchange membranes for the specific application. Electrodialysis with ultrafiltration membranes (EDUF) was also investigated for the separation of mixtures containing positively charged Arg. Separation was achieved but the product purity was compromised due to the lack of selectivity of the ultrafiltration membranes. Membranes with smaller pores or nanofiltration membranes, could reduce the co-transport of uncharged ions to the receiving compartment. Therefore it is worth to determine the benefits and limitations of electrodialysis with ultrafiltration/nanofiltration membranes.

Of utmost importance is the improvement of mixed matrix membranes for process intensification. To obtain a mixed matrix membrane with the desired thickness, pore size and enzymatic activity should the main goal. On one hand, a polymeric matrix with smaller pores would lead to less water transport through the membrane and an improved stability. In this way, its coupling with an ion exchange membrane could be avoided. On the other hand, an engineered enzyme carrier is essential. Relizyme EP403/S was studied in the present work, appears to have the optimum functional group density, enabling maximum immobilization yield without compromising enzyme activity. Because of the large pore size of Relizyme EP403/S, diffusion limitations can be neglected and further milling of the particles does not result in an activity increase. Nevertheless, particles not bigger than 40 – 60 μm are needed to produce thin mechanically stable membranes. Therefore, engineered resin carriers, with optimum functional group density and particle size are needed to achieve MMMs that show a satisfactory performance for enzymatic conversion and electrodialysis.

Related to this, other membrane types and immobilization routes might be investigated. For example immobilizing the enzyme directly on the membrane [2-5] or on polymeric brushes [6-8]. For the specific case of electrodialysis with MMMs where immobilization takes place on the

surface of the membrane only, the enzyme needs to be attached only on one side of the membrane, so that reaction takes place after the first separation. For example for the case of Asp and Glu separation, if reaction takes place before the first separation stage, Glu would be converted into GABA, the valuable product, and GABA would remain in the feed compartment, together with the remaining neutral amino acids. Another possibility is the immobilization of enzymes on carbon nanotubes [9]. Carbon nanotubes can be incorporated in ion exchange membranes and have the ability of increasing the limiting current density [10].

Although different biobased sources with high protein content are available in sufficient quantities, they are not suitable to be fed directly to an electrodialysis stack due to the presence of multiple components in complex mixtures. First extraction of the proteins and their hydrolysis needs to be carried out to make the amino acids available for further conversion and separation. Studies on protein extraction have been carried out. Plant proteins can be extracted using acid, alkaline and enzyme assisted extraction [11]. Extraction at alkaline conditions gives higher recovery than when acidic conditions are applied [11]. Proteins from *Jatropha curcas* can be obtained in recoveries of around 82% using counter current multistage extraction [12]. Enzyme assisted protein extraction shows an increased extraction yield for soybean (extraction yield = 90%), rapeseed and algae meals (extraction yields = 50 – 80%) [11]. After extraction, further solubilization of proteins (hydrolysis) can be performed in acidic, neutral or basic media at different temperatures [13] or in the presence of enzymes [14]. The suggested approach need to be applied and the resulting amino acid mixture should be fed to the ED system to show the feasibility of the process to separate complex biobased mixtures.

8.3 Epilogue

The potential of an integrated membrane process, which combines enzymatic modification and electrodialysis for the production of biobased chemicals is high. Proper optimization of the process is needed though. When successful to real feed mixtures, the suggested approach is ready to be applied in the biorefinery concept.

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Summary

This research aims to contribute to the shift from a conventional refinery to the biorefinery concept. It deals with the application of electrodialysis, a membrane process that uses the potential difference as driving force, for the separation of amino acids based on the differences in their charge behavior with respect to pH. Amino acids can be found in biomass and when isolated they can be used for the production of chemical intermediates. Enzymatic conversion of specific amino acids towards an interesting chemical intermediate is considered to obtain pronounced differences in the charge behavior and guarantee successful isolation with electrodialysis.

Chapter 2 shows the proof of principle. First, electrodialysis is applied for the separation of the acidic amino acids, L-glutamic acid (Glu) and L-aspartic acid (Asp). Second, enzymatic modification of Glu to γ - aminobutyric acid (GABA, a precursor of important industrial chemicals) is assumed. By carrying out a final electrodialysis stage GABA and Asp can be successfully isolated from each other. This chapter includes a detailed description and discussion of the technology used and the phenomena occurring during the electrodialysis process such as pH changes that limit the maximum amino acid recovery.

Subsequently, the concept is applied to other amino acid groups. **Chapter 3** deals with the separation of basic amino acids and evaluates the performance of the process when external pH control, such as acid/base dosing or the use of a buffer, is applied. To achieve the separation the modification of L-lysine (Lys, a basic amino acid) into 1,5 – pentandiamine (PDA) is considered. As expected, external pH control represents the addition of smaller molecules to the system that compete with the amino acids and do not improve the process significantly. Therefore, for the isolation of the modification product of L-serine (Ser, neutral amino acid), ethanolamine (Etn), a novel membrane concept is introduced and presented in **Chapter 4**. Here the limitations in the process due to pH changes do not relate to transport of the target amino acids but to the co-transport of unwanted molecules, such as the neutral amino acid L-alanine (Ala), which becomes charged due to the decrease in pH. A segmented bipolar membrane (sBPM) that consists of a continuous cation exchange layer and a perforated anion exchange layer, is used to enhance water splitting while at the same time allowing amino acid transport. The application of the sBPM for pH control results in a pure product stream at the same recoveries with improved process performance.

To validate the concept, the application of electrodialysis for the fractionation of a complex amino acid mixture consisting of the three groups, acidic, basic and neutral, is evaluated. The work shows that the basic amino acid L-arginine (Arg), when positively charged, has a poisonous effect on commercially available cation exchange membranes and restricts the separation of Lys and Arg from the amino acid mixture. This phenomenon is related to the steep decrease in the swelling degree of the membranes in the presence of Arg. **Chapter 5** deals with the application of electrodialysis with ultrafiltration membranes (EDUF) for the separation of Arg and Lys. Though successful, the product purity is compromised. The use of tailor made cation exchange membranes made from sulfonated poly ether ether ketone (SPEEK) and with a higher swelling degree however results in high recoveries without compromising the product purity.

Chapter 6 describes the integration of mixed matrix membranes (MMMs) that contain immobilized enzyme in the electrodialysis process as a step towards process intensification. This approach is applied for the separation of the acidic amino acids Glu and L-aspartic acid (Asp) considering the enzymatic conversion of Glu to GABA. With the integrated MMMs lower overall conversion of Glu to GABA is obtained relative to the use of native enzyme. Nevertheless, the results prove the strength of the concept and indicate that with the proper optimization the use of MMMs could be beneficial for the specific application.

To finalize the research, a preliminary process design and economic evaluation is carried out and presented in **Chapter 7.** Though preliminary, it indicates the directions to further optimize the process. Focus for future research should be on increasing the amino acid flux and the decreasing the cost of the membranes to make this approach cost effective.

Samenvatting

Dit onderzoek wil een bijdrage te leveren aan de transitie van conventionele raffinaderijen naar bio-raffinaderijen. Het behandelt de toepassing van elektrodialyse, een membraanproces dat gebruik maakt van een potentiaalverschil als drijvende kracht, voor de scheiding van aminozuren. Aminozuren komen voor in biomassa en kunnen na isolatie gebruikt worden voor de productie van chemicaliën. Voor deze scheiding is het gedrag van specifieke aminozuren als functie van de pH van de oplossing essentieel, aangezien die de lading van het specifieke aminozuur bepaald. Enzymatische omzetting van specifieke aminozuren naar interessante chemicaliën kan tevens worden aangewend om de lading van specifieke aminozuren te beïnvloeden om zo een meer succesvolle scheiding te garanderen.

In **Hoofdstuk 2** wordt het principe van de scheiding van aminozuren met behulp van electrodialyse aangetoond. Het concept van enzymatische omzetting van het aminozuur L-glutaminezuur naar γ-aminoboterzuur (GABA, een precursor voor belangrijke chemicaliën) gecombineerd met de scheiding van aminozuren met behulp van electrodialyse is voor de eerste keer toegepast. Dit hoofdstuk bevat een gedetailleerde beschrijving en discussie van de gebruikte technologie en de verschijnselen die plaatsvinden tijdens elektrodialyse, zoals veranderingen in de pH die de maximale opbrengst van de aminozuren beperken.

Vervolgens wordt dit concept toegepast op andere groepen aminozuren. **Hoofdstuk 3** behandelt de scheiding van basische aminozuren en evalueert de prestatie wanneer externe pH-controle, zoals dosering van zuren/basen of het gebruik van een buffer, wordt toegepast. Om een scheiding te bewerkstelligen is de modificatie van L-lysine (Lys, een basisch aminozuur) naar 1,5-pentadiamine (PDA) uitgevoerd. Zoals verwacht levert externe pH-controle door de toevoeging van kleine moleculen aan het systeem die concurreren met de aminozuren, geen significante verbetering op in het proces. Daarom is voor de isolatie van ethanolamine, het gemodificeerde product van L-serine (Ser, een neutraal aminozuur), een nieuw membraanconcept geïntroduceerd en gepresenteerd in **Hoofdstuk 4**. De pH gerelateerde wijzigingen die limiterend zijn voor het proces zijn hier niet gerelateerd aan transport van het gewenste aminozuur, maar aan het transport van ongewenste moleculen, zoals het neutrale aminozuur L-alanine (Ala), dat geladen is bij een verlaging in de pH. Een gesegmenteerd bipolair membraan (sBPM) dat bestaat uit een continue kation-uitwisselende laag en een geperforeerde anion-uitwisselendelaag, is gebruikt om water splitsing te bevordering en gelijkertijd aminozuurtransport mogelijk te maken. Het

toepassen van deze sBPM voor het controleren van de pH resulteert in een productstroom met dezelfde opbrengst en verbeterde procesprestaties.

Om het totaalconcept te valideren is de toepassing van elektrodialyse voor de fractionering van een complex aminozuurmengsel bestaande uit drie groepen, zure, basische en neutrale aminozuren, geëvalueerd. Wanneer het basische aminozuur L-arginine (Arg) echter positief geladen is, heeft het een vergiftigde werking op het commercieel verkrijgbare kation-uitwisselingsmembraan. Gevolg hiervan is dat de scheiding van Lys en Arg uit het aminozuurmengsel gelimiteerd wordt. Dit verschijnsel is gerelateerd aan de scherpe daling van de zwellingsgraad van het membraan in de aanwezigheid van Arg. **Hoofdstuk 5** behandelt de toepassing van elektrodialyse met ultrafiltratiemembranen (EDUF) voor de scheiding van Arg en Lys. Hoewel de scheiding succesvol is, verslechterd de zuiverheid van het product. Het gebruik van kation-uitwisselingsmembranen met een hoge zwellingsgraad, gemaakt van gesulfoneerd poly-ether-ether-keton (SPEEK), resulteert daarentegen in hoge opbrengsten zonder de productzuiverheid aan te tasten.

Hoofdstuk 6 beschrijft de integratie van mixed matrix membranen (MMMs) met geïmmobiliseerde enzymen in het elektrodialyseproces. een stap vooruit te zetten naar procesintensivering. Deze benadering is toegepast op de aminozuren Glu en Asp waar Glu aan het MMM enzymatisch wordt omgezet tot GABA. In vergelijking met het proces met niet-geïmmobiliseerde enzymen, resulteert het proces met MMMs in een lager omzetting van Glu naar GABA. Desalniettemin bewijzen de resultaten de kracht van het concept en tonen deze aan dat met de juiste optimalisatie het gebruik van MMMs gunstig kan uitpakken voor deze specifieke applicatie.

Tenslotte is een voorlopig procesontwerp geschetst en een economische evaluatie uitgevoerd zoals gepresenteerd in **Hoofdstuk 7**. Hoewel het een voorlopig ontwerp betreft, wijst het de richting voor verdere optimalisatie van het proces. Toekomstig onderzoek zou zich moeten richten op het verhogen van de aminozuurflux en het verlagen van de membraankosten om deze aanpak rendabel te maken.

About the author

Olga Kattan was born on the 14th of April, 1981 in San Salvador, El Salvador. After receiving the high school diploma at the Deutsche Schule San Salvador, she started studying chemical engineering at the Universidad Centroamericana José Simeón Cañas, in El Salvador. In August 2005 she obtained her Bachelor of Science in Chemical Engineering after a successful semester abroad at the Technische Universität Hamburg - Harburg in Hamburg, Germany, where she carried out her Bachelor Thesis on supercritical extraction of vegetable oils. In October 2005 she continued studying Biotechnology at the same university. Related to her studies, she performed her 6 months internship on fundamental requirements for the production and stability of plant-protective formulations based on oil dispersions at BASF AG, in Ludwigshafen, Germany, in 2007. In August 2008 she obtained her Master of Science in Biotechnology after successfully defending her Master Thesis on the separation of phytosterols dissolved in CO_2 with supercritical adsorption. From October 2008 to October 2012 she joined the Membrane Technology Group at the University of Twente as a PhD student, focusing on the research topic presented in this dissertation: electrodialysis of amino acids for the production of biochemicals. Since October 2012 she works at Membrane Technology Group as a Post-Doctoral researcher in gas separation.

Conferences and courses

October 2008	NPS8. Veldhoven, The Netherlands.
November 2008	Posterdag. Antwerpen, Belgium.
March 2009	NanoMemCourse EA1: Nanostructured materials and membranes for energy. Skeikampen/Lillestrøm, Norway. Participant with grant. **Poster presentation.**
June 2009	PERMEA 2009. Prague, Czech Republic. **Poster presentation.**
September 2009	NanoMemCourse EA3: Nanostructured materials and membranes for food processing. Cosenza/Cetraro/Rende, Italy. Participant with grant. **Poster presentation.**
June 2010	NYM12. Lappeenranta, Finland. **Oral presentation.**
September 2010	PERMEA 2010. Tatranské Matliare, Slovakia. **Oral presentation.**
October 2010	NPS10. Veldhoven, The Netherlands. **Oral presentation.**
July 2011	ICOM 2011. Amsterdam, The Netherlands. **Oral presentation.** *ICOM 2011 Organizing Committee.
October 2011	NPS11. Arnhem, The Netherlands. **Oral and poster presentation.**
November 2011	International conference on biobased feedstocks for fuels and chemicals. Singapore.
June 2012	2012 Membrane Symposium and 13th Poster day. Shell Technology Center Facility. Amsterdam, The Netherlands. **Poster presentation.**
September 2012	Euromembrane 2012. London, UK. **Oral presentation.**

February 2013 NL Guts. Hoogvliet, NL. **Invited speaker.**
April 2013 ECCE9. Den Haag, NL. **Oral presentation.**

Publications

O.M. Kattan Readi, H. J. Mengers, W. Wiratha, M. Wessling, K. Nijmeijer, On the isolation of single acidic amino acids for biorefinery applications using electrodialysis. *J. Membr. Sci.* **(2011)**, 384 (1-2), 166- 175.

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Submitted manuscripts
O.M. Kattan Readi, J.H. Kuenen, H.J. Zwijnenberg, K. Nijmeijer, Novel membrane concept for internal pH control in electrodialysis of amino acids using a segmented bipolar membrane (sBPM). Submitted to *J. Membr. Sci.*

O.M. Kattan Readi, E. Rolevink, K. Nijmeijer, Mixed matrix membranes for process intensification in electrodialysis of amino acids. Submitted to *J. Chem. Technol. Biotechnol.*

In preparation for publication
O.M. Kattan Readi, Y. Wu, A.G.J. van der Ham, K. Nijmeijer, Process design and economic evaluation of electrodialysis for the separation of amino acids.

Honors and awards

- **Best oral presentation award**
  International Congress on Membranes and Membrane Processes, ICOM 2011, Amsterdam, The Netherlands. Title of the presentation: "Membranes in biorefinery applications"

- **Best oral presentation award**
  11th Annual Netherlands Process Technology Symposium, NPS11, Arnhem, The Netherlands. Title of the presentation: "Isolation of single amino acids for biorefinery applications using electrodialysis".

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Surely a lot of other things are behind science. **Greet**, I hope that saying it one more time does not hurt you: You are the best secretary ever! Besides your ability to never (or maybe only once) forget something and always manage to arrange – even last minute – things, you are always there for each of us. I am happy to celebrate my birthday on the exact same day as you and less happy to share the back problems. Thank you for always listening and trying to solve our complains, frustrations and difficulties, for always sharing our happiness with our achievements and our plans or simply greeting us with a smile every day.

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This is certainly a very nice group and that thank to all the nice persons involved. And I will start with the person that gave me the warmest welcome in the group when I went to him with my travel declaration at around 10:12 am on October 02, 2008. **Antoine**, I have no clue what you were screaming to me and Kitty and I certainly did not care. You could not manage to hide your big and pure heart for longer than that morning. And it was good like that. **Karina**, you received me in the office and together with **Wika** we started having nice conversations right away. You are great listeners and both, yes, **Wika** as well, great talkers. I would have been lost without you at the start and probably I would still be. Thank you for making the difficult days seem not so difficult, for giving me courage by showing it yourselves and by giving me happiness by never stopping smiling. 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As you see I thought it would be more interesting to just mention everybody I know. **Lydia** for your help with HPLC and UV. **Izabela S.**, when you were there we talked a lot and we managed to visit you afterwards. It was not so nice when you left but it was certainly the best decision for you. **Katja**, I could manage not to forget my German for a while thanks to the every day's traveling with you. Although you left some time ago it is always nice to see you in important events and to know that our friendship did not get lost. Also thanks to you and **Christoph** for taking me home after parties even though that meant an extra 40 min ride for you guys. During the ride we talked a lot and your advices were always welcome. This has nowadays turned into a tradition: **Can&Laura** thanks for always offering me a ride whenever you want to know a detail about my personal life. **Ikenna** I would love to say that your jokes were not funny but they actually were. Coming to the office and not being able to use the computer made me think of you right away and I had to smile immediately. It was nice having you around. **Mayur&Tina,** I still recall the last evening you spent in Enschede and cannot forget Laura being about to cry. Saying good-bye to cool people is always sad, but having shared nice moments together is worth it, especially when those moments make you laugh out loud. The image of you running around tables while waiting for experiments is just priceless. **Jigar** I am sure that you haven't given up trying to teach people your special moves. You and **Falguni** are one of the couples that immediately show how perfect you are for each other. Now your family grew and it is lovely. **Anne Corine**, we started almost at the same time and learned things together. Your time here was not easy but you showed you are made of iron. We had fun in the labs, during our "secret" talks in the fourth floor with **Jeroen** or when travelling to NPS together with **Katja**, when it became obvious why we called it "the cool car". **Al-Hadidi**, my lovely neighbor, we had eternity-long talks and you always picked the wisest words to make me feel better. I publicly forgive you for forcing me to eat until I cried. I am glad things worked out fine for you and, as you said yourself, you are happily a real father now. **Szymon**, honest, funny, dedicated and smart as every Polish person I know. And an excellent friend. You&**Danuta** and **Wojtek&Joanna** rescued (or should I say kidnapped?) me on one of the most difficult days I have had in my life. You guys did not succeed in making me eat but you certainly made me feel better. Big big thank you to you!

At some point with so many people leaving the energy of the group went down a bit. Luckily not for long thanks to new very valuable additions: **Wojtek** for having your own online dictionary, for teaching us not to pronounce specific letters in English words and for walking looking to the roof. **Sinem**, for your calm, sweet and helpful

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My dear friend **Enver**, despite of my hard efforts to make you enjoy partying without success I still think you deserve a paragraph. From the start you have been there for me 24/7 unless you have stomach problems and even then I am sure you would have been there if I would have needed you. Thank you for the time you invested in this thesis, for never letting me test your membranes, for never listening to what I say, for moving to Tilburg and then complaining about it, for never finishing your lunch, for having a white phone cover and for always offering your help if needed despite of all the things you usually have to do ☺.

Jeroen, you were lucky enough to be given a task immediately when you started: helping me analyzing the amino acid content in my samples. We gave up at some point with the analysis but luckily not with our friendship. Thanks for all the trips together, for all the parties, all the coffees (always on your card), our "half hour of Dutch" tradition, plenty of beers and vodka, our discussions about people, for translating my summary, for your lack of coordination and for your 20 min ___ breaks but especially, for forgiving me for not writing a whole page of acknowledgements as promised. In my heart you have many more than one (crying already?). Our friendship continued growing and you are one of the persons that knows me the most, for good or bad. I am curious about my stukje. Yes I know, **Enver** and **Herman** are taking care of it….

Spanish speaker additions to the group were always welcome. **Nazely**, I first heard of you in June 2010 at a conference in Prague and I met you a couple of months later during a course in Italy. I was lucky enough to have you as office mate. You did stay for only 18 months, but even if you would have stayed twice as long (or more) it would have felt short. You were my confident back then and we had fun inside and outside working hours. You are an amazing person! **Ivonne**, similar story: I met you in Finland during NYM and later you came to us. Again I was lucky to share the office with a Spanish speaker. But three months, seriously? It was not even enough to understand who you actually meant by "los muchachos" ☺….

A relatively new tradition – at least for me – are our legendary borrels. **Jeroen**, **Beata**, **Enver, Krzystof**, **Sinem**, **Yali**, **Antoine** thanks for joining us sometimes. **Can**, **Erik**, **Harmen** and **Dimitris** thanks for being legendary all the time. **Damon&Saghar**, thanks for never being there but somehow always make us think that you actually are. **Laura**, thanks for being Can's girlfriend (now wife), joining our borrels and taking **Pavlina** with you.

A remarkable event was possible thanks to the efforts of **Harro&Imre** to get me the house I live in at this moment ☺. With the help of **Wojtek**, **Erik**, **Enver**, **Can**, **Harro** and later **Jeroen** and **Arturo** under the condition of an invitation to a dinner (I should accept that it will never happen…) I moved to Enschede in February 2011, almost at the start of a year that by far would be the highlight of my PhD *journey*. I shared the house with the best roommate one could ever wish for. **Nazely**, I still miss our dinners together and of course the tea ceremony afterwards. My house will never be so clean again ☹ and certainly never such a home-like place as it used to be, at least not for now.

And as days were passing a big event was approaching. Probably many of you still do not know how it feels to turn 30! Well, let me elaborate on this a bit: it is tough. Unless you celebrate it with the best company you could have. **Nazely** thanks for organizing a dinner and ordering my favorite food: sushi! **Jeroen**, thanks for coming early and ruining the surprise. **Enver** thanks for not having things to do on that day and actually being there. **Harro&Imre** thanks for joining and bringing your own food. And the next day I travelled to Switzerland, *my safe place*….

Girls, we have known each other almost since the moment we were born and I am still constantly amazed by having you always by my side. **Clau**, **Nai**, **Karin**, the dinner could not have been any better. INNOBO – and I am sure el **Bebo** contributed to your skills as cooks ☺ - is with no doubt a big thing. And so was the whole organization of the surprise you prepared for me. I could not have wished for cooler intruders in those pictures we were attempting to take on that street in Zürich close to that bar where we just had a couple of "Voll Mond" beers. **Leti&Aldo** that was simply amazing and never expected. **Leti**, I met you not so long ago through friends in common, hard and happy days and lots of dancing and alcohol our friendship grew incredibly fast. You are my favorite TV star and I am

blessed to have you in my life. Thanks for being there thinking that it was your birthday celebration and blowing up the candles together with me (or instead of me?). **Nikki**, you were also part of that day and I actually cannot recall a single day when I needed a friend that you were not there for me. Big thank you for always making sure that I was doing ok. You deserve the best and I wish you and **David** all the happiness in the world. Big thanks also to the rest of the crew: **Alex**, weil du immer noch die Haare schoen hast ☺. **Anita, María** and **Dani** because you knew exactly the best way to cheer me up all the time (like the unicorn picture). **Martin**, for knowing every single song of the Mariachi's during Claudia's birthday on that same year. **Moni** and **Loni** for being so unique and so much fun. **Inti**, for being our personal sightseeing guide. Last but not least, **Rhina** and **Christian**, thank you for your hospitality, for your never ending beer supplies, for making me feel at home and for contributing to assign to Zurich the *"my safe place"* adjective.

But my birthday celebration was not over: **Nai** thanks for organizing that awesome trip to Prague, for the cool pictures and for arranging the tickets to that amazing opera. **Nazely**, thanks for crying in the opera and agreeing to come with us. **Gabriela** thanks for joining me in "keeping" the beer glasses as souvenirs. **Leti&Aldo** thanks for leaving the glasses in the hotel, for the awesome dinner the last night and for letting **Nai** and me party and play the piano in your room. As **Leticia** once said, we should be everyday as happy as we were during that trip!

That same year two prizes came to my hands and with one I decided to attend a conference in Singapore. I took the opportunity to visit Prof. Tony Fane's group at Nanyang Technological University. **Xing Yang** thanks for welcoming me, showing me around and giving me some tips on where to go in Singapore. I appreciated it a lot! During my stay, Prof. **Tai-Shung Neal Chung** also welcome me for a visit to his group at the National University of Singapore. A very detailed lab tour was organized for me and I had lunch together with the whole group. I especially thank **Sun Shipeng** for his kindness and taking care of all arrangements of my visit.

I had the possibility to attend several other conferences during my PhD time. I would like to thank everybody that I met during all conferences especially **Sonia** and **Sara** for our nice trip to Wien, Tatranské Matliaré and Bratislava. **Ekain** and **Enrique** for attending almost all conferences I also attended and having recurrently fun together. **Aleksandra**, for the time we shared in Italy, for our long chats and for offering me your house in Tarragona last year. I hope to meet you all again in the future. Also the Winterschools organized by the group from Aachen and **Steffi K.** for hosting Karina's first good bye party.

As everything, also good things come to an end and also good years. 2011 was over and 2012 did not start that smooth. Special thanks to **Kasia, Nayeli, Daniela F.M., Maite, Iza W., Dagmara, Nancy** for all the support and the time you invested in trying to cheer me up. You were of great help and honestly, I do not know how I would have ended without your words.

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Can&Laura, you blamed it on me that I made your families cry with the movie but I am not sorry for that. I was myself never so close to crying than when preparing it. Thank you for inspiring that movie, for giving us the opportunity to have so much fun when organizing, together with **Gerard**, such a party for you and for being an

example of kindness, love, perseverance, honesty, courage, friendship and all what it takes to succeed in all areas in life. Thank you for being always willing to help everybody in any situation no matter how busy you are (like taking me to the doctor when I did not feel half of my face), and, as **Kadir** said, for making of both of your weddings the highlights of 2012. **Laura**, as I already wrote once, thanks for keeping **Can** here until I finish and **Can**, thanks for bringing **Laura** into our lives. My time in Enschede would certainly not have been the same without you both.

Luckily, a bad start does not automatically mean a bad end. The end of 2012, extensive to the start of 2013, turned into one of my best holidays ever. I would like to thank my friends that not only make from every holiday in El Salvador a special one but that from the far have been following my steps all this time and giving me their support during my PhD. **Daniela B**., **Daniela O.**, **Patty**, **Bianca**, **Silvana**, **Nai**, **Clau**, **Karin**, **Peter**, **Flo** and the friends I stole from my sister **Bobby**, **Morris**, **Miri**, **Mariane&Sven**, **Ingrid&Kafie**, **Mel**, it is always nice to see you, catch up and know you are there. My friends from the university, **Gaynell**, **Delmy**, **Claudia&Antonio**, **Alejandro**, **Rommel**, time passes by and it feels like yesterday when we were taking lectures together. Thanks for always comforting me when needed and being willing to drink some beers on a Friday night or share a Sunday at the beach. **Jupa** and **Juancho**, thanks for always having the right energy to party. The **Velázquez Parker** family & sub-families, for always inspiring me in many ways like **Lucía**'s music or **Ricardo**'s quotes. The **Humanum Tempore** family and especially **Estela Mena**, for always welcoming me with open arms and a big open heart. **Gaby** and **Katya**, for never understanding why I decided to do PhD but, together with **Elsita**, still supporting me all the time. **Sixto** for sharing the "margarita quería churros españoles" enthusiasm with me. **Bernardo** for agreeing to go to play squash with your uncle when we had agreed to meet and for getting lower grades than me in Física 1. **Fide**, **Beba** and **Carmen** for having many cool party dresses to lend and such a pure heart. **Maria Renee** and **Gracia** for that afternoon at my grandparents place where we created an unforgettable legend (BS – M – G – O). **Chato**, for being the only person that calls me "ingeniero". **Diego** for singing "Tabaco y chanel" 14 times in a single night. **Beita** for sharing the same interests and taste about dancing, partying, "el depor", travelling, and many other things. You are one of my favorite persons in the whole world and an excellent friend. **Manuel** for being one of the only persons that actually know how to dance. **Chamba** for waking us up screaming at 6 am when we actually went to bed at 5:53 and for coming to my house with a Chihuahua. **Leti&Aldo** for always managing to make every day I share with you a remarkable one. I could not be any happier about you guys having met. You are just perfect for each other and it should also stay like this ☺. **Jorge**, I could not avoid mentioning you here. Having met you all those years ago was definitely a highlight in my life, and now, so many years later, you still somehow inspire me. Thanks for sharing the enthusiasm of this PhD with me, for your advices, for your support, for teaching me to dance bachata and to be critical with myself, for all those vallenatos we danced and for always delighting us with your music.

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Selin, we were basically the only two foreigners in the MSc in Biotechnology at TUHH what contributed to our start as friends. I will never forget the time when we were both almost unable to stand up from bed, you with surgery in your knee, me having just had surgery on my back, but still we were planning to move together the following month. I am wondering how that house would have looked like. Without your help with explanations and collecting material for the examinations in that period I would not have been able to finish my studies. I learned a lot from you and not only related to enzymes. You are a very decided, perseverant and straight forward person. I am happy to have had you as a colleague, to have you as a friend and to have you standing next to me on this very important day in my life. I wish you lots of success and happiness in your career and your personal life with **Peter**.

And now I look to the other person standing next to me: **Karina**, on your last months of your PhD you showed extreme courage. And now you are showing it again by having started a job in Saudi Arabia. Have I ever told you how much I admire you? And, moreover, how much you deserve to be as happy as you want? Dear **Karina**, I could always rely on you, in any sense, at any time, on every day. We cried and laugh in that office, several times. You know it already of course, but you are amazing. And, like you, there is only one thing I regret about our friendship: that it started too late. But it started, and it keeps on going, and that is what matters.

Hold on…I almost finished…..except that I still have to thank my whole family, and it is big.

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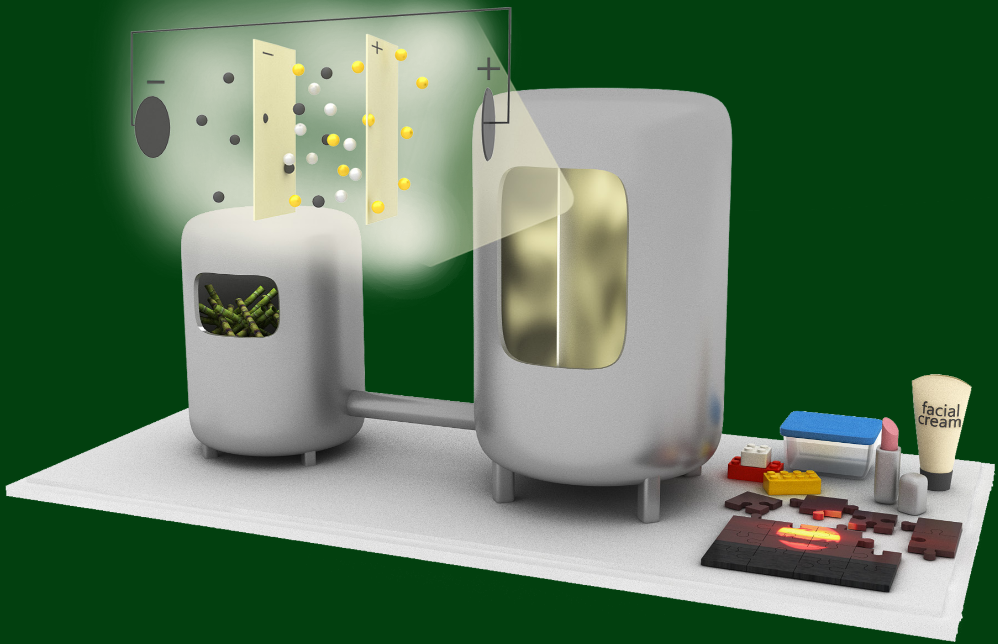
Olga K.

PS: I could promise a beer to the ones I forgot but I would never pay that (like the never-happening housewarming party). You can still let me know and make me feel guilty forever about it.



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