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On the isolation of single acidic amino acids for biorefinery applications using electrodialysis

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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.

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 (Fig. 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 Fig. 1.

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 (Fig. 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 (PVP) [2,3]. 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

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Table 1
Comparison between the different amino acids, their iso-electric points and their charge at neutral pH.

Amino acid	p*I*	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

product recovery by process parameters such as current density and pH.

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, and can consequently be applied for the separation of charged amino acids (Fig. 2). 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, based on differences in their p*I*'s. Amino acids are positive, neutral or negative depending on the surrounding pH. Table 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.

In principle, ED should be able to separate amino acids as long as there is a difference in their corresponding isoelectric points or

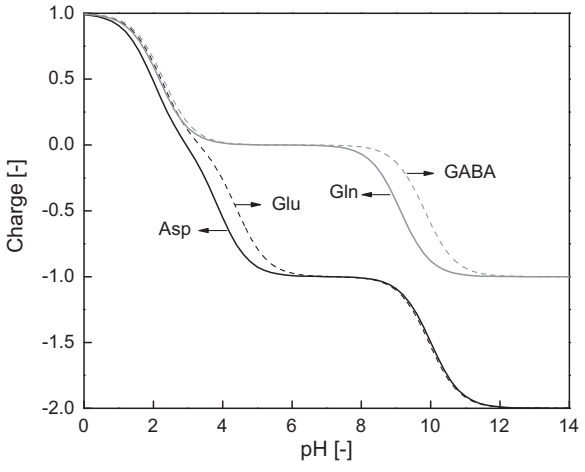

Fig. 1. Charge behavior of Glu and Asp (acidic amino acids), Gln (neutral amino acid) and GABA (modification product of Glu) with respect to pH.

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 polyether 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 (PVP) 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