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Obtaining the zwitterionic form of L-lysine from L-lysine monohydrochloride by electrodialysis

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Abstract. The process of electromembrane transformation of L-lysine monohydrochlorides into their zwitterionic form in four- and two-chamber electrodialysis apparatus was investigated. The process of transformation at various concentrations of lysine monohydrochloride (0.1-0.6 mol.L-1) was studied and it was established that at the optimum density of current optimal concentrations of lysine hydrochloride during electrodyalisis was in the range of 0.2-0.4 mol.L-1. It was determined that the process of total transformation was accomplished when pH of the lysine solution achieved 10. Changes of concentrations of Cl^- ions and lysine diffused into the neighboring chamber were determined depending on the time. The method developed by us allows adjusting the removal coefficient of Cl^- ions during transformation to a maximal value, the losses of lysine diffused into the next chamber after its return to the technological cycle being less than 1.0 %. The specific energy consumption during the process of transformation in two- and four-chamber electrodialyzers was 0.19 and 0.205 A.h.kg-1 and the current efficiency was 75.9 and 73.1 %, correspondingly. Study of the process of electromembrane transformation allowed obtaining zwitterionic form of L-lysine from L-lysine monohydrochloride with minimal reagent and energy consumption.

Keywords: electrodialysis; L-lysine monohydrochloride; zwitterion of L-lysine; transformation

1. Introduction

In microbiological production basic amino acids (L-lysine, L-ornithine, L-arginine, L-histidine) are mainly produced in the form of monohydrochlorides. Since the salts of basic amino acids (aspartate, glutamate, nicotinate, citrate, tartrate, phosphate, sulfosalicylate, etc.) used in medicine and food industry are mainly obtained by interaction of the zwitterionic form of basic amino acid with the appropriate acid (Petrosyan *et al.* 2005, Petrosyan *et al.* 1999, Tateba and Michio 1993), the determination of optimal parameters of transformation of basic amino acid hydrochlorides into their zwitterionic form is an urgent problem of theoretical and practical significance.

Due to high solubility of zwitterionic form of L-lysine and L-ornithine, these amino acids are mainly obtained from their monohydrochlorides by ion-exchange method which, however, has a number of disadvantages, namely it is a multi-stage process, requiring huge consumption of reagents, burdened with large volumes of acidic and regeneration effluents.

The method for production of lysine in the zwitterionic form from its hydrochloride by means

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of electrophoresis is well-known (Szilasi *et al.* 1991). According to this technology, lysine hydrochloride is passed through ion-exchange resin and the resultant ammonia eluate after the vacuum evaporation is subjected to electrophoresis at the direct current voltage of 200-1000 v. The lysine solution is collected at pH = 9.74. Though the method allows obtaining lysine in a zwitterionic form, it is labor-consuming and expensive.

In work (Aghajanyan 2001) to obtain L-lysine and L-ornithine in the zwitterionic forms, the solutions of technical crystals of these amino acids were decolorized and acidified solutions were passed through sulfo-cationite CU-2 × 8 in NH_4^+-form. After water rinsing, the collected ammonia eluates of basic amino acids were evaporated. To remove residual inorganic ions, solutions of amino acids were passed through sulfo-cationite in the form of the appropriate basic amino acid, resin yielding pure solution of basic amino acid.

The method for obtaining lysine in the zwitterionic form from pure crystals of D,L-lysine hydrochloride is described in work (Shostenko *et al.* 1995). According to this method, D,L-lysine solutions were passed through sulfo-cationite in H^+-form and the collected ammonia eluates were evaporated. Ether alcohol (10:1) was then added to the solution. The precipitate formed was separated and dried.

In work (Miroslav *et al.* 1996) to obtain L-lysine in the zwitterionic form from its hydrochloride, the solution of lysine hydrochloride was added in the stoichiometric quantity to the NaOH solution (10-50 %), the obtained mixture was cooled to 20°C. Then the lysine solution was desalted by means of electrodialyzer and evaporated to the necessary concentration.

The similar approach for production of lysine in the zwitterionic form from its hydrochloride is discussed in work (Chen *et al.* 2011). The work presents all parameters of the process including concentration of L-lysine hydrochloride, molar ratio of L-lysine HCl: NaOH, pH value of the solution and the current voltage. It should be mentioned that at the optimal ratio of lysine hydrochloride-alkali (3:5) chosen by the authors, it is necessary to remove the excess of OH^- and Na^+ ions that create additional difficulties for fulfillment of the transformation process. In particular, the excess of OH^- ions in the lysine solutions reduces the velocity of Cl^- ions transport through the membrane. In addition, in an excess of OH-ions the charge of lysine will be changed, which will lead to its diffusing from dilution chamber into the concentration one.

The afore-mentioned methods for production of lysine in the zwitterionic form are multi-stage and labor-consuming.

To desalinate amino acid solutions and transfer them from one ionic form into another one, electrodialysis with ion-exchange membranes is the most selective (Garcia-Garcia *et al.* 2000, Grib *et al.* 1998). This method unlike the known ones is economically more efficient and ecologically safe.

There are some publications devoted to application of the electromembrane method for purification of solutions of amino acids (Liu *et al.* 2009, Shen *et al.* 2005) , their separation from each other (Bukhovets *et al.* 2009, Elisseeva *et al.* 2002, Zhang *et al.* 2007), isolation and purification from fermentation solutions (Aghajanyan *et al.* 2008).

The goal of the present research is to study the possibility of applying the method of electrodialysis with ion-exchange membranes to transform lysine hydrochlorides into zwitterionic form with minimal reagent and energy consumption and determine the optimal technological parameters of the transformation process.

2. Experimental

Home-made two- and four-chamber electrodialyzers were used in this study. The electrode chambers of two-chamber electrodialyzer were separated with anion-exchange membrane AM-40, consisting of average basic anionite EDE-10P (Russia). The chambers of four-chamber electrodialyzer were separated from each other by CM-40 cation-exchange membranes made of sulfo-cationite CU-2-8 (Russia) and AM-40 anion-exchange membranes (Fig. 1). A 3×10^{-3} m inter-membrane distance was used in chambers. While desalting, the constant current density was supported with the current regulator.

Strips of platinized titanic sheet were used as electrodes. The working surface of membranes was 5.3×10^{-3} m^2, the volume of the circulating solution was 5×10^{-4} m^3, the linear speed of the liquid stream through chambers was 6.5×10^{-2} m/s. The hydraulic mode was circulating. The speed of feeding by solutions was adjusted by a Masterflex (USA) peristaltic pump.

To effectively perform the process of electromembrane transformation of basic amino acid hydrochlorides into their zwitterionic form, the values of the limiting current density (LCD) in the investigated system were determined by the graphic method of Cowan and Brown (Cowan and Brown 1959) in the coordinate system of the dependence of the solution resistance on the reciprocal value of the current strength. The LCD equaled ~18 mA.cm^{-2}, at concentration of lysine monohydrochloride in diluate chamber –0.3 mol.L^{-1}.

The specific energy consumption (EC) is calculated by the formula indicated in work (Chen *et al.* 2011).

$$EC = \frac{\int IUdt}{m} \tag{1}$$

Where m is the mass of Cl^- removed (kg); I the current (A); t the time (h) and U the voltage (V).

The current efficiency is calculated by the formula

$$\eta = \frac{I_t}{I_p} \tag{2}$$

In which I_t and I_p are the theoretical and practical currents, respectively.

The concentration of amino acids in the solution was determined by thin-layer chromatography (TLC) and amino acid analyzer AAA-339 (Czech Republic).

The concentration of zwitterionic form of lysine in the solution was determined from the Diagram of dependence of mole fraction of lysine various ionic forms on the pH of the solution (Saeki and Sakata 1982) as well as by determination of the concentration of Cl^- ions.

3. Results and discussion

To reduce the number of stages in production of basic amino acids from their hydrochlorides in the zwitterionic form and to eliminate the use of organic solvents, we have employed reagent-free electrodialysis.

Four-chamber electrodialyzer with the initial solution of basic amino acid hydrochloride circulating between two anion-exchange membranes (Fig. 1) was used in this work.

During the electrodialysis, chlorine ions migrated through the anion-exchange membrane into the water chamber under the action of the electric potential gradient and hydroxyl ions being formed in the cathode chamber as a result of water electrolysis migrated into the lysine

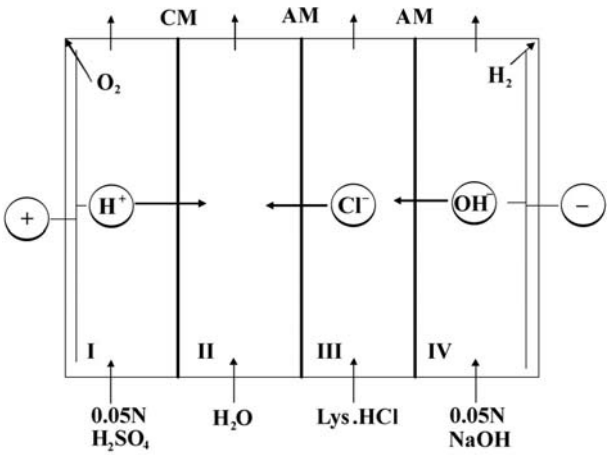

Fig. 1 Scheme of a four-chamber electrodialyzer

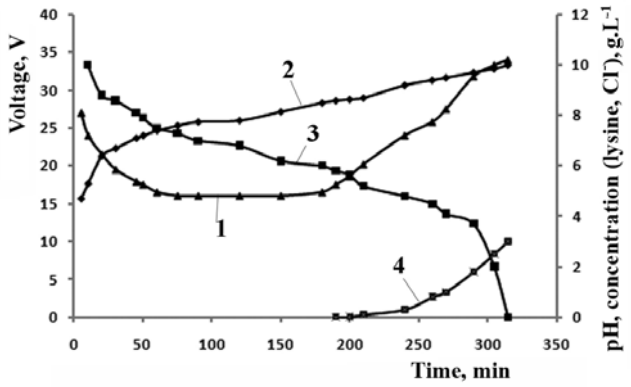

Fig. 2 Changes of voltage in the circuit, pH and concentration of Cl^- ions in the lysine solution and the contents of lysine in the water chamber in the process of hydrochloride (concentration 0.3 mol.L^{-1}) transformation into zwitterionic form in a four-chamber electrodialyzer depending on time.
1–Voltage; 2–pH of the lysine solution; 3–Concentration of Cl^--ions in the lysine solution; 4–Concentration of lysine in the water chamber

hydrochloride chamber and attached to the cationic lysine. Thus, the so-called process of continuous ion exchange is realized in the course of which some ions are replaced by other ones. Cl^- ions that migrated into the water chamber, being attached to H^+ ions formed in the anode chamber as a result of water electrolysis, diffuse through the cation-exchange membrane into the water chamber. The formed diluted hydrochloric acid can be used for preparation of ion-exchange membranes or in other technological cycles. Thus, the process of transformation becomes a wasteless process.

As follows from Fig. 1, which schematically presents the arrangement of membranes, the process of electromembrane transformation of lysine from hydrochloride into zwitterionic form is carried out due to the diffusion of mobile inorganic ions (Cl^-, OH^-, H^+) into the neighboring chamber. This allows conducting the transformation process with relatively less consumption of energy.

The dependence of changes in the circuit voltage, pH and concentration of Cl^--ions in the lysine hydrochloride solution as well as of the lysine concentration in the water chamber during electrodialysis is presented in Fig. 2.

As follows from Fig. 2, at the initial stage of electrodialysis voltage in the circuit gradually falls due to diffusion of Cl^- and H^+-ions into the water chamber, then it stabilizes and the transformation process proceeds under reduced voltage (~16 v) for more than half of the time. When a sharp decrease of the concentration of Cl^- ions in the lysine solution begins (curve 3) attended by transition of lysine from hydrochloride into zwitterionic form, current strength in the circuit begins to lower and to preserve current density at the maximum level (~18 $mA.cm^{-2}$), and the voltage in the circuit has to be raised. At the end of the process it equals 36 v (curve 1). In the process of transformation, pH of the lysine solution increases (curve 2) and the process proceeds up to complete removal of Cl^- ions from the lysine solution.

Experiments have shown that this can be achieved when pH of the lysine solution was not lower than 10.

During transformation, availability of lysine in the water chamber was measured depending on time. It was established that in the process of electodialysis when the solution contained lysine in the cationic (pH < 7) or zwitterionic (pH = 9.6) form it did not diffuse into the water chamber (Saeki and Sakata 1982). When pH of the solution was more than 10 and concentration of Cl^- ions in it was minimal (0.03 $g.L^{-1}$) or zero, the lysine transformed from zwitterionic into anionic form and started to diffuse into the water chamber to ensure electroconductivity in the circuit.

Experiments have shown that when pH of the lysine solution had reached 10 there were no Cl^- ions in the lysine solution (curve 3) and the concentration of lysine in the neighboring water chamber achieved 3.0 $g.L^{-1}$ (curve 4). The transformation process was considered complete at that. During transformation the lysine did not diffuse into the electrode chambers. At the end of transformation process the concentration of zwitterionic lysine in diluate chamber was 0.285 $mol.L^{-1}$, without accounting the lysine returning into the technological cycle from water chamber.

The diffused lysine in the form of hydrochloride can be extracted from the solution of water chamber by vacuum evaporation and isohydric crystallization followed by the lysine return to the cycle of the transformation process. Such technological approach provides yield of lysine at the stage of transformation up to 99 %.

The process of transformation of lysine hydrochloride into the zwitterionic form at various concentrations of hydrochloride in the range of 0.1-0.6 $mol.L^{-1}$ in a four-chamber electrodialyzer was studied.

Experiments have shown that when lysine hydrochloride was used in concentration up to 0.4 $mol.L^{-1}$ the transformation process proceeded to the end. The curves of the dependence of voltage in the circuit, concentration of Cl^- ions and pH of the lysine solution on the time differed slightly from each other. In the case of increasing hydrochloride concentrations in the initial solution the transformation process did not come to an end.

The dependences of changes of voltage in the circuit, pH and concentration of Cl^- ions in the lysine solution at 0.6 $mol.L^{-1}$ initial concentration of hydrochloride are presented in Fig. 3. In this experiment LCD was ~35 $mA.cm^{-2}$.

Comparing the curves presented in Fig. 2 and Fig. 3 it is evident that they differ from each other. Thus, for example, pH of the lysine solution increases only to 9.4 at 0.6 $mol.L^{-1}$ concentration of hydrochloride. This is evidently connected with insignificant quantity of OH^- ions formed in the cathode chamber. Due to this, the concentration of Cl^- ions in the lysine solution

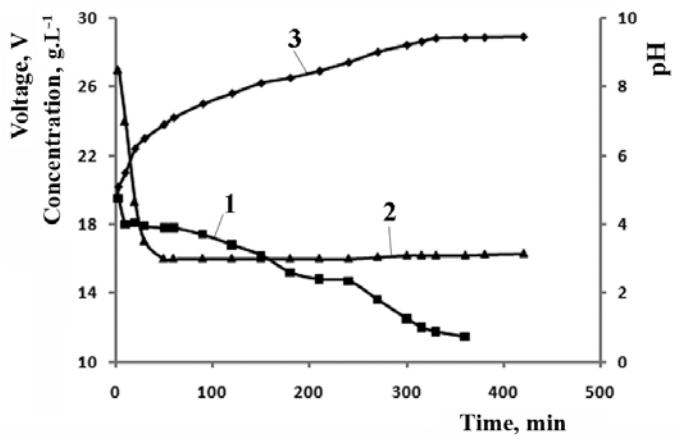

Fig. 3. Changes of voltage in the circuit, pH and concentration of Cl^- ions in the lysine solution depending on time in the process of lysine transformation from hydrochloride into basic form at 0.6 mol.L^{-1} concentration of lysine hydrochloride in the initial solution.
1–Concentration of Cl^- ions in the lysine solution; 2–Voltage; 3–pH of the lysine solution

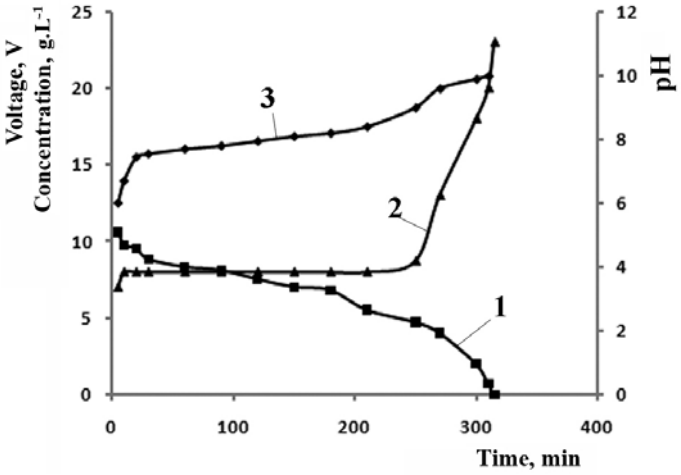

Fig. 4 Changes of voltage in the circuit, pH and concentration of Cl^- ions in the lysine solution in the process of hydrochloride (concentration 0.3 mol.L^{-1}) transformation into zwitterionic form in a two-chamber electrodialyzer depending on time.
1–Concentration of Cl^--ions in the lysine solution; 2–Voltage; 3–pH of the lysine solution

decreases by ~50 % as compared with their initial quantity. In the process of transformation voltage in the circuit practically does not change throughout the whole process and equals 16 v (curve 2) that proves incomplete transition of lysine from hydrochloride to zwitterionic form.

At the end of the process the concentration of Cl^- ion in the diluate chamber equaled 1.6g .L^{-1} and concentration of lysine in the water chamber achieved 5.7 g.L^{-1}.

The process of transforming lysine hydrochloride into zwitterionic form in a two-chamber electrodialyzer with the use of anion-exchange membrane AM-40 was also explored. In the process of electrodialysis the solution of lysine hydrochloride circulated through the cathode

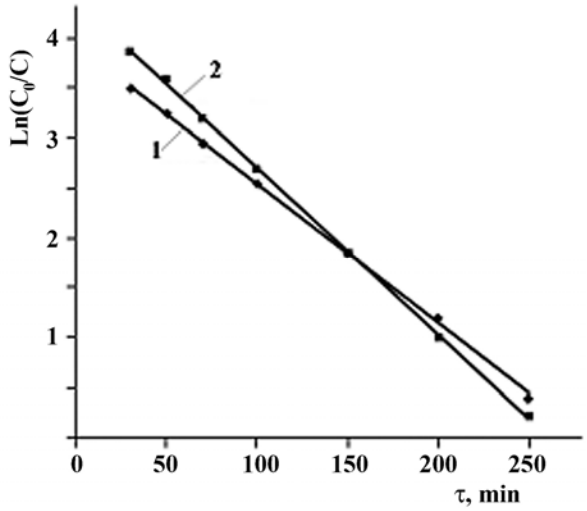

Fig. 5 Kinetic curves of lysine exchange from monohydrochloride to zwitterionic form in the process of electromembrane transformation.
1–Four-chamber electrodyalizer; 2–Two-chamber electrodyalizer; C_o–Concentration of lysine hydrochloride in the initial solution, mol.L^{-1}; C–Concentration of lysine in the zwitterionic form in the solution, mol.L^{-1}.

chamber. Changes of voltage in the circuit and pH of the solution in the course of lysine hydrochloride transformation in a two-chamber electrodialyzer are presented in Fig. 4.

As evident from Fig. 4, the process of lysine transformation from salt into zwitterionic form as distinct from four-chamber electrodialyzer proceeds under reduced voltage (~8 v). At the end of the process voltage in the circuit starts to rise sharply due to transition of lysine into zwitterionic form. The latter is a bad current conductor that is why to keep current density at the ultimate level, voltage in the circuit should be raised up to 19 v.

Experiments have shown that while using a two-chamber electrodialyzer (unlike a four-chamber electrodialyzer), lysine does not diffuse into the neighboring chamber during transformation. However, the disadvantage of the use of a two-chamber electrodialyzer compared with the four-chamber electrodialyzer is that the Cl^- ions diffused into the anode chamber have less recovery potential than oxygen of water. This results in formation of the gaseous chlorine, utilization of which creates additional difficulties.

The order of the exchange reaction of lysine from monohydrochloride to zwitterionic form was studied. The results of $Ln(C_o/C)$ dependence on in the coordinate system are presented in Fig. 5.

The diagram presented in Fig. 5 has a linear form, which proves that the proceeding exchange reaction relates to the irreversible reaction of the first order tg being equal to 0.018 min^{-1} and 0.0166 min^{-1} in the case of carrying out the exchange process in two- and four-chamber electrodialyzers, respectively.

During the process of lysine transformation from hydrochloride into zwitterionic form in two- and four-chamber electrodialyzers, the specific energy consumption calculated by formula 1 was 0.19 and 0.205 A.h.kg^{-1} or, 1.85 and 3.82 kW·h·kg^{-1} correspondingly. The current efficiency in two- and four-chamber electrodialyzers, calculated by Eq. (2), was 75.9 and 73.1 %, respectively.

Proceeding from the afore-mentioned kinetic (Fig. 5) and energy data, it is preferable to carry

out the process of lysine transformation from monohydrochloride into zwitterionic form in a two-chamber apparatus.

Experiments have shown that the obtained regularities of lysine hydrochloride transformation into its zwitterionic form are completely reproducible during transformation of ornithine hydrochloride into its zwitterionic form.

Thus, study of the process of electromembrane transformation allowed obtaining zwitterionic form of basic amino acids from their hydrochlorides with minimal reagent and energy consumption.

References

Aghajanyan, A.E. (2001) "Method for obtaining of the base of basic amino acids", Armenian Patent N904 A2.

Aghajanyan, A.E., Hambardzumyan, A.A., Vardanyan, A.A. and Saghiyan, S.S. (2008), "Desalting of neutral amino acids fermentative solutions by electrodialysis with ion-exchange membranes", *Desalination*, **228**(1-3), 237-244.

Bukhovets, A.E., Saveleva, A.M. and Elisseeva, T.V. (2009) "Separation of amino acids mixtures containing tyrosine in electromembrane system", *Desalination*, **241**(1-3), 68-74.

Chen, Y., Zhang, Y., Yue, M. and Zhou, Y. (2011), "Production of L-lysine from L-lysine monohydrochloride by electrodialysis", *Desalination and Water Treatment*, **271**(1-3), 163-168.

Cowan, D.A. and Brown, J.H. (1959), "Effect of turbulence on limiting current in electrodialysis cells", *Ind. Eng. Chem*., **51**(12), 1445-1448.

Elisseeva, T.V., Shaposhnik, V.A. and Luschik, I.G. (2002), "Demineralization and separation of amino acids by electrodialysis with ion-exchange membranes", *Desalination*, **149**(1-3), 405-409.

Garcia-Garcia, V., Montiel, V., Gonzalez-Garcia, J., Exposito, E., Iniesta, J., Bonete, P. and Ingles, M. (2000), "The application of electrodialysis to desalting an amino acid solution", *J. Chem. Educ*., **77**(11), 1477-1479.

Grib, H., Bonnal, L., Sandeaux, J., Sandeaux, R., Gavach, C. and Mameri, N.J. (1998), "Extraction of amphoteric amino acids by an electromembrane process. pH and electrical state control by electrodialysis with bipolar membranes", *J. Chem. Technol. Biotechnol*., **73**(1), 64-70.

Liu, L.F., Yang, L.L., Jin, K.Y., Xu, D.Q. and Gao, C.J. (2009), "Recovery of L-tryptophan from crystallization wastewater by combined membrane pracess", *Sep. Purif. Technol*., **66**(3), 443- 448.

Miroslav, B., Zbynek, P. and Vladinir, J. (1996), "Sposob pripravy velmi ciste baze L-Lyzinu", Czech Patent N 279309.

Petrosyan, A.M., Karapetyan, H.A., Sukiasyan, R.P., Aghajanyan, A.E., Morgunov, V.G., Eravchenko, E.A. and Bush, A.A. (2005), "Crystal structure and characterization of L-arginine chlorate and L-arginine bromate", *J. Mol. Struct*., **752**(1-3), 144-152.

Petrosyan, A.M., Sukiasyan, R.P., Terzyan, S.S. and Burbelo, V.M. (1999), "Interaction of lysine with iodic acid", *Acta Cryst*., **B55**(2), 221-225.

Saeki, M. and Sakata, Y. (1982), "Some physicochemical properties of amino acids", *Ferm. and Ind*., **40**(10), 906-016. (in Japan).

Shen, J.Y., Duan, J.R., Liu, Y.S., Yu, L.X. and Xing, X.H. (2005), "Demineralization of glutamine fermentation broth", *Desalination*, **172**(2), 129-135.

Shostenko, U.V., Sheyn, A.T., Lapkina, U.I., Kovalev, I.P. and Konev, F.A. (1995), "Method for obtaining of L, D-lysine base, Inventors Certificate of USSR N1518948.

Szilasi, J., Pataky, A. and Sova, O. (1991), "Sposob vyroby chemicky L-lyzinu", Inventors Certificate of Czechosm, SSR, N 265509.

Tateba, T. and Michio, Sh.(1993), "Process for producing crystals of salt of acidic amino acid and basic

amino acid", US Patent N 5, 227, 007.
Zhang, X.Y., Lu, N.H. and Ren, H.Y. (2007), "Recovery of glutamic acid from isoelectric supernatant using electrodialysis", *Sep. Purif. Technol*., **55**(2), 274-280.

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