Scientific Paper

Recovery and Purification of Rosmarinic Acid from Rosemary Using Electrodialysis

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Received 15-12-2004

Abstract

Recovery and purification of rosmarinic acid from rosemary was performed using lab-scale electrodialysis unit. Electrodialysis of rosmarinic acid model solution was performed to validate its applicability for rosmarinic acid separation. Positive influence of ethanol addition on process performances was analysed in the experiments with dried rosemary extract. Under optimum process conditions (addition of 30% ethanol) ion flux reached 5.9 10^{-1} g m^{-2} min^{-1}, specific energy consumption was 3.9 kWh kg^{-1}, and current efficiency achieved 80.1%.

Key words: electrodialysis, rosemary, rosmarinic acid

Introduction

Rosmarinic acid (RA, $C_{18}H_{16}O_8$, cream crystalline solid at 25 °C, *MW* = 360.3, melting point 171–175 °C) derived from caffeic acid and (*R*)-(+)-3-(3,4-dihydroxyphenyl)lactic acid represents one of the most common caffeic esters in plant material and is accumulated constitutively.[1] It is a well-known natural product from rosemary (*Rosmarinus officinalis*), lemon balm (*Melissa officinalis*), and other *Lamiaceae* as well as another plant families, e.g. the medical plants like thyme, oregano, savory, peppermint, sage.[2–4] RA exhibits various pharmacological activities including prevention of oxidation of low density lipoprotein, inhibition of murine cell proliferative activity and of cyclooxygenase, and anti-allergic action.[5] The biological activity of RA is described as antibacterial, antiviral, and antioxidative.[6,7] Its activity especially against rheumatic and inflammatory conditions makes it a sought-after substance for use in phytotherapy.[8] More recently, rosmarinic acid or its salts were reported to have anti-HIV activities.[9]

Rosmarinic acid can be synthesized from their constituents, e.g. caffeic and (*R*)-(+)-3-(3,4-dihydroxyphenyl)lactic acids, as the racemate by low-yield chemical synthesis.[10] It has been produced by fermentation from suspension cell cultures of *Coleus blumei*,[6,11] *Salvia miltiorrhiza*,[9] *Anchusa officinalis*,[12] and by chemo-enzymatic synthesis in the presence of rosmarinic acid synthase.[8] Furthermore, batch fermentation of rosmarinic acid using *Coleus blumei* cell suspension followed by isolation and purification of rosmarinic acid consisted of centrifugation, extraction and adsorption, was developed as large scale production process.[13]

Application of mono-polar electrodialysis for recovery and purification of rosmarinic acid from rosemary extract was demonstrated in this work. Electrodialysis is a charge dependent ion separation process in the electric field and in comparison with other separation processes it offers some basic advantages, e.g. simple technology, separable products, low environmental impact and operating costs, and high process efficiency.[14] It represents one of the most important membrane processes for environmentally clean technology in the biochemical industries.

Electrodialysis has been widely applied for the production of different organic acids[15–17] which was the main motivation to use it for recovery and purification of rosmarinic acid from rosemary extracts. Furthermore, extraction and adsorption processes commonly used in the separation of rosmarinic acid from rosemary extract generate large quantities of waste streams, e.g. solvents in the extraction and highly concentrated salts solutions for the regeneration of ion-exchange resins in the adsorption. The waste streams will increase the treatment costs and weaken the competitiveness of the process. Therefore, electrodialysis, which can minimize the production of waste streams, has been considered as the promising alternative process.[18]

Experimental

Feed solutions

The model solution used in the electrodialysis experiments was 1.5 g dm^{-3} rosmarinic acid (purum, w_{RA} ≥ 97%, CAS: 20283-92-5, Fluka, Switzerland; VITIVA, Slovenia) in demineralised water. In experiments with rosemary extracts (VITIVA) two different kinds of extract were used; aqueous rosemary extract (c_{RA} = 5 g dm^{-3}) and dried rosemary extract (w_{RA} = 25%), respectively. 0.5 mol dm^{-3} Na_2SO_4 (p.a., CAS: 7783-20-2, Kemika, Croatia) aqueous solution was used as the electrode rinse solution in all experiments.

Mono-polar electrodialysis

Homo-polar electrodialysis consisted of a cell stack (Type 02, Berghof Filtrations - und Anlagentechnik GmbH & Co. KG, Germany) with three cell pairs, a power supply (Model IPS-3610D, ISO-TECH, UK), three centrifugal pumps (Model MD-6-230GS, Iwaki Co., Ltd, Japan) and three solutions tanks (1.5 dm^3) holding the feed, the concentrate and the rinse solutions, respectively. The cell stack was separated into the feed and the concentrate compartments by alternating cation (PC SK, PCA GmbH, Germany) and anion (PC 400D, PCA GmbH) exchange membranes (Figure 1). Each membrane sheet had an effective membrane area of 36 cm^2 to give a total membrane area of 252 cm^2.

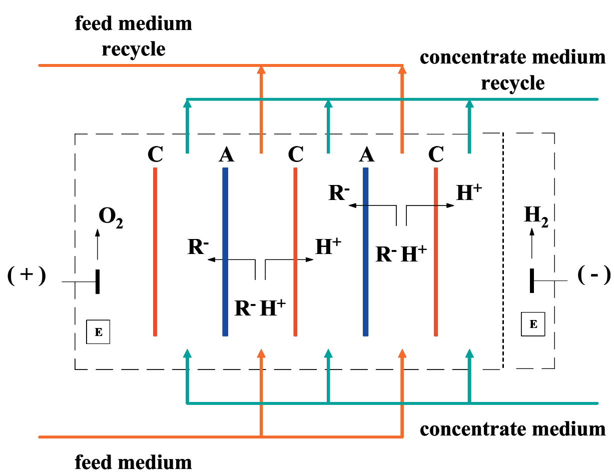

Figure 1. Basic principle of mono-polar electrodialysis. The electrode solution (E) was circulated continuously to transfer the electric current and to remove gases produced by the electrode reaction. A, anion-exchange membrane; C, cation-exchange membrane; R^-, rosmarinate ion; H^+, hydrogen ion.

ED-operations

Initially, the concentrate compartment was filled with demineralised water. All streams were circulated through compartments and recycled to the reservoir before electric power was supplied to electrodialysis stack. The voltage drop in the system was measured by voltmeter. The pH of concentrate compartment and conductivity of each compartment were measured using pH meters (Handylab pH 11, SCHOTT Instruments GmbH, Germany) and conductivity meters (Handylab, LF 11, SCHOTT Instruments GmbH), respectively. A 2 mL sample was taken from each compartment in a predetermined schedule for analysis of rosmarinic acid concentration.

All experiments were performed in the constant voltage mode of 34.3 V (current range varied between 0.05 and 0.25 A). The experiments were conducted in the batch mode at ambient temperature (25 °C). Each experiment was performed in the triplicates and on 90 % confidence interval results have no statistical difference.

HPLC analysis

The concentration of rosmarinic acid was measured using the HPLC. Determination of the rosmarinic acid concentration involves the use of 125-4 HIBAR® RT LICHROSORB® RP 18 column (Merck, Germany). The separation was performed with mixture of acetonitrile and 0.03 % triflouroacetic acid solution in the ratio of 25:75% v/v. Eluent flow rate was 0.5 cm^3 min^{-1} (pump S1000, Sykam Chromatografie Vertriebs GmbH, Germany) and the detection was at a wavelength of λ = 280 nm (detector S3300, Sykam Chromatografie Vertriebs GmbH). The signal was analyzed with an integrator (C-R6A, Sykam Chromatografie Vertriebs GmbH) and the sample volume used was 100 µL. An external standard of rosmarinic acid (w_{RA} = 25%, Sigma-Aldrich, Germany) was used for the calculation of results. The HPLC method was validated and on 95% confidence range results had no statistical differences.

Results and Discussion

Electrodialysis of rosmarinic acid model solution

Electrodialysis of rosmarinic acid model (synthetic) solution was performed in order to validate prospects for this technology as a basis for recovery and purification of RA derived from rosemary extract. Low solubility of RA at room temperature observed in the preliminary experiments (approximately 1 g dm^{-3}) was overcome by its dissolving in the 0.4% acetone. Initial volume of both, the feed medium compartment and of the concentrate medium compartment, were 0.5 dm^3. Experimental results are plotted in Figure 2 showing the variation of RA concentration in the both, feed and concentrate compartments. The properties of electrodialysis process performed with RA model solution were investigated by comparing some characteristic electrodialysis process parameters, e.g. ion flux (F), current efficiency (μ), current density (i) and specific energy consumption (E), respectively, achieved in the electrodialysis experiments with pyruvic acid and citric acid model solutions (Table 1).

In comparison with results obtained in the experiments with pyruvic[17] and citric acid, unsatisfactory performances were achieved in electrodialysis experiment with RA model solution. For example, ion flux was 5 fold lower than in the experiment with pyruvic acid performed using the same experimental set-up. It means necessity to install 5 fold bigger membrane

Table 1. Comparison of some characteristic electrodialysis process parameters for the electrodialysis experiments with model solutions of different organic acids.

Compound	*F* [g m^{-2} min^{-1}]	*μ* [%]	*j* [A m^{-2}]	*E* [MJ kg^{-1}]
Pyruvic acid	6.1	93.3	39.7	4.9
Citric acid	5.0	78.3	19.8	5.9
Rosmarinic acid	1.3	32.9	2.4	1.4

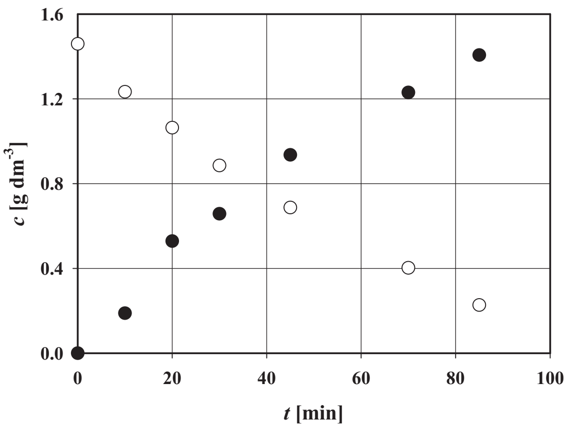

Figure 2. Changes of rosmarinic acid concentration during the electrodialysis experiments of RA model solution; RA concentration in the feed compartment - o; RA concentration in the concentrate compartment - •.

area to transport the same amount of rosmarinic acid if this increase will not cause additional mass transfer resistance, and further decrease of available driving force – current density. Furthermore, low current efficiency obtained in the electrodialysis experiment with RA model solution clearly indicates significant mass transfer resistance and energy consumption caused by the splitting of water molecules into hydrogen and hydroxyl ions.[17] Relatively low specific energy consumption achieved cannot neglect other electrodialysis process parameters which proved to be clearly negative.

Main reasons for insufficient results achieved in the electrodialysis experiments with RA model solutions are its low dissociation equilibrium constant and with it connected low conductivity. If an anionic species will migrate in the electric field from the dilute to the concentrate side, an undissociated acid will be transferred in the same direction if its concentration on the concentrate side exceeds its concentration on the dilute side. The anionic species will migrate the opposite direction (back diffusion) if the concentration of undissociated acid on the concentrate side is greater than that on the dilute side. Clearly, if molecule has low dissociation equilibrium constant, which is in the case with RA, undesirable back diffusion will be dominating mechanism in electrodialysis process (instead of electro migration) leading to its unsatisfactory performances.[19]

Electrodialysis of aqueous rosemary extract

Despite the fact that electrodialysis of RA model solution was not enough efficient, having in mind high price value of the pure RA, further electrodialysis experiments were performed using rosemary extract (VITIVA, Slovenia). As a feed medium two different kind of rosemary extract were used, aqueous rosemary extract and rosemary extract in 20 % ethanol. Initial volume of the feed medium compartment was 1.0 dm^3 and initial volume of the concentrate medium compartment was 0.25 dm^3. Experimental results are summarized in the Figure 3 and in the Table 2.

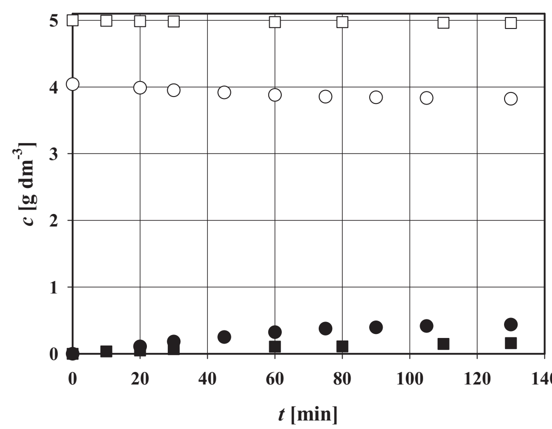

Figure 3. Changes of rosmarinic acid concentration during the electrodialysis experiments of rosemary extract; aqueous rosemary extract (feed compartment - ▫; concentrate compartment - ▪); rosemary extract in 20% ethanol (feed compartment - o; concentrate compartment - •).

Current density in the electrodialysis experiment with aqueous rosemary extract was significantly higher than in the experiment with RA model solution (Table 1). At the same time other electrodialysis process parameters, e.g. ion flux, current efficiency and specific energy consumption, were even worse indicating undesirable electro transport of salts and/or other acids present in the aqueous rosemary extract. As experimental results show transport of RA was minor compared to the results obtained in the experiment with RA model solution. Electrodialysis was stopped after approximately 2 hours of operations because flow rate of the feed stream was negligible indicating fouling of electrodialysis membranes by organic substances present in the aqueous rosemary extract.

Table 2. Comparison of some characteristic electrodialysis process parameters for the electrodialysis experiments with aqueous rosemary extract and rosemary extract in 20% ethanol.

Solvent	*F* [g m^{-2} min^{-1}]	*μ* [%]	*j* [A m^{-2}]	*E* [MJ kg^{-1}]
Water	4.8 10^{-2}	11.5	7.9	69.7
20% ethanol	2.7 10^{-1}	75.1	1.6	9.2

To prevent fouling of electrodialysis membranes new electrodialysis experiment was performed with rosemary extract in the 20% ethanol. The main impact of the ethanol is prevention of fouling of electrodialysis membranes by organic substances which are not soluble in water and therefore responsible for membrane fouling during long-term ED operation. To improve process characteristics additionally, water, initially used as acceptor medium in the concentrate compartment, was replaced with 20% ethanol. Ethanol was chosen because pharmaceutical and food industry as mayor consumer of rosmarinic acid, can accept traces of ethanol in their products and intermediates.

By this change significantly better results were obtained in the performed electrodialysis experiment. Although obtained current density was 5 fold lower than in the experiment with aqueous rosemary extract, significant improvement of the other parameters characterizing electrodialysis process was observed. Ion flux was increased 5 fold, while in the same time current efficiency was even higher than in the experiment with RA model solution. The obtained parameters are in the range with results of electrodialysis of pyruvic and citric acid. It should be emphasized that the specific energy consumption was decreased 7 fold just by adding some ethanol in the feed and in the concentrate compartments.

Electrodialysis of dried rosemary extract

Positive effect of the ethanol addition on the electrodialysis performances was further analysed in the experiments with dried rosemary extract. Dried rosemary extract was dissolved in aqueous mediums containing different concentration of ethanol, namely 30%, 40% and 50% and those solutions are used as the initial mediums in the feed compartment of the electrodialysis unit. Initial medium in the concentrate compartment was 20% ethanol. Initial volume of the feed medium compartment was 1.0 dm^3 and initial volume of the concentrate medium compartment was 0.5 dm^3. Experimental results of those electrodialysis experiments are summarized in the Figure 4 and in the Table 3.

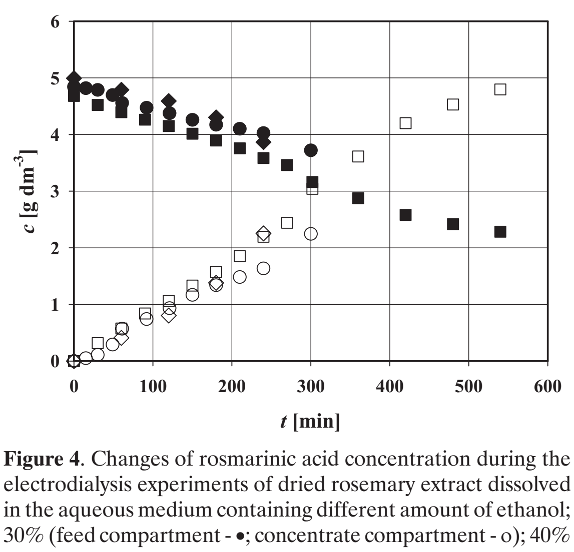

Figure 4. Changes of rosmarinic acid concentration during the electrodialysis experiments of dried rosemary extract dissolved in the aqueous medium containing different amount of ethanol; 30% (feed compartment - •; concentrate compartment - o); 40% (feed compartment - ▪; concentrate compartment - ▫); 50% (feed compartment - ⬩; concentrate compartment - ◊).

Table 3. Comparison of some characteristic electrodialysis process parameters for the electrodialysis experiments with dried rosemary extract dissolved in the aqueous medium containing different amount of ethanol.

Solvent	F [g m^{-2} min^{-1}]	μ [%]	j [A m^{-2}]	E [MJ kg^{-1}]
30% ethanol	5.9 10^{-1}	80.1	1.5	3.9
40% ethanol	7.1 10^{-1}	75.7	1.8	5.2
50% ethanol	7.2 10^{-1}	78.1	1.6	4.4

Increase of the ethanol concentration slightly improves performances of electrodialysis process, but this improvement hardly can cover costs of used ethanol. Increase of ethanol concentration from 20% (Table 2) to 30% (Table 3) improved significantly parameters characterizing electrodialysis process. In all performed experiments with dried rosemary extract recovery of RA was less than 50%, which was consequence of long-term processes. The reason wasn't some experimental problems and/or membrane fouling. To improve process conditions additionally, membrane area should be increased (which was not possible with available lab-scale electrodialysis-unit) and rosemary extract should be purified and concentrated with other separation processes prior to start-up electrodialysis. Furthermore, improvement of membrane properties, e.g. use of anion-exchange membranes especially designed for large molecular cations (like highly water-swollen types cation-exchange membranes) could be next step in the development of the electrodialysis process of rosemary extract. It is known that commercial anion membranes, like is PC 400D used in this work, prepared using hydrophobic polymers as the base material, are not the best choice for the separation of large organic acids because they have relatively small free volume in the membrane. Therefore, several problems, such as significant energy consumption and organic fouling, which was exactly the case in the performed experiments, possibly occur during the separation of large molecular organic acids.[18]

Conclusions

The use of electrodialysis has shown that this approach is not suitable as a single unit operation for the recovery and purification of rosmarinic acid from the aqueous and dried rosemary extract. The main reason is low dissociation equilibrium constant for RA corresponding to its low conductivity. There are also some practical problems, such as, fouling of the membranes by organic compounds present in the rosemary extract, small membrane area of the used lab-scale unit and inappropriate anion-exchange membrane, which was found during experiments. Therefore, additional purification and concentration of rosemary extract should be performed with other separation processes prior to start-up electrodialysis with other membranes.

Acknowledgements

The authors gratefully acknowledge to the VITIVA d.o.o. for the financial support. The work was partially funded by the Croatian Ministry of Science, Education and Sport, contract grant number 0125-021.

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Povzetek

V delu sta prikazani izolacija in koncentriranje rožmarinske kisline iz rožmarina na laboratorijski elektrodializni celici. Za validacijo postopka elektrodialize na primeru rožmarinske kisline je bila uporabljena modelna raztopina rožmarinske kisline. Kljub dejstvu, da je bila elektrodializa modelne raztopine rožmarinske kisline nezadovoljiva v primerjavi z elektrodializo nekaterih drugih organskih kislin, so bili narejeni nadaljnji poizkusi z vodnim in suhim ekstraktom rožmarina. V primeru suhega ekstrakta rožmarina, je dodatek etanola pozitivno vplival na potek elektrodialize. Pri optimalnih procesnih pogojih (dodatek 30% etanola) je bil ionski fluks 5.9 10^{-1} g m^{-2} min^{-1}, poraba specifične energije 3.9 kWh kg^{-1} in dosežen izkoristek 80.1%.