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Extraction and Liquid Membrane Preconcentration of Rosmarinic Acid from Lemon Balm (*Melissa Officinalis L.*)

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Abstract: Liquid membrane separation technique was applied for the recovery and preconcentration of rosmarinic acid from aqueous extract of dried leaves of Balm lemon (*Melissa officinalis L.*). Among several studied organic solvents, diisopropyl ether and ethylacetate appeared to be appropriate membrane liquids for recovery and selective preconcentration of the acid. The difference in pH values between the two aqueous solutions was the driving force in this case. An integrated process coupling the extraction of rosmarinic acid from ground Melissa leaves with a simultaneous stripping of membrane and accumulation of the extracted solute was demonstrated using a laboratory bulk liquid membrane contactor. The process provided an almost complete (96 percent) exhaustion of the herbal mass and highly enriched final extract, containing 87 percent RA after strip solution drying.

Keywords: Liquid membranes, pertraction, lemon balm, rosmarinic acid, integrated process

INTRODUCTION

It is known that the most active dietary antioxidants belong to the family of phenolic and polyphenolic compounds. They are used in the food industry

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to delay the oxidation process (1, 2) Among them, rosmarinic acid deserves special attention due to its thoroughly studied antioxydative effect and availability.

Pure rosmarinic acid or (R)-2-(3,4-dihydroxytrans-cinnamoyloxy)-3-(3,4-dihydroxyphenyl) propionic acid (Fig. 1), represents rosmarinic acid crystals, with melting point 168°C, soluble in methanol, ethanol, and organic ether, esters of the aliphatic carboxylic acids. Its solubility in water is $>15\,g/L$ at 25°C (3).

Rosmarinic acid as a phenolic acid is not stable at temperatures above 50°C (4) and sensitive towards the UV light. As known, on exposure to UV radiation or daylight, the phenolic acids are transformed from *trans* isomers into *cis* isomers (5).

Natural sources of rosmarinic acids are several botanicals of the families Lamiaceae as *Rosmarinus officinalis*, Chicory (*Cichorium intybus*) and Lemon balm (*Melissa officinalis L.*). The latter, usually containing 2–4 percent rosmarinic acid is widely used for treatment of several diseases such as headaches, nervousness, gastro-intestinal disorder, etc (6). The highly antioxydative activity of the aerial parts of this plant or its extracts are attributed, as confirmed in the literature, to the contained phenolic acids, mainly rosmarinic acid (7, 8).

There are various extraction methods for rosmarinic acid recovery from the plant. For this purpose a large number of solvents were tested, including CO_2 in the case of supercritical fluid extraction (SFE) (9, 10). In this study, however, we shall try to apply a liquid membrane (pertraction) technique, looking for appropriate membrane liquids and operational conditions.

Species separation by liquid membranes is an attractive and a promising method for selective recovery of valuable or toxic substances from various liquid sources, usually representing their dilute aqueous solutions (11, 12). The pertraction process combines in time and space extraction of feed solution using organic membrane and stripping of the latter with a second, aqueous solution. The two aqueous phases, the donor (feed) and the acceptor (stripping) solutions, are separated by the liquid "membrane" phase, practically insoluble in both aqueous solutions. If the thermodynamic conditions at the donor/membrane interface favor the solute extraction and at the other interface—membrane/acceptor the conditions favor the

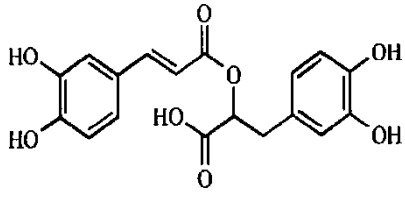

Figure 1. Rosmarinic acid.

stripping process the solute can be transferred and accumulated into the acceptor phase.

The number of published studies on pertraction of natural organic products, derived from plant materials is very limited. For example, Yu et al. (13) and Wang and Li (14) studied the transfer of nicotine, while in our laboratory another alkaloid was a target of recovery and preconcentration from native plant extracts (15).

Recently, special attention is devoted to the integration of two or more processes. Typical examples are the combinations of fermentation processes with operations for simultaneous removal of the reaction products, hindering or blocking fermentation. In this way ethanol, butanol, L-isoleucine, etc. are removed from the fermentation broth by pervaporation or liquid membrane separation (16–18).

Considering the advantages, offered by the process integration schemes, we applied such scheme in the studied case—extraction of rosmarinic acid from dried aerial parts of lemon balm (*Melissa officinalis L.*), with a simultaneous preconcentration of the acid by pertraction.

EXPERIMENTAL

Materials Used and Method of Analysis

Hexane, diisopropyl ether (for synthesis, Merck, Germany), chloroform (p.a., POCh, Gliwice, Poland), n-butanol, diethyl ether, ethylacetat (p.a., Valerus, Bulgaria), 1-octanol (pure, Reachim, Russia) were used as solvents—possible extractants of rosmarinic acid.

Hydrochloric acid (p.a. Marvin, Bulgaria), sodium hydroxide (p.a., Chyema, Poland), K_2HPO_4 (p.a. Fluka, Germany) and KH_2PO_4 (>98%, ALDRICH, Germany) were used for buffer solutions preparation.

Pure rosmarinic acid analytical standard was obtained from Extrasynthese, France.

Plant material—dried aerial parts (leaves) of *Melissa officinalis L.*, grown in the region of Karlovo/Kazanlak, Bulgaria and collected in July 2004, was ground, homogenized, and stored in a dark place.

For analysis of rosmarinic acid, a HPLC system consisting of a pump "Knauer", a variable length wave UV-Detector "Knauer", an integrator C-R6A Chromatopak "Shimadzu" and a reverse phase C18 Nucleosil 100-5 column of 300 mm was used.

The mobile phase used was mixture of methanol (super gradient, Labscan, Ireland) and water (80:20 v/v) with pH = 2.5. Formic acid (for analysis, Ferak Laborat GmbH, Germany) was used to adjust the pH-value of the mobile phase. Its flow rate was 0.4 mL/min and injection volume 10 μL. UV spectra were recorded at 280 nm. All analyses were carried out at temperature of 20°C.

The pH-values of the aqueous solutions were measured with a laboratory pH meter Radelkis OP-211/1.

Equipment Used

In the pertraction studies a simple laboratory "bulk" type pertractor, shown in Fig. 2, was used.

The device consists of an outer glass cylinder (1), 100 mm in diameter, equipped with four baffles (2). A second, internal cylinder (3), 50 mm in diameter rotates inside the external one by means of a pulley (4), mounted on its top and driven by a controlled speed electromotor. An immobile narrow tube (5) equipped with a baffle was located along the contactor axes, playing the role of acceptor phase sampler. There was another sampling capillary tube (6) for the external, feed phase F, occupying the bottom annular space between the two cylinders. The rotating internal cylinder contains the acceptor solution R. The "membrane" liquid M, covering both aqueous solutions, was common for both phase compartments.

Rosmarinic acid partition coefficients were obtained during the equilibrium experiments carried out in laboratory separator funnels of 100 mL.

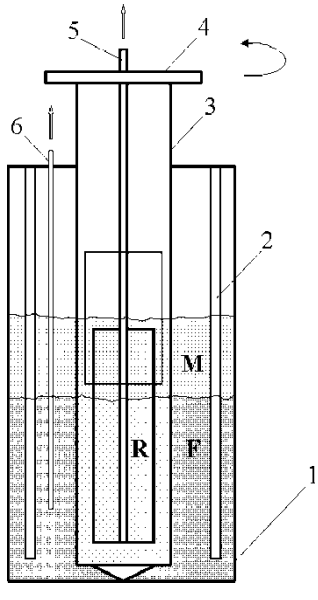

Figure 2. Laboratory glass pertractor: 1. outer vessel. 2. baffles. 3. internal rotating vessel for strip phase with lateral windows. 4. pulley, driven by electric motor. 5. immobile baffled axes sampling tube for the strip solution. 6. sampling capillary for stripping phase.

RESULTS AND DISCUSSION

Equilibrium Study

In order to select an appropriate membrane solution several organic solvents were tested. The equilibrium studies were carried out in the above-mentioned separator funnels. For this purpose a fresh, aqueous plant extract was obtained by percolation with hot (60°C) distilled water during 6 hours. The native extract was cooled to room temperature, filtered, and used as aqueous feed phase in the experiments.

The rosmarinic acid partition between equal volumes (20 mL) of the aqueous feed phase and the organic solvent was determined analyzing both liquids, after 20 min of vigorous shaking and subsequent complete phase separation at 20°C. In some of these preliminary experiments the pH values were modified by phase acidification with hydrochloric acid. The distribution coefficients obtained, as well as the corresponding pH values of the aqueous phase at equilibrium are shown in Table 1.

From the data presented in Table 1, one can conclude that ethylacetate and diisopropyl ether offer better opportunities than the other five tested solvents. Diisopropyl ether provides better conditions for solute extraction during the first transfer step—the RA extraction from the feed into the membrane, but the acid stripping at the second interface (M/R) when the acceptor solution R is neutral or slightly alkaline, is not complete. This is not the case when ethylacetate is used as a membrane liquid: although the

Table 1. Rosmarinic acid partition coefficients

Organic phase	pH_{eq}	Partition coefficient
n-Hexane	5.71	0.71
	1.83	0.009
1-Octanol	5.67	0.008
	1.95	0.47
n-Butanol	5.71	0.9
	2.02	0.14
Diethyl ether	5.71	0.73
	1.85	0.6
Diisopropyl ether	5.71	0.5
	1.95	2.46
Ethylacetate	5.63	0.07
	1.97	0.99
Chloroform	5.71	0.64
	1.80	0.41

RA partition coefficient at the first interface (F/M) is lower, the solute stripping from the membrane is almost complete when $pH_R > 6.5$.

More detailed presentation of the aqueous phase pH-value effect on the equilibrium distribution of rosmarinic acid is shown in Fig. 3. It should be mentioned, however, that carrying out pertraction in pH-regions outside the range $1.5 > pH < 9.0$ could be harmful for the transferred solute as well as for the ethylacetate. The latter will be saponificated in strong alkaline medium. Considering these limitations we chose to use in the pertraction experiments an acidified feed of $pH = 2.0$ and an acceptor phase, buffered at $pH = 7.5$.

The extraction/stripping processes provide a good purification of rosmarinic acid at the above mentioned conditions when ethylacetate is used as a membrane phase. The rosmarinic acid concentration in the solid residue after strip solution drying was found to be about 87 percent, while its content in the dried initial feed solution (the native extract) was less than 8 percent.

Integrated Process Kinetics

The integrated process combines the operations of solvent extraction of lemon balm leafs and pertraction of the extracted rosmarinic acid. It was

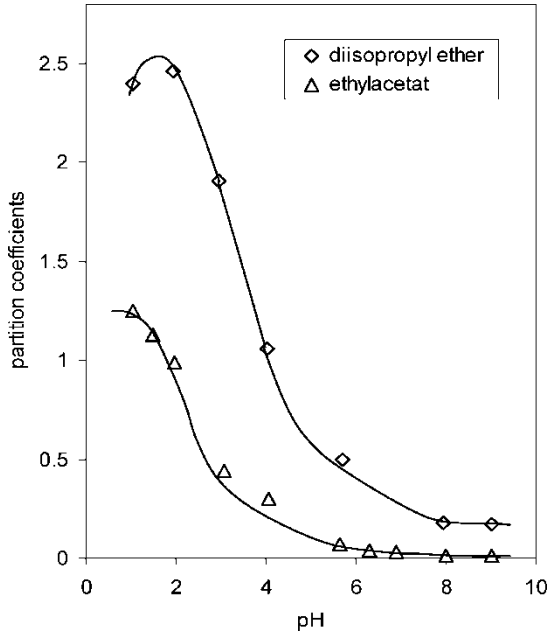

Figure 3. Effect of aqueous phase pH-value on rosmarinic acid partition coefficient at 20°C (◇) diisopropyl ether/water binary system; (△) ethylacetate.

carried out in the laboratory glass pertractor, shown in Fig. 2. For this purpose a suspension of 1.75 g finely ground dry leaves in 350 mL 0.01 M solution of hydrochloric acid in distilled water (pH = 2.0) was prepared *in situ* in the F-phase compartment of the device just before the start of the experimental run. The stripping liquor R in the internal cylinder was 50 mL 0.06 M K_2HPO_4/KH_2PO_4 solution of pH = 7.5. The ethylacetate membrane M, contacting both aqueous phases F and R was 150 mL.

The experiments were carried out during 7 hours at 20°C and at a constant speed of the inner cylinder rotation—160 rpm. This rotation intensity was sufficient to keep the solid phase in a suspended state and at the same time to not disturb drastically the contact interfaces.

In order to reveal the effect of the suspended particle size—a possible rate controlling factor, two size fractions were used $0.4 < d_p < 1.0$ mm and $0.1 < d_p < 0.4$ mm.

Figure 4 shows the evolutions of rosmarinic acid concentrations in the feed, membrane, and stripping phases upon the time. As one can see, the solute concentration increases in the feed solution during the first 90 minutes and then steadily decreases because the solid phase is partially exhausted and the solute continuously transferred into the membrane. After some time lag, the concentration of rosmarinic acid in the stripping phase increases rapidly, while its concentration in the intermediate liquid, the membrane, practically does not change.

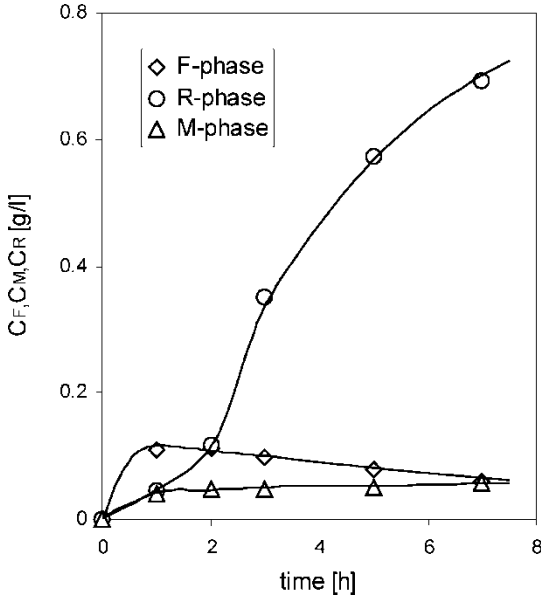

Figure 4. Evolution of rosmarinic acid concentrations in the feed (◇), membrane (△) and stripping (○) phases upon the time.

The reduction of rosmarinic acid content in the solid particles is shown in Fig. 5, where the solute amount in each phase, including the solid phase, is given. The latter, presented as a dotted curve, is calculated on the ground of initial rosmarinic acid content in the plant (G_{solid}at $t = 0$) and the measured current concentrations in all liquid phases. The only point on this curve obtained by direct analysis is the final one, analyzing the dried solid residue after the experimental run is completed.

The data in Fig. 5 show that after 7 hours of continuous stirring only half of the rosmarinic acid is accumulated in the stripping phase, the remained amount is still in the feed and membrane solutions, and only less than 4 percent in the solid particles. Obviously, the rate controlling factors are the relatively low mass transfer coefficients around the two liquid-liquid interfaces and mainly the very low contact areas between the three liquid phases in the laboratory pertractor used. For certain, a larger volume of the receiving phase will increase the amount of the acid in the stripping liquor, but the preconcentration effect will be reduced. Rosmarinic acid was almost completely recovered from the solid phase due to the relatively small size of the particles (fraction 0.4–1.0 mm) and to the continuous removal of the solute from the feed solution.

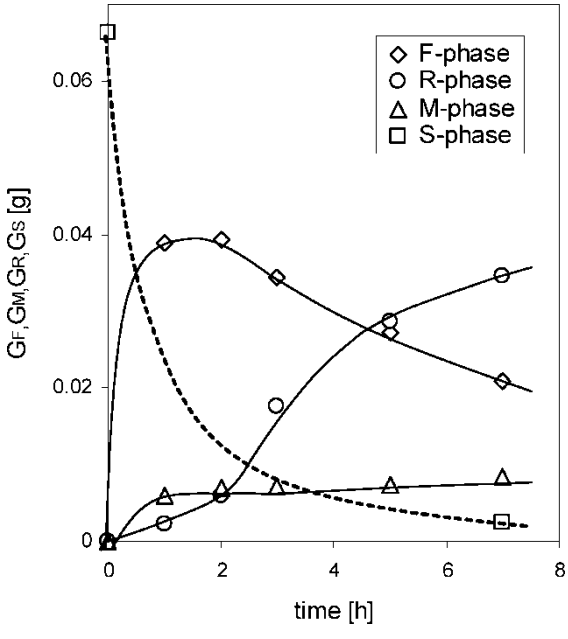

Figure 5. Variation of rosmarinic acid content in all four phases vs. time: (◇) feed phase, (△) membrane, (○) stripping solution and (□) solid phase (calculated) upon the time.

Table 2. Concentrations of rosmarinic acid in the stripping phase for two size fractions of the solid particles

	Concentration of rosmarinic acid in the stripping phase (C_R) in g/L	
t [h]	Fraction 0.1–0.4 mm	Fraction 0.4–1.0 mm
0	0	0
3	0.346	0.352
5	0.552	0.573
7	0.733	0.693

The contribution of the rate of solid-liquid extraction step on the overall rate of the integrated process was elucidated by changing the size of the solid particles. Table 2 illustrates that the solute accumulation in the stripping solution follows practically the same paths for both particle size fractions $d_p = 0.1–0.4$ mm and $d_p = 0.4–1.0$ mm. Obviously, the contribution of the solid-liquid extraction rate on the overall rate of the integrated process is not significant.

CONCLUSIONS

Among the seven organic solvents tested for rosmarinic acid recovery from its aqueous plant extracts, diisopropyl ether and ethylacetate were found to be suitable for liquid membrane (pertraction) preconcentration of rosmarinic acid. It is shown that the product obtained after strip solution evaporation and drying contains 11 times more rosmarinic acid than in the dried residue of the native lemon balm aqueous extract.

An integrated process, combining extraction of rosmarinic acid from ground plant leaves and selective recovery of the acid from the liquid extract by pertraction, was introduced and studied. The solute was extracted from the solid particles by slightly acidified (pH = 2.0) aqueous solution, transferred into the organic membrane and finely stripped from the latter by slightly alkaline acceptor solution. Performing simultaneously these two operations, solid-liquid extraction and separation by pertraction, one can achieve almost complete removal of the valuable solute from the botanical.

It was established that the size of the solid particles, up to 1.0 mm has no significant effect on the overall rate of the integrated process. The latter is controlled by the mass transfer rates across the two consecutive liquid-liquid interfaces.

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