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Solvent extraction of rosmarinic acid from lemon balm and concentration of extracts by nanofiltration: Effect of plant pre-treatment by supercritical carbon dioxide

G. Peev[a,*], P. Penchev[b], D. Peshev[a], G. Angelov[b]

[a] Department of Chemical Engineering, University of Chemical Technology and Metallurgy, 1756 Sofia, Bulgaria
[b] Institute of Chemical Engineering, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

ABSTRACT

The present work investigates a complex scheme of valuable components extraction from herbs and extracts concentration by nanofiltration. Two ethanol–water mixtures (50:50 and 80:20 (v/v)) have been used for solvent extraction of rosmarinic acid (RA) from dry lemon balm aerial parts and high recovery (up to 94%) has been obtained in three steps scheme. A positive impact of a preliminary treatment by supercritical CO_2 (in order to utilize the plant essential oil) on the successive RA extraction has been observed. The cross-linked polyimide membrane Duramem™ 200 has performed RA rejection of over 99% and reasonable flux at dead end nanofiltration pressure of 30 bar. This high rejection being independent of ethanol content in the solvent, supercritical pretreatment and RA concentration of the extracts has allowed to obtain nearly saturated retentate solutions (up to 19 g/L RA) and to reuse the permeates for lemon balm extraction in place of pure solvent. A RA content in the extracts dried total solid mass of 28 ± 2 % has been achieved.

The additional resistance due to osmotic pressure difference and concentration polarization has been determined as a power function of retentate concentration with power value near to unity and approximately similar for the extracts and a model solution of RA.

The study has confirmed the potential benefits of nanofiltration implementation in herbal extracts processing.

Keywords: Lemon balm; Rosmarinic acid; Solvent extraction; Nanofiltration

1. Introduction

Rosmarinic acid (RA) is a natural polyphenol carboxylic acid isolated for the first time by Scarpati and Oriente (1958) from *Rosmarinus officinalis* L. It has a number of interesting biological activities, e.g. antiviral, antibacterial, anti-inflammatory and antioxidant. For this reason it is an important constituent of many medicines, e.g. Neurex™ (Smart), Persen™ (Lek Pharmaceuticals d.d.), nutritional additives, e.g. PAX+™ (Arcopharm), Life Extension™ (Herb soul), or preservatives e.g. Aquarox™ (Vitira). Usually RA is introduced in these preparations as ground dried leaves (aerial parts) of some natural plants, known to have human beneficial and health promoting effects, or powders obtained by evaporation of liquid extracts from these plants. Among them the most significant are the Laminaceae herbs such as lemon balm, rosemary, oregano, sage, thyme and peppermint (Clifford, 1999). Several investigations have revealed that lemon balm is very rich in RA. Its content varies from 0.5 to 6.8% of the dried herb mass depending on the geographical area and time of its collection (Lamaison et al., 1990, 1991; Janicsák et al., 1999; Caniova and Brandsteterova, 2001; Zgórka and Glowniak, 2001; Žiaková et al., 2003; Wang et al., 2004).

Different aspects of RA solvent extraction from dried lemon balm have been object of research. Major attention has been paid to the use of water–ethanol mixtures as commercial solvent due to ethanol safety and low price, as well as their higher capacities than water or ethanol alone. Concentrations of alco-

* *Corresponding author*. Tel.: +359 2 8163293; fax: +359 2 8685488.
E-mail addresses: georgiapeev@gmail.com (G. Peev), petko.penchev@yahoo.fr (P. Penchev), d.peshev@uctm.edu (D. Peshev), gang@bas.bg (G. Angelov).

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Nomenclature

A	membrane surface (m^2)
a	experimentally determined coefficient in Eq. (3)
b	experimentally determined coefficient in Eq. (3)
C_e	concentration of rosmarinic acid in the extract (kg/m^3)
C_f	concentration of rosmarinic acid in the feed (kg/m^3)
$\overline{C}_p$	mean-mixed concentration of rosmarinic acid in the permeate (kg/m^3)
C_{pe}	permeate concentration at the end of nanofiltration (kg/m^3)
C_R	concentration of rosmarinic acid in the retentate (kg/m^3)
C_s	concentration of rosmarinic acid in dried extract residue (mass%)
J_p	flux of permeate (L/m^2 h)
J_s	flux of solvent (L/m^2 h)
k, n	coefficients in Eq. (8)
R_1	rejection of membrane, Eq. (2a)
R_2	rejection of membrane, Eq. (2b)
R_3	rejection of membrane, Eq. (2c)
R_m	resistance of membrane to solvent flow ((bar m^2 h)/L)
R_o	resistance of membrane due to osmotic pressure difference ((bar m^2 h)/L)
R_p	resistance of membrane due to concentration polarization ((bar m^2 h)/L)
t	time of nanofiltration (h)
V_f	feed volume (L)
V_R	retentate volume (L)
V_p	permeate volume (L)
Y	yield of rosmarinic acid, % (kg/kg dried leaves mass) × 100
ΔP	applied pressure difference (bar)

hol from 0 to 96% (v/v) have been investigated, and higher extraction degree of RA (more than 88%), has been observed within the range 30–60% ethanol, while for water it is about 40% and for ethanol—about 20% (Penchev, 2010). A moderate grinding of raw material to an average size fraction of about 1 mm and temperature increase up to 40 °C have been found favourable for the extraction rate (Angelov et al., 2007; Boyadzhiev et al., 2009). Liquid to solid ratio has been varied in wide limits, from 4 to 10 L/kg (Herodež et al., 2003) to large excess of solvent (Angelov et al., 2007). The lower the ratio the higher RA concentration is in the extract. However, as shown by Boyadzhiev et al. (2009), even after a centrifugal separation of phases at 900 rpm, the remaining quantity of liquid in the wet plant material is approximately 1 L per kg dry mass. This requires a number of consecutive treatments with solvent in order to recover RA from the retained extract and to obtain higher RA yield from the plant. For that reason the extracts obtained after the lemon balm treatment with ethanol–water mixtures have relatively low concentration and their traditional thermal processing to dry powder is expensive. For example, the alcohol has to be removed from the liquid extract for economic reason (solvent recuperation and recycling), but also for technological reasons (e.g. process safety during the spray drying operation). Although the distillation is carried out at lower temperature under vacuum, it may cause some changes of RA which is unstable over 50 °C. The same is valid for the drying process. Therefore utilization of alternative methods for RA extract concentration is of great interest.

Boyadzhiev and Dimitrova (2006) have studied the applicability of liquid membrane separation technique to this aim. They have obtained RA content of 0.7 g/L in the stripping phase using as a feed aqueous extract of lemon balm, prepared at 60 °C. In the presence of ethanol in the solvent, however, the choice of an appropriate liquid membrane will be very difficult. The reported degree of concentration is very low as compared to those obtained applying nanofiltration for the same purpose (Vincze and Vatai, 2004; Peshev et al., 2010; Tylkowski et al., 2010). The nanomembrane selection is facilitated by the development of organic solvent resistant membranes, some of them already tested for flux and rejection of RA extracted from rosemary by ethanol (Peshev et al., 2010). This process has additional advantages (operation at ordinary temperature, possibility of permeate reuse for extraction, etc.), discussed in details elsewhere (Tzibranska et al., 2009).

Besides the bioactive constituents of lemon balm, among which RA is considered as the most important, the plant contains essential oil of high quality with application in cosmetics (Carnat et al., 1998; Patora et al., 2003). The possibility for supercritical fluid extraction of oil components from dried lemon balm has been studied, and encouraging results have been reported (Ribeiro et al., 2001; Rozzi et al., 2002; Marongiu et al., 2003, 2004; Diaz-Reinoso et al., 2006). Selective recovery of essential oil, waxes and some other non-polar constituents has been achieved operating at 40–50 °C and moderate pressure below 150 bar. Two separation stages have been used and precipitation of waxes has been accomplished in the first one (Marongiu et al., 2003). A study of the antioxidant activity in the supercritical residue has confirmed that at these mild conditions flavonoids, triterpenoids, and organic acids remain unextracted (Ribeiro et al., 2001). Moreover, the absence of light and oxygen during the supercritical treatment prevents oxidation reactions thus conserving biological properties of the substances remaining in the residue. Based on these results, a complex utilization of lemon balm has been proposed: first step – essential oil extraction with supercritical fluids at moderate pressure followed by a second step – conventional solvent extraction of the solid residue (Ribeiro et al., 2001; Marongiu et al., 2004). This idea has been developed regarding the residue as a source of rosmarinic acid (Penchev, 2010), and the results about the first step of the complex process has been reported (Angelov et al., 2010). In the present study accounting for the recent advance in the organic solvent nanofiltration and the importance of the second step we set the following aims:

- To investigate the RA extraction kinetics from lemon balm previously treated by supercritical carbon dioxide and to compare it to that of non-treated material, using in both cases ethanol–water mixtures;
- To process selected extracts by nanofiltration in order to assess its applicability for production of concentrated solutions, as well as to determine possible effects of the previous supercritical fluid treatment on the nanofiltration process;
- To prove the use of permeates for extraction instead of pure solvent, which is advantageous for process economy.

The realization of these aims would bring important information and contribution to the idea for complex lemon balm

utilization confirming a successful application of organic solvent nanofiltration as third technological step and estimating the impact of supercritical pretreatment. This approach could also be used for processing of other plants.

2. Experimental

2.1. Materials

Dried aerial parts (leaves and stems) of *Melissa officinalis* L., grown in the region of Karlovo/Kazanlak, Bulgaria and collected in July 2007 was used as raw material. It was ground and then classified by sieving. The fraction of size 0.63–1.25 mm was used in all experiments. Ethanol p.a. was supplied by Valerus, Bulgaria, methanol, LiChrosolv grade—from Merck, Germany, formic acid, p.a.—from Ferak Laborat GmbH, Germany, and rosmarinic acid, purum >95%—from Fluka. The Duramem^{TM} modified polyimide membrane, Evonik MET, UK with a molecular weight cut off of 200 Da was used for nanofiltration. It was selected by a membrane screening in a previous investigation (Peshev et al., 2010).

2.2. Analytical methods

Rosmarinic acid concentration in extracts, permeates and retentates was determined by means of high performance liquid chromatography (HPLC). The system consisted of a pump "Knauer", a variable wavelength UV-detector "Knauer", an integrator C-RGA Chromatopak "Shimadzu" and a column Discovery^{R} C18 (25 cm × 4.6 mm, 5 μm) Supelco. Calibration solutions of RA in methanol were prepared in the concentration range 0.002–0.45 g/L. The mobile phase was mixture of methanol and water 80:20 (v/v) with pH fixed at 2.5 using formic acid. Injection volume of 20 μl and flow rate of 0.4 mL/min were utilized. UV spectra were recorded at 280 nm. All analyses were carried out at a constant ambient temperature of 20 ± 0.5 °C in duplicate.

The total solid content in the samples was determined gravimetrically after evaporation of a given volume and drying its residue at 105 °C.

2.3. Extraction

Parts of the dried plant material were treated at various supercritical conditions (pressure, temperature and CO_2 mass flow rate), the procedure and results being described elsewhere (Angelov et al., 2010; Penchev et al., 2010). Also, in some runs ethanol was used as co-solvent. Samples of the raw material and residues after supercritical processing (8 g/run) were subjected to solvent extraction at equal conditions. The process was performed in glass flasks incubated in water bath shaker at moderate mixing (120 pulses/min) and temperature 40 ± 0.1 °C. In all cases the first extraction step was realized at a solvent to dry solid ratio 10 mL per gram. Samples from the liquid phase were taken during the process with a micropipette equipped with a microfilter (0.45 μm). The extraction time of 110 min was chosen according to kinetic data in Fig. 1 showing a saturation plateau after this process duration. Then the phases were separated by ordinary filtration. The wet solid phase was treated twice with the same volume of pure solvent and for the same period of time as in the case of dry sample extraction. The liquid phase of each of these three steps was subjected separately to nanofiltration. The same procedure was applied when permeates were used instead of pure solvent.

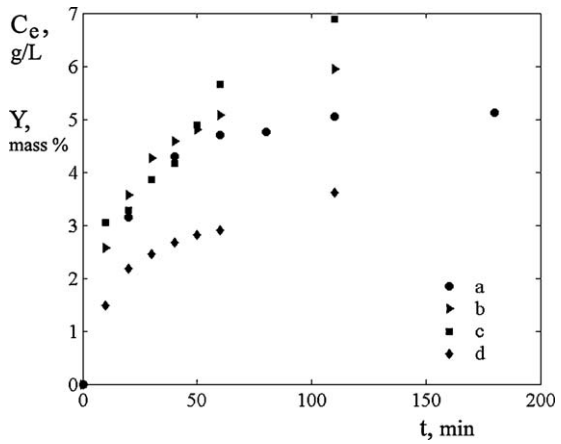

Fig. 1 – Extraction kinetics with 80:20 (v/v) ethanol–water mixture: a—untreated plant material; b–d—with pretreatment; b—CO_2 flow rate Q_1 = 5 g/min, P = 90 bar, T = 50 °C; c—Q_1 = 30 g/min, P = 280 bar, T = 60 °C; d—Q_1 = 5 g/min and ethanol with $Q_2 = 0.15Q_1$, P = 280 bar, T = 60 °C.

Two ethanol–water mixtures were tested: 50:50 (v/v) selected from the interval, wherein an existence of optimum yield of RA was reported (Wang et al., 2004; Angelov et al., 2007), and 80:20 (v/v). The latter was chosen on the base of two reasons: recommendation of membrane manufacturer to avoid operation at high water contents and information about increasing of the total solid content of the extracts at higher water concentration (Boyadzhiev et al., 2009).

2.4. Nanofiltration

Dead end nanofiltrations were performed utilizing an equipment (a 270 mL stirred cell and pressure regulator) supplied by Evonik Membrane Extraction Technology, UK. The cell effective membrane area was 54 cm^2 and stirrer revolutions were kept constant at 350 rpm, in order to minimize concentration polarization (MET, 2008). The runs were carried out at a constant ambient temperature of 21 ± 0.5 °C and pressure of 30 bar, maintained by high purity nitrogen (99.996%), supplied from a cylinder. The operational pressure was chosen on the base of membrane screening experiments (Peshev et al., 2010), confirming the conservation of satisfactory flux with ethanolic extracts from rosemary at high RA rejection. The membrane conditioning was realized by initial filtration of 300 mL 80 vol.% ethanol at 30 bar, as to avoid a compression effect in the later stage of experiments and to remove the oil used to protect the active membrane layer. The volume of the extracts processed by nanofiltration was usually 45 mL. Permeates were continuously collected in a cylinder and times for accumulation of given volumes were measured. At the end of the process a small sample was taken from the out flowing liquid and analyzed for RA content. The average concentrations in the permeates and retentates were also determined. The feed to final retentate volume ratio was almost equal to 3. Details for the nanofiltration set-up can be found elsewhere (MET, 2008; Tylkowski et al., 2010). Firstly, a model solution of 4.6 g/L RA in 80 vol.% ethanol was processed in order to confirm the Duramem^{TM} 200 high rejection, reported in a previous investigation (Peshev et al., 2010). Afterwards, extracts obtained with the two tested solvents were subjected to nanofiltration using

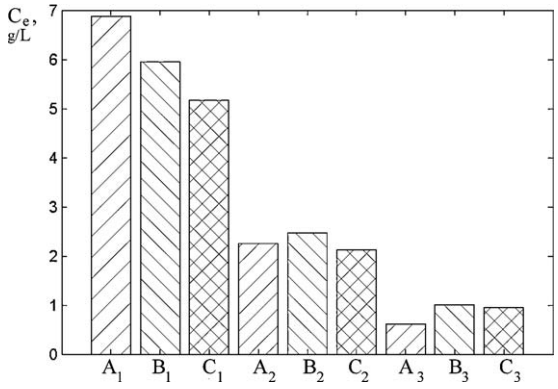

Fig. 2 – Rosmarinic acid concentration in the first (1), second (2) and third (3) extracts: A with 50:50, B and C with 80:20 ethanol to water solvent; A and C—untreated plant material, B—plant material after supercritical pretreatment.

the same membrane. In the intervals between two successive series of nanofiltration the membrane was kept submerged in the solvent used to produce the next extract.

3. Formularization, results and discussion

3.1. *Solvent extraction*

The kinetics of solvent extraction process without and with supercritical extraction pretreatment is illustrated in Fig. 1. The RA concentration of the extract C_e in g/L, which at the chosen solvent to solid ratio is equal to the RA yield in (g RA/g herb) × 100 increases with the time in a manner typical for this process. A rapid yield increase in the beginning and slow solute recovery at the end have been already observed (Herodež et al., 2003; Angelov et al., 2007; Boyadzhiev et al., 2009) when treating dried lemon balm with ethanol and ethanol/water mixtures. Our data could be used for determination of the internal diffusion coefficient following the procedures applied in (Herodež et al., 2003), but this will be skipped here because of the variation of this parameter depending on the place and time of the plant collection.

The statistical analysis of data displayed in Fig. 1 reveals that the maximum and average absolute errors are 0.2 and 0.07 g/L, respectively. For relative errors these values are 4 and 1.5%.

According to Fig. 1, the process duration can be fixed at 110 min. which is in agreement with that selected by Herodež et al. (2003), Angelov et al. (2007) and Boyadzhiev et al. (2009). The solvent with ethanol to water ratio 50:50 (v/v) shows some higher extraction capacity than that obtained with 80:20 (see Fig. 2) confirming the information reported by Wang et al. (2004) and Angelov et al. (2007).

The herb pretreatment using supercritical CO_2 with 15% ethanol as co-solvent decreases considerably the RA yield (Fig. 1—compare points d to a, b and c). The most likely explanation of this effect is an increased RA removal during the pretreatment due to the higher polarity of the added co-solvent (Penchev, 2010). This result rejects such pretreatment as obviously inconvenient regarding the proposed two step processing of lemon balm (Ribeiro et al., 2001; Marongiu et al., 2004), but it suggests to use ethanol as a solvent modifier if supercritical extraction of RA will be performed as a second step. Stepwise increase of extraction pressure and modifier concentration has been used to recover separately the essential oil and more hydrophilic substances from various herbs (Diaz-Reinoso et al., 2006). Implementation of extraction solely with CO_2 seems to help the successive separation of RA from the herb with the ethanol–water mixture (Fig. 1—compare points b and c to a). The concentration of the extracts increases from 5.17 (a—untreated) to 5.94 (b) and 6.9 g/L (c) after the respective pretreatment. This effect can be attributed to the increased concentration of RA and modified solvent capacity after elimination of some compounds with the supercritical solvent. Indeed, about 5–10% reduction of herb mass after CO_2 processing has been reported (Penchev et al., 2010). So, by unloading some "ballast" compounds (waxes, lipids, essential oil, chlorophyll, etc.) the supercritical pretreatment changes the structure of the plant matrix and makes the internal cells more accessible to the solvent (facilitated solvent penetration to internal cells). The data shown in Fig. 1 suggest that RA remains unextracted at harsher conditions than those proposed in Ribeiro et al. (2001), Marongiu et al. (2003), Marongiu et al. (2004), and Diaz-Reinoso et al. (2006). The concentration of 6.9 g/L, however, has been obtained at operational conditions unfavorable for the process economy (higher energy and solvent consumption—pressure 280 bar, temperature 60 °C, mass flow rate of CO_2 30 g/min), while the value 5.94 g/L—at moderate conditions (90 bar, 50 °C, 5 g/min).

Large amount of extract was retained in the herb after a gravitational filtration (about 40% of solvent initial mass). Two successive treatments of the wet herb with pure solvent were realized for a better RA recovery. The extracts concentrations at the end of each step are displayed in Fig. 2. The analysis of data from this figure based on RA material balance reveals that washing has a dominating effect in the second and third treatment. Some additional RA extraction takes place, but it is more pronounced with 80 vol.% ethanol partially compensating for the lower degree of extraction with this solvent in the first step. Finally, the total yield of RA from the dry plant material reaches approximately the same value with both solvents. At this circumstance the use of 80% ethanol has some advantages over 50% ethanol:

- The higher RA solubility in the first solvent will allow a higher extracts concentration by nanofiltration;
- The lower total solid content at lower water concentration will result in a higher RA content in the product obtained after the extract drying;
- Better membrane durability will be achieved according to the recommendations of the manufacturer (Evonik MET, UK).

The calculation of optimum extraction steps is a complicated problem needing additional information not accessible to the authors. However, the analysis of this problem follows to the conclusion that at equal conditions, the optimum number of extraction steps will be higher when using permeates instead of pure solvent, because the costs for solvent regeneration will be lower. Respectively, the total yield of extracted bioactive substances will be higher. In our study, the introduction of fourth step will add less than 6% to the yield of the three cases considered in Fig. 2 and seems useless.

3.2. *Nanofiltration*

3.2.1. *Material balance of rosmarinic acid*

The material balance was used as a measure of accuracy of each experiment. The error was calculated by the ratio of the

difference between the two sides of Eq. (1) and its left part

$$V_f C_f = V_R C_R + V_p \bar{C}_p. \quad (1)$$

It was found to be 3% in average (near to that of the extraction experiments in Fig. 1), right-hand part being always smaller due to some volume losses in the membrane, outgoing tube and on the cell walls (see Table 1).

3.2.2. *Rejection calculation and degree of extract concentration*

The membrane rejection of RA was calculated in three different ways:

$$R_1 = 1 - \frac{\bar{C}_p}{C_f} \quad (a); \qquad R_2 = 1 - \frac{C_{pe}}{C_R} \quad (b);$$

$$R_3 = \frac{lg(C_R/C_f)}{lg(V_f/V_R)} \quad (c); \quad (2)$$

The results obtained from the expressions (2a) and (2b) were in very good agreement, R_1 being smaller than $R_2 (R_2 - R_1 \leq 0.01)$. In the further calculations the arithmetic mean value of R_1 and R_2 was admitted. In this way all four analyses of RA concentration were accounted for. In most cases the results for R_3 were smaller than $R_1 (R_1 - R_3 \leq 0.02)$, the difference being dependent on the material balance error.

All arithmetic mean values obtained were over 99%, confirming the excellent selectivity of the membrane Duramem^{TM} 200. At this high rejection, retentates concentrations of RA were up to 3 times higher than those in the respective extracts and reached values in the range 15–19 g/L for the first extracts from the plant. These values were close to RA solubility (15 g/L in water and 25 g/L in ethanol at 25 °C; Scarpati and Oriente, 1958). Some of the most encouraging results are given in Table 1. No remarkable influence of the feed water content or RA concentration and plant supercritical CO_2 pretreatment upon the rejection is observed. This allows the extracts from the second and third steps to be nanofiltrated up to the retentate RA content obtained from the first extract. Retentates with these concentrations could be directly used as preservatives, antioxidant food supplements, medicines against herpes etc., or processed cheaper to powder.

3.2.3. *Flux determination and modelling*

Some representative data about the permeate cumulative volume against the time for its collection are shown in Fig. 3. All curves indicate a tendency for continuous decrease of the current flux with the time, which can be modelled by a second order polynomial

$$\frac{V_p}{A} = at + bt^2 \quad (3)$$

Eq. (3) with values of a and b identified by the least square method fits the curves with a correlation coefficient varying from 0.997 to 0.999.

The flux at a given time can be calculated from the expression obtained after differentiation

$$J_p = \frac{dV_p}{A\,dt} = a + 2bt \quad (4)$$

Some illustrative results for the flux J_p in the course of time are shown in Fig. 4. As it can be seen in Fig. 4a, the flux for

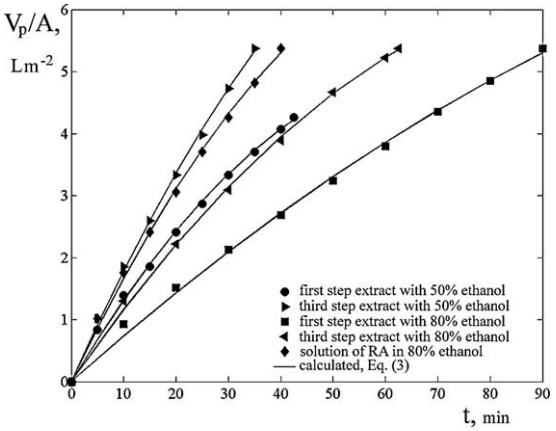

Fig. 3 – Cumulative permeate volume per unit membrane surface versus the time of its collection: points—experimental data, curves—calculation via Eq. (3).

50% ethanol extract increases from the first to third extraction step due to the decreasing RA concentration. The same trend is observed with 80 vol.% ethanol extracts of pretreated herb (Fig. 4b). In both cases the corresponding lines are nearly parallel. Comparing the fluxes of the model RA solution in 80% ethanol and the first plant extract with the same solvent it becomes evident that the other constituents extracted from the lemon balm slow down the flux. Perhaps this is the reason for some different behavior of 80% ethanol extracts (Fig. 4b). In this case some additional extraction of such constituents takes place during the consecutive steps, provoking a steeper descent of higher step lines. At approximately equal RA concentrations the fluxes with the solvent containing more water are significantly higher. Herb pretreatment results in somewhat lower flux.

3.2.4. *Process resistance*

The influence of RA extracts concentration on the process resistance due to osmotic pressure and concentration polarization was evaluated in the following manner.

As far as membrane fouling and cake formation was not observed, the permeate and solvent fluxes were expressed by Eqs. (5) and (6), respectively

$$J_p = \frac{\Delta P}{R_m + R_o + R_p} \quad (5)$$

$$J_s = \frac{\Delta P}{R_m} \quad (6)$$

The additional resistance due to current osmotic pressure difference in the membrane, R_o, and concentration polarization in the retentate, R_p, was found by combining Eqs. (5) and (6)

$$R_o + R_p = \Delta P \left(\frac{1}{J_p} - \frac{1}{J_s} \right) \quad (7)$$

As far as the permeate concentration was very small due to the high membrane rejection, R_o at each moment should be proportional to the current retentate concentration, found from Eq. (2c) using data for the collected permeate volume at this moment. The current permeate flux, J_p, was calculated through Eq. (4) and the solvent flux, J_s, was measured at the end of membrane conditioning. With this information the resistance sum $(R_o + R_p)$ was determined from Eq. (7). In Fig. 5 it

Table 1 – Illustrative data for extraction and nanofiltration of rosmarinic acid.

Extract of step no.	Solvent (ethanol/water, v/v)	C_f (g/L)	V_f/V_R	C_R (g/L)	$\overline{C}_p$ (g/L)	C_{pe} (g/L)	$(R_1+R_2)/2$	Mass balance error (%)
1	80:20	5.17	3	15.39	0.025	0.030	0.997	−0.4
1	50:50	6.68	3	19.58	0.080	0.094	0.992	−1.5
1[a]	80:20	5.94	2.7	15.55	0.024	0.029	0.997	−1.6
2[a]	80:20	2.47	2.9	6.96	0.012	0.013	0.996	−2.7
Solution of RA	80:20	4.60	3	13.6	0.074	0.077	0.99	−0.5

[a] Pretreatment with supercritical CO_2.

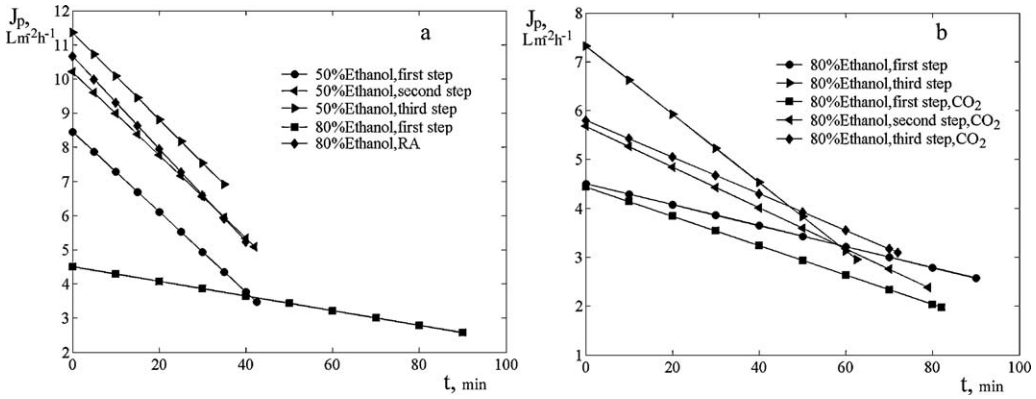

Fig. 4 – Results for the current permeate flux versus time during the filtration runs.

is plotted against the current retentate concentration in case of 80% ethanol solvent ($J_s = 19$ L/m^2 h). As seen, straight lines are obtained in double logarithmic scale, suggesting a relation of the form

$$R_o + R_p = kC_R^n \quad (8)$$

with n being the line slope.

The values of k and n found by least square method are given in Table 2. It is seen that n values obtained for the extracts and RA solution are approximately equal and in average near to 1, which corresponds to the prediction of Van't Hoff equation for the osmotic pressure, valid at low solute concentrations (Wankat, 1990). Different values of the intercept (represented by k) are obtained, and it is probably due to the different total solid content. In our agitated system (small liquid volume, high revolution) the concentration polarization should be kept at low level, so $R_o \gg R_p$, and the additional resistance is mainly due to the osmotic pressure. When the mixing is stopped (after the second reading of permeate volume), the sum $(R_o + R_p)$ begins to increase quicker because of increased concentration polarization (see the solid triangles in Fig. 5). It might be concluded that the liquid in the cell is well homogenized at 350 rpm of the stirrer, so the n values obtained are not surprising. The slope of lines in Fig. 5 increases from the first to third extract of the raw material. For the pretreated samples the slope is nearer to that of the modelling solution (Table 2). Perhaps the pretreatment reduces the content of substances which are the reason for lower n value.

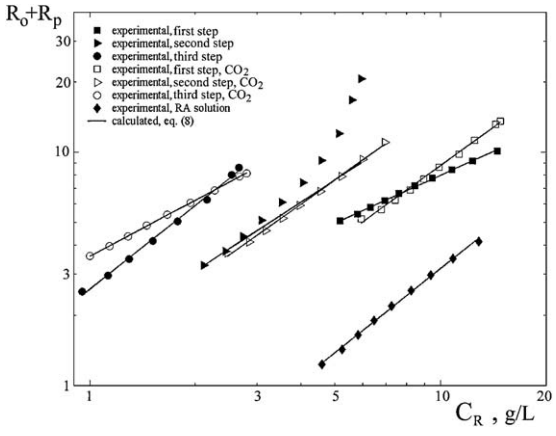

Fig. 5 – Additional resistance due to osmotic pressure difference and concentration polarization against the current retentate concentration: solvent—80 vol.% ethanol, empty points—plant pretreated with supercritical CO_2, diamonds—model solution of rosmarinic acid.

Equal membrane rejections for RA modelling solution and extracts from Rosemary with the same solvent have already been reported (Peshev et al., 2010). This study, however, reveals an additional advantage of applying modelling solutions—a possibility to predict approximately the membrane flux at different concentrations of the key component in the retentate after several initial measurements by drawing the extract line by means of the slope found from the line of the modelling solution. In order to generalize this observation it is interesting to investigate the behavior of other systems (membrane, plant, solvent, and substance).

3.2.5. *Use of permeates for extraction*

The high rejections obtained predetermined a successful use of permeates in place of pure solvent for RA extraction from lemon balm. For experimental confirmation of this statement three-step extraction–nanofiltration scheme was used. Each permeate produced after nanofiltration of the extract from a given extraction step was used as solvent in the same step of dry herb mass treatment.

The results are illustrated in Fig. 6. As seen, RA content in the extracts obtained with permeates are even slightly higher than these with pure solvent. This could be explained with the initial presence of some RA in the permeates, increase of their

Table 2 – Experimentally determined coefficients of Eq. (8).

No.	Characteristic of nanofiltration feed (extracts with 80% ethanol)	Range of retentate concentration, C_R (g/L)	Slope of the line, n	Line intercept, k	Coefficient of linear correlation, R
1	Raw material, first extraction	5.17–14.50	0.68	1.65	0.998
2	Raw material, second extraction	2.12–2.74	0.98	1.56	–
3	Raw material, third extraction	0.95–2.67	1.19	2.58	0.997
4	Pretreated material, first extraction	5.94–14.80	1.09	0.71	0.997
5	Pretreated material, second extraction	2.47–6.95	1.07	1.35	0.998
6	Pretreated material, third extraction	1.00–2.80	0.80	3.58	0.999
7	Modelling solution of RA in 80% ethanol	4.60–12.86	1.20	0.20	0.998

water content due to partial evaporation during the processing, casual inaccuracy related to variation of RA content in the plant, analytical inaccuracy, etc.

The total solid content (TSC) of the extracts and the concentration of rosmarinic acid in it are shown in Fig. 7. As expected, TSC decreases from the first to third extraction step, but RA content remains approximately constant -28 ± 2% (w/w) of the extracted solid phase. This is another indication that the second and third treatments of lemon balm have mainly washing functions and no selective extraction of RA takes place. The TSC of the first extract corresponds to 20.5% (w/w) of the initial herb mass. In Boyadzhiev et al. (2009) at some lower solvent to solid ratio (7.29 L/kg) 24% have been extracted with 15 vol.% ethanol.

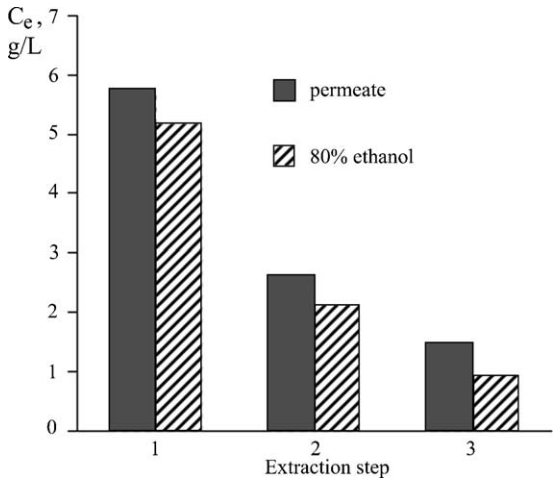

Fig. 6 – Rosmarinic acid concentration in the extracts obtained with pure solvent and permeates, 1–3—extraction steps.

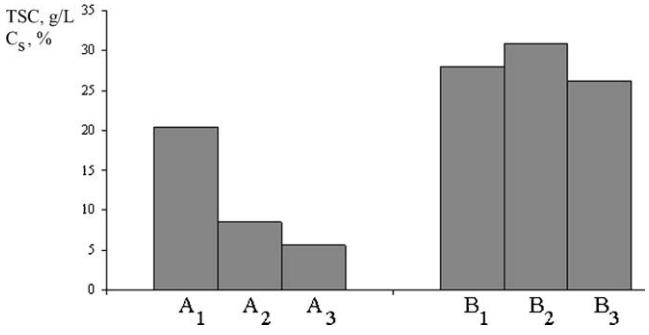

Fig. 7 – Total solid content (A) of the extracts and concentration of rosmarinic acid in the dry extract (B), utilizing permeates as solvent, 1–3—extraction steps.

The confirmed use of permeates instead of pure solvent in all extraction steps may have an important impact on the process economy decreasing significantly the cost of solvent regeneration.

4. Concluding remarks

The present investigation has confirmed the possibility to rationalize the production of rosmarinic acid by extraction with ethanol–water mixtures either from lemon balm or from its residue after an initial supercritical extraction of essential oil from the plant, using organic solvent nanofiltration as an advanced alternative of thermal evaporation.

Both mixtures used in this study can be applied for RA extraction from dried lemon balm, each one having some advantages over the other. The solvent with ethanol–water ratio 50:50 (v/v) offers higher extraction capacity and nanofiltration flux. That with 80:20 (v/v) allows higher RA concentration in the retentates and in the final dried product. Three steps extraction scheme is sufficient for very high RA recovery from the herb (over 94%).

A pretreatment with supercritical CO_2 has a positive impact on the successive extraction increasing RA concentration of the plant material due to elimination of ballast components and allowing better solvent access to internal plant cells because of possible modification of the plant matrix. It seems that ethanol as co-solvent enhances considerably the direct extraction of RA from lemon balm with supercritical carbon dioxide.

High rejection (over 99%) is obtained for RA extracted with both examined solvents and it is found independent of herb pretreatment and RA concentration of the retentates. This result allows to use the permeates of extracts nanofiltration instead of pure solvent in all steps of the extraction scheme with positive impact on the process economy. No membrane fouling has been observed and satisfying flux has been realized at pressure of 30 bar up to retentate concentration near to RA solubility.

The nanofiltration resistance due to the solute osmotic pressure and concentration polarization is found to be a power function of retentate concentration. The power value is approximately equal for the extracts and for a model solution of rosmarinic acid, which may extend the importance of the modelling experimentation.

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