

Solid Liquid Extraction of Phenolic and Flavonoid Compounds from *Cotinus coggygia* and Concentration by Nanofiltration

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Extraction kinetics of polyphenols and flavonoids from plant material and their separation and concentration by nanofiltration were investigated. The kinetics experiments were carried out with *Cotinus coggygia*. The influence of the extraction solvent on the extraction rate was defined. Four different extraction solvents or mixtures were applied – methanol, azeotropic ethanol, 50/50 ethanol/water mixture, and pure water. The optimum extraction rate of flavonoids and polyphenols was achieved by using 50 % ethanol as a solvent for extraction. This solvent mixture was used for generating extracts for the nanofiltration experiments. Organic solvent nanofiltration membranes from DuraMem™ series with different pore sizes (200, 300 500 and 900 Da) were tested in dead-end and cross-flow filtration systems. Flux and rejection data were obtained for every membrane type. Very good separation and respectively concentration of the extracted useful compounds was achieved (rejection for polyphenols and flavonoids is 91 and 93 %, respectively). During a feed/extract concentration experiment, the constant polyphenols and flavonoids rejection was measured. The results suggest that the nanofiltration technology could be combined successfully with solid-liquid extraction for natural sourced valuable compounds enrichment.

Key words:

solid-liquid extraction, kinetics, *Cotinus coggygia*, nanofiltration

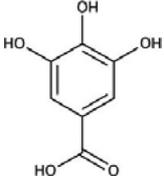
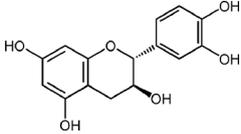
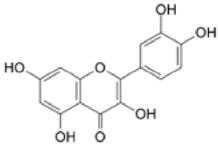
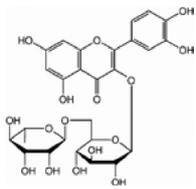
Introduction

Cotinus coggygia is a deciduous shrub of the family *Anacardiaceae*, widespread in Southern Europe, the Balkans, and Southwestern and Central Asia. Extracts from the leaves, twigs, wood, and inflorescences of the plant are used in the ethnomedicine of Eastern and Southeastern Europe and China as antidiarrhoeic, anti-inflammatory and anti-paradentosis^{1–3}. It has been proven to contain a high amount of polyphenols, which relates to its medical use⁴. A few of the common compounds are listed in Table 1.

By extract preparation, the useful components (polyphenols) from plant raw material are transferred into a liquid phase (solvent) and this process is limited by the transfer inside the pores of the solid phase (plant material)^{5–9}. Each experimental extraction kinetics curve includes, in a hidden way, all factors influencing the diffusion process velocity, such as: polydispersion, anisotropy, solid particles form, characteristic change of concentration in the liquid phase.

The extracting solvent, the ratio between the solvent and the solid plant material (liquid-solid ra-

Table 1 – Common polyphenolic compounds in *Cotinus coggygia*

Name	Chemical structure	Molecular weight, g mol ⁻¹
Gallic acid		170.1
Catechin		290.3
Quercetin		302.5
Rutin		610.5

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tio – ζ), and the process temperature are the most important parameters for obtaining optimum extraction conditions. A quantitative measure for this is the valuable compounds extraction yield from the plant material.

Once obtained, the extract needs to be further treated in order to maintain and ideally improve its medical properties before use, e.g. boost the concentration of valuable compounds by reducing solvent volume. The conventional evaporation techniques are not always applicable due to thermo-sensitivity of the most natural compounds. The organic solvent nanofiltration is a relatively new alternative in this field and its capability was studied in this work.

Nanofiltration itself is a membrane-based and pressure-driven process used for separation of molecules or other complex species with a molecular weight between 200 Da up to 1000 Da^{10–13}. The technique has found a variety of applications in recent years, including solvent exchange, catalyst recovery and recycling, purification and concentration. However, the application of nanofiltration in many areas has been limited due to the instability of membranes in many common solvents used in pharmaceutical, chemical and natural product industries. More recently, novel cross-linked OSN membranes have been developed^{14–17}. They show remarkable stability in organic solvents and thus have enabled the use of nanofiltration for the separation and purification of natural product extracts as well, since many extraction processes involve organic solvents.

The aim of this work is twofold – 1) to test the ability of OSN membranes to retain/separate valuable compounds in plant extracts in order to obtain a more concentrated/refined product without heat application, and 2) to determine experimentally the optimum extraction solvent or solvent mixture (by other fixed process conditions) for maximum extraction yield from solid plant material.

Experimental

Solid-liquid extraction from plant material

Plant material

Leaves from *Cotinus coggygria* were used as a solid phase in the kinetics experiments. The plant was harvested in Bulgaria, its leaves were dried and grounded.

Extraction design

The kinetics experiments were performed in a stirred vessel. The ground raw material was put in a reactor and poured on with solvent (water, ethanol, methanol or mixture of the two). Process tempera-

ture was kept constant and the mixture was stirred continuously. To ensure limiting internal diffusion, the angular velocity of the mixer was controlled. After a certain time of extraction, samples from the liquid were taken, filtrated through plaited filter paper (to ensure removal of any residual solids) and prepared for analysis.

Experimental conditions

The kinetics study was carried out by batch extraction from *Cotinus coggygria* in a stirred vessel. Several experiments for the investigated system were carried out in order to obtain the optimum conditions for extraction, and only part of them are presented in this work. Since stirrer speed of above 320 min⁻¹ eliminates external mass transfer resistance, the velocity of the stirrer was maintained at a minimum of $n = 320$ min⁻¹. The experiments were performed at $T = 60$ °C, using different solvents or solvent mixtures (water, ethanol, methanol, ethanol-water mixture) – (see Table 2). The concentration of valuable compounds in the liquid phase (C_1) during the extraction was measured. Each point of the kinetics curve was established, based on the average value of three independent experiments. Each extraction experiment lasted 2 hours in order to reach equilibrium state of the process.

Table 2 – Extraction process conditions

	Solid/liquid ratio (ζ), m ³ kg ⁻¹	Solvent	Process temperature, °C
Kinetics study 1	0.03	Water	60
Kinetics study 2	0.03	50 % EthOH/ 50 % Water	60
Kinetics study 3	0.03	Azeotropic Ethanol	60
Kinetics study 4	0.03	Methanol	60

Analytical methods

A small amount of the liquid phase was taken periodically during the extraction process. The extracts were filtered through filter paper (Whatmann, Germany, Grade 1) prior to analysis to ensure full solid phase separation. Samples were analysed by HPLC and UV-absorption:

Single compounds determination by HPLC

It was previously reported that gallic acid, rutin and quercetin are present in *Cotinus coggygria*¹⁸. An HPLC method for simultaneous determination of those compounds was implemented using Agilent 1100 HPLC system with DAD detector. The separation was performed on a reverse-phase C18

column^{19–21} (Zorbax SB-C18, 100 x 3.0 mm i.d, 3.5 μm particles size), oven temperature 48 $^{\circ}\text{C}$, detection wavelength 330 nm. A binary gradient from methanol (solvent A) and buffer solution (solvent B) were used as a mobile phase. The buffer solution contained potassium dihydrogen phosphate (40 mmol L^{-1}) in water with pH 2.3 (adjusted with orthophosphoric acid). The gradient started with 5 % solvent A to 42 % solvent A over the first 35 minutes, followed by isocratic elution with 42 % solvent A for 3 minutes; flow rate 1 mL min^{-1} ; injection volume 10 μL ²².

Analytical standards of gallic acid, rutin and quercetin were purchased from Sigma Aldrich, Germany. Ethanol mixtures of those compounds were used as stock solution for calibration. The obtained calibration curves are presented in Fig. 1.

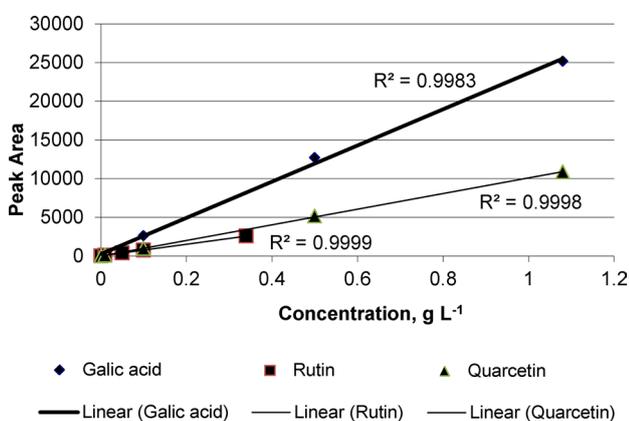


Fig. 1 – Calibration curves for Gallic acid, Rutin and Quercetin using Agilent 1100 HPLC system

Total phenols and flavonoids analysis

Extraction samples were analysed for total phenols and flavonoids content by Folin-Ciocalteu and aluminium chloride colorimetric assays, respectively²³. Analytical standards of gallic acid and catechin dissolved in ethanol were used for obtaining the calibration curves (Fig. 2). The light absorption was measured using the DAD of Agilent 1100 HPLC system and therefore is represented by peak area of the read signal. When the concentration of analysed samples was higher than the maximum measured at calibration, they were diluted accordingly. This way, the measured concentration was always within the calibration limits.

OSN membranes and equipment

Used were organic solvent stable membranes DuraMemTM 200 (DM 200), DuraMemTM 300 (DM 300), DuraMemTM 500 (DM 500) and DuraMemTM 900 (DM 900) (product of Evonik MET, UK). They

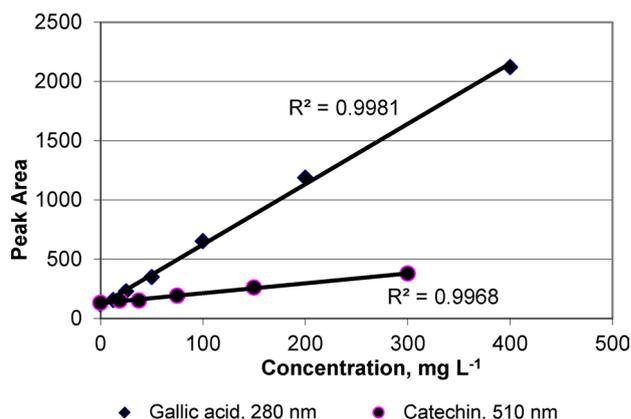


Fig. 2 – Calibration curves for Gallic acid and Catechin following the Folin-Ciocalteu and aluminium chloride colorimetric assays, respectively

are nanofiltration polymeric membranes with molecular weight cut-off 200 Da, 300 Da, 500 Da and 900 Da, respectively.

Feed concentration experiments were performed in METcell dead-end filtration equipment (from Evonik MET, UK). The METcell is a stainless steel, high-pressure, stirred cell capable of performing a wide range of membrane separations. It uses membrane sheets with an area of 51 cm^2 , placed at the bottom of the cell. The pressure for driving the liquid through the membrane was obtained by high-pressure nitrogen gas from a gas cylinder. The equipment was provided with a magnetic stirrer rotating close to the membrane surface in order to minimize the concentration polarization effect. A laboratory magnetic stirrer plate was used to generate the stirring/mixing required. A general schematic of the setup of the METcell system is given in Fig. 3.

Initial membranes screening was performed in order to investigate the ability of different membranes to retain and/or separate the valuable compounds in *Cotinus coggygria* extract. METcell cross-flow system (equipment from Evonik MET, UK) was used for this test. The filtration apparatus consisted of an 800 mL feed vessel and a pumped recirculation loop through four or more cross-flow cells connected in series. The cross-flow system is shown schematically in Fig. 4. Mixing in the cross-flow cells was provided by flow from the gear pump – the flow was introduced tangentially to the membrane surface at the outer diameter of the membrane disk and followed a spiral flow pattern to a discharge point at the center of the filtration cell/disk. The nanofiltration membrane disks were conditioned with pure solvent at operating pressure and temperature for at least 30 minutes, to ensure washing out of any preservatives/conditioning agents from the membrane, and achievement of maximum compaction of the membrane at operating pressure.

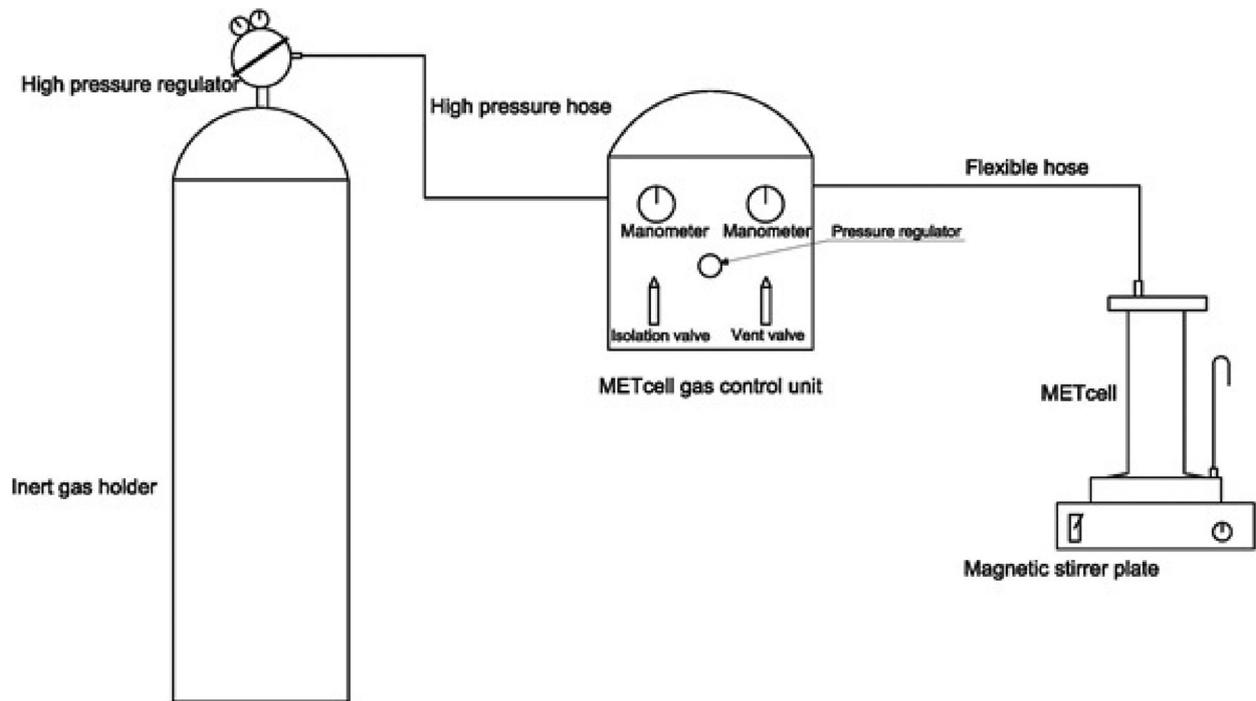


Fig. 3 – Schematic of METcell dead-end filtration system

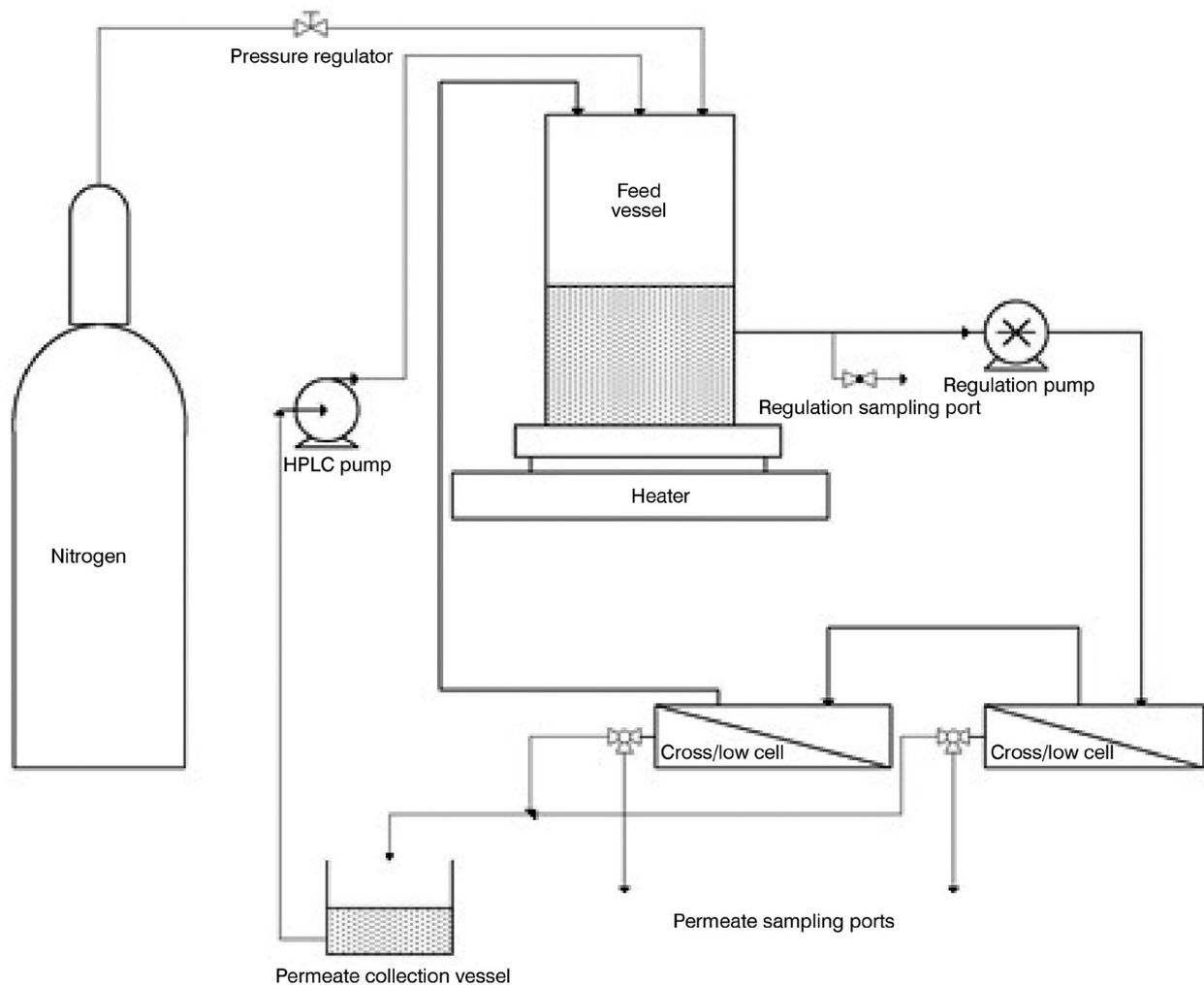


Fig. 4 – Schematic of METcell cross-flow filtration system

The test mixture/feed solution (extract) was then permeated across each conditioned membrane disk at the desired operating temperature and pressure. Samples of permeate and retentate solutions were collected for analysis after 4h of filtration.

Results and discussion

Extract composition – analytical issues

Broad literature research on phenolic compounds present in *Cotinus coggygria* and their analysis was done. However, no single compound (gallic acid, rutin or quercetin) using the implemented HPLC method could be determined. Due to the very good calibration of the analytical apparatus to analytical standards of those compounds, equipment or analytical method incapability was eliminated as the cause for this. Rather, the observed phenomenon was related to the single compounds concentration, i.e. the concentration of those compounds in the extract was not high enough to be detected on the HPLC.

Total phenols and total flavonoids analysis performed on extracts in this work are aligned with previously reported values in the literature, and therefore were used for extraction kinetics description and nanofiltration evaluation.

All kinetics experiment were carried out at 60 °C and liquid-solid ratio $\zeta = 0.03 \text{ m}^3 \text{ kg}^{-1}$, maintaining similar stirring (mixer rotation around 320 min^{-1}) for all studies.

Kinetics curves and optimum extraction conditions

From Fig. 5, it is clear that the highest equilibrium concentration is achieved using 96 % ethanol as extraction solvent. The results for 50 % ethanol

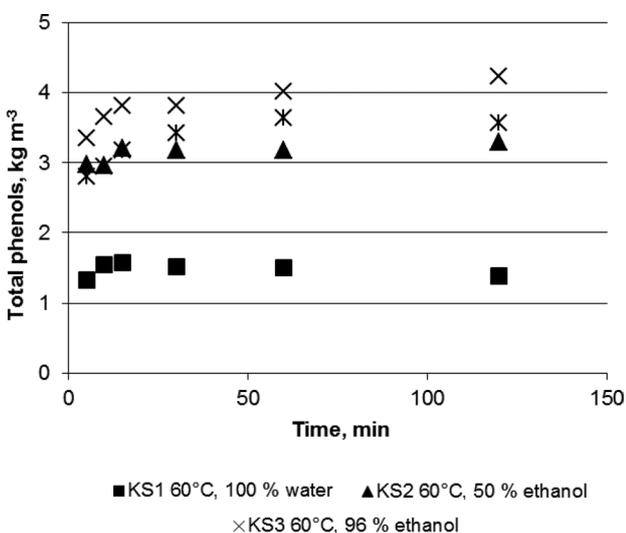


Fig. 5 – Total phenols extraction kinetics; KS – kinetics study

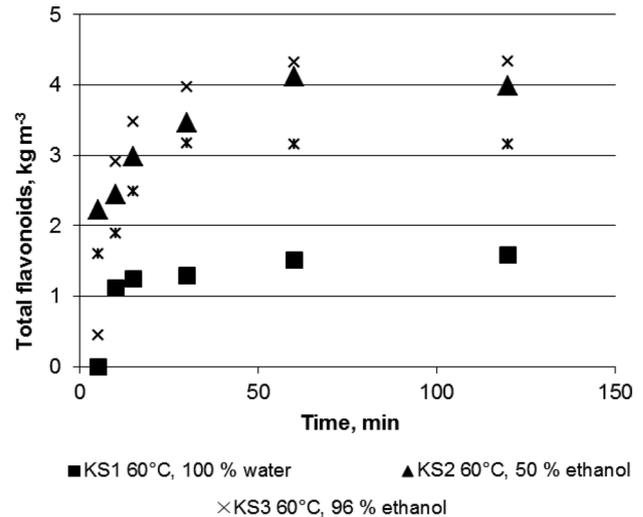


Fig. 6 – Total flavonoids extraction kinetics; KS – kinetics study

and 100 % methanol are similar and slightly lower than those obtained with azeotropic ethanol.

By comparing Figs. 5 and 6, we can conclude that the extraction of phenols and flavonoids varies with different solvents. Flavonoids appear to be very well extracted with 50 % ethanol/water solution.

Due to the good extraction rate of both target groups of compounds (total phenols and flavonoids) with 50 % ethanol/water mixture, this solvent combination was chosen for extract preparation used in the OSN filtration experiments. Furthermore, the use of azeotropic ethanol is not commercially feasible. The higher cost of pure ethanol as extraction solvent is not justified by the difference in target compounds yield.

Nanofiltration

A cross-flow nanofiltration system with six filtration cells was used for the initial membrane screening. Three types of membranes (DM 300, DM 500 and DM 900 – two coupons each) were tested in series to obtain the ability of those membranes to retain phenolic compounds. Final flux and rejection data are summarized in Table 2. A feed solution of 1:1 water/ethanol extract from *Cotinus coggygria* was filtered through the membranes at 20 bar and 30 °C. Samples from retentate and permeate of each filtration cell were taken for analysis after 4 h of filtration. The values in Table 3 are obtained by averaging the data from two identical membranes tested. Fig. 7 presents the flux change during the experiment for all three tested membranes.

The flux of all tested membranes decreased over time along with their compaction until it reached constant value (usually after 3-4 hours of filtration). The significant flux change of DM 900

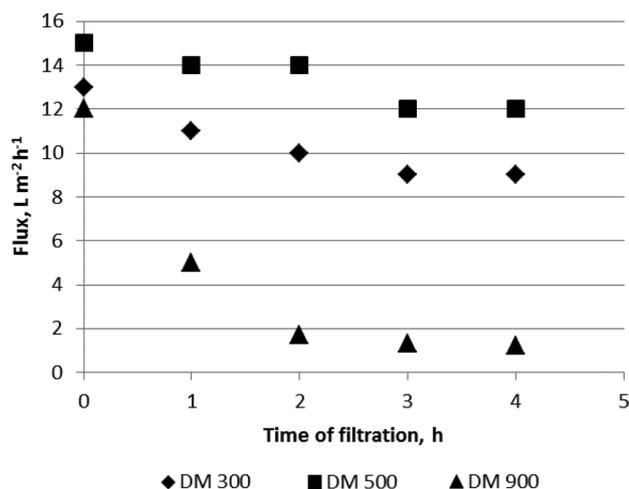


Fig. 7 – Flux change during filtration with 50 % ethanol/water *Cotinus coggygia* extract

Table 3 – Membranes screening summary

	Membrane		
	DM 300	DM 500	DM 900
Flux after 4 h of filtration, L m ⁻² h ⁻¹	9	12	1.2
Total phenols rejection, %	91	89	88
Total flavonoids rejection, %	93	90	87

membrane was unexpected and could be related to the composition of test solution and its influence on membrane performance. That made the membrane DM 900 unsuitable for the current application.

It is clear that the valuable compounds are well retained by the membranes and the tighter the membrane the better the rejection. Therefore, an even tighter membrane (DM 200) was applied in the next step – feed concentration experiment. It was expected that this membrane would have similar or higher rejection of phenols and flavonoids than that measured with DM 300.

A fresh feed solution (1:1 water/ethanol extract) was prepared and filtered in METcell dead-end filtration apparatus in order to demonstrate total phenols concentration increase by feed volume reduction.

The experiment was performed at 30 bar and 30 °C. 25 % of the initial feed volume was collected as permeate (was taken out of the system). A constant total phenols rejection of 91 % was recorded and the solution was concentrated 1.3 times as a result. The flux changed from 13 to 2 LMH over the whole filtration process (Figure 8). The experiment was carried out over a period of 8 – 9 hours.

The significant flux decrease observed during the experiment was found to be due to a fouling on the membrane surface. This phenomenon is well

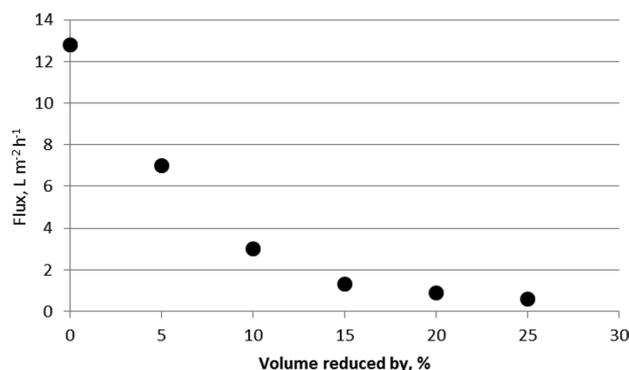


Fig. 8 – DM 200 flux decrease during concentration of 50 % ethanol/water *Cotinus coggygia* extract

known and quite common in membrane processes and could be minimized (and ideally avoided) with better mixing of the feed solution. On a larger scale (membrane modules), this is provided by ensuring certain feed velocity which prevents concentration polarization and fouling on membrane surface. Therefore, a filtration using a cross-flow system, where feed mixing is driven by a circulation pump (much better than stirrer mixing in METcell dead-end), is much closer to real process configuration and the obtained results are much more representative.

Due to the relatively small membrane area used, the trial was performed over several days by which the system was pressurized and depressurized several times. This process can be considered as one of the greatest “challenges” for membrane properties and function. However, all tested membranes were found to be stable in the process solution (ethanol/water extract) – no surface or structural change after completion of the experiments and constant rejection performance.

Conclusions

The extraction kinetics of polyphenols and flavonoids from *Cotinus coggygia* was experimentally obtained at $T = 60$ °C using different extracting solvents (96 % ethanol, 50 % ethanol in water mixture, water and methanol). The higher extraction rate for flavonoids was achieved with azeotropic ethanol and 50 % water in ethanol solution. For the polyphenols, the best results were achieved with methanol and azeotropic ethanol, and the water/ethanol mixture provides very good target compounds yield as well. Therefore, this solvent combination was considered as optimal for the investigated system and was used in extract preparation for the nanofiltration experiments ensuring maximal concentration for both groups of valuable compounds in the solutions and easier membranes performance evaluation, respectively.

Two types of OSN filtration experiments were performed – cross-flow nanofiltration for the initial membrane screening, and dead-end filtration for extract concentration. Three types of membranes (DM 300, DM 500 and DM 900 – two coupons each) were tested in the first step. The best retention of polyphenols and flavonoids was obtained with the tightest membrane (DM 300) as expected – 93 % for flavonoids and 91 % for polyphenols. Using an even tighter membrane (DM 200), the extract was successfully concentrated by reducing its volume by permeating out the solvent and retaining the majority of valuable compounds in a dead-end filtration system. Both DM 200 and DM 300 membranes can be successfully used for concentrating the extract.

The obtained data lead to the conclusion that a combination of solid-liquid extraction and OSN could be a very good way of producing highly concentrated natural sourced valuable compounds under mild conditions, i.e. without applying heat and keeping the desired properties of the substances. This study could be seen as an initial step, and further optimization is possible and needed for total process evaluation.

Symbols used

- n – stirring velocity, min^{-1}
 T – temperature, $^{\circ}\text{C}$
 ζ – liquid-solid ratio, $\text{m}^3 \text{kg}^{-1}$

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