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M. Lazarova[a]; K. Dimitrov[ab]
[a] Institute of Chemical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria [b] Laboratoire ProBioGEM EA, Polytech'Lille, USTL, Lille, France

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M. Lazarova[1] and K. Dimitrov[1,2]
[1]Institute of Chemical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Laboratoire ProBioGEM EA, Polytech'Lille, USTL, Lille, France

Abstract: Selective recovery of aporphine alkaloids from *Glaucium flavum Crantz* was studied. The alkaloids were successfully recovered from aqueous solutions, including native extracts of *Glaucium flavum Crantz*, applying pertraction in a rotating discs contactor. As a liquid membrane *n*-heptane and as receiving solution diluted phosphoric acid were used. Pertraction was also coupled to solid-liquid extraction in order to simultaneously purify the extract obtained from the plant. This integrated extraction-pertraction process was very simple, rapid, and efficient. The permeation of alkaloids through the liquid membrane was very selective and their purity in the receiving solution was 88.7%.

Keywords: Alkaloids, extraction, glaucine, *glaucium flavum crantz*, integrated process, liquid membrane, pertraction

INTRODUCTION

The aporphine alkaloid glaucine ($C_{21}H_{25}NO_4$), which is the predominant one in the yellow horn poppy (*Glaucium flavum Crantz*), is well known as an analgesic (1–2) and strong antitussive agent (3–7) and is being largely used together with other biologically active substances in the production

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Address correspondence to K. Dimitrov, Laboratoire ProBioGEM EA 1026, Polytech'Lille, USTL, Lille, France. Tel.: +33 3 287408; Fax: + 33 3 28767401. E-mail: krasimir.dimitrov@polytech-lille.fr

of antitussive drugs (8). It also shows good antioxidative (9–12), antibacterial (13–15), and anti-inflammatory (16–18) properties.

Usually, the alkaloids are extracted from raw plant material by appropriate solvents, but their content in the obtained native extracts is rather low because of the large amount of co-extracted species. For selective recovery of the target alkaloids additional purification of the extracts, based mainly on solvent extraction, has been applied (8,19,20).

Recently the interest of liquid membrane processes (also called pertraction processes) as a perspective alternative of the conventional extraction for isolation of valuable species from native aqueous extracts has been demonstrated (21–23). Ma et al. have recovered rutaecarpine from *E. rutaecarpa* var. *officinalis* extracts by using bulk liquid membranes (21). The pertraction technique has been successfully applied by Boyadzhiev and Yordanov for recovery and preconcentration of the indole alkaloid vincamine from native extracts of *Vinca minor L.* (22), whereas Dimitrov et al. have selectively recovered tropane alkaloids from extract solutions of *Atropa Belladonna L.* (23).

In the liquid membrane processes, the extraction by a suitable organic solvent and the stripping of the loaded solvent are simultaneously performed in one apparatus which enables a selective recovery of the target specie (24). The three-liquid-phase system consists of two aqueous solutions: F (feed) and R (receiving), separated by an organic phase (M) which is insoluble in both aqueous solutions and acts as a liquid membrane (24–27). The thermodynamic conditions at the interfaces F/M and M/R are different and carefully chosen in order to ensure the solute transfer from the feed across the membrane into the receiving solution. The main advantage of the pertraction process over conventional solvent extraction is the possibility to maintain a maximal driving force throughout the whole experimental run. Even if the distribution coefficients at the interface F/M are relatively low, as in the case of many alkaloids, the target solutes could be completely or almost completely removed from the feed because of the continuous liquid membrane stripping (regeneration) (23,24). By conventional solvent extraction this is only possible with multiple extraction-stripping operations (28). Due to the incessant regeneration of the organic liquid membrane, pertraction processes also give the opportunity to use as liquid membranes solvents which are less powerful, but more selective, less toxic, less harmful, and less expensive than the solvents used in conventional liquid-liquid extraction.

An integrated process based on simultaneous solid-liquid extraction and liquid membrane purification of the obtained native extract has been proposed for recovery and isolation of valuable species from vegetal sources (29–31). The coupling of solid-liquid extraction with pertraction provides simple and rapid obtaining of products of high purity and complete exhaustion of the solid material.

The main objectives of this work were to selectively recover the alkaloids from native aqueous extracts of yellow horn poppy grass, as well as to demonstrate the possible implementation of a process, integrating in one single stage the solid-liquid extraction of alkaloids from *Glaucium flavum Crantz* and their simultaneous purification by a pertraction technique.

EXPERIMENTAL

Materials and Methods

Standard glaucine hydrobromide (>97%) was kindly supplied by Dr. Filipov (Inst. of Org. Chem., Sofia, Bulgaria) and was used as received without further purification. The aerial part of *Glaucium flavum Crantz*, grown in the region of Burgas in Bulgaria (collected in the blooming period, 2004) was ground, homogenized, and stored in a cool, dark, and aerated place.

The organic solvents *n*-heptane (Reachim, Russia), chloroform (POCh, Gliwice, Poland) and diisopropyl ether (Merck), phosphoric acid (Fluka) and the dipotassium hydrogen phosphate (Sigma-Aldrich) used for pH-adjustment of the aqueous solution, as well as the acetonitrile (Labscan) and potassium dihydrogen phosphate (Sigma-Aldrich) used in the HLPC analysis were all of AR grade.

When model aqueous solutions of pure glaucine were used, alkaloid concentration in the aqueous solutions was determined at a wavelength of 280 nm by UV analysis (UV spectrophotometer UNICAM Helios β) and by a HPLC system, consisting of an UV-detector "Knauer," an integrator C-R6A Chromatopack "Shimadzu," and a reverse phase column C18 Nucleosil 100–5. The mobile phase used was a 74:26 (v/v) mixture of 0.03 M KH_2PO_4 and CH_3CN. The flow rate was $1.0\,cm^3 \cdot min^{-1}$. The glaucine amount in the organic solutions was obtained from the mass balance.

Concentrations of glaucine, as well as total aporphine alkaloids amount in the extracts of yellow horn poppy grass were obtained by HPLC, after preliminary purification. This sample preparation consisted in the alkalization of the extract up to pH ~ 9.0, followed by threefold consecutive extraction with diisopropyl ether and back extraction of the alkaloids from the collected organic solution with 0.07 M H_3PO_4. Total alkaloids content in the purified samples was also evaluated as glaucine by UV-analysis. Since, for the analyses of aporphine alkaloids in the extracts large sample volumes were required, the initial and final alkaloids concentrations in the feed solution and in the organic liquid membrane were determined only. The high alkaloids purity in the receiving solution allowed a direct UV-measurement of their concentration in

this solution during liquid membrane purification of the extract in the cases of batch pertraction and integrated process extraction-pertraction. The final alkaloids concentrations in the receiving solutions were determined by both methods and a disagreement was not observed.

The amount of all species dissolved in the initial and final aqueous solutions was determined after solvent evaporation and the alkaloids percentage in the extracts was obtained.

Hydrogen ion concentration in the aqueous solutions was measured by a digital pH meter OP-211/1 (Radelkis, Hungary) equipped with a combined electrode.

Equipment and Procedures

The alkaloids extraction from yellow horn poppy grass by distilled water, as well as by slightly acid and slightly basic aqueous solutions was studied. In each experiment 2.0 g of the ground aerial part of the plant were introduced in 50.0 cm^3 aqueous solution and the obtained suspension was agitated for 4 h by a magnetic stirrer. The obtained native extracts were filtered and the alkaloids concentrations in the extracts were determined by both UV analysis and HPLC after sample purification.

Multifold solid-liquid extraction of the alkaloids was carried out as well. For this purpose 1.0 g of ground aerial part of *Glaucium flavum Crantz* was put in 30.0 cm^3 of dilute H_3PO_4 aqueous solution (pH $\sim$ 3.0) and the suspension was stirred for 3 h. The obtained extract was separated and the solid phase was newly contacted with a fresh portion of 30.0 cm^3 of the same aqueous solution. This procedure was repeated several times till complete exhaustion of the plant material. The native aqueous extracts were collected and the content of alkaloids in this total extract, respectively in the initial plant material, was determined.

Liquid-liquid extraction equilibrium experiments were carried out by contacting in separating funnels organic solvents and model glaucine aqueous solutions in volume ratio 1:1. A moderate shaking of 15 minutes was found to be sufficient to attain equilibrium distribution of glaucine between the organic and the aqueous solutions. After settling and phase separation, the alkaloid concentrations in the aqueous solutions were spectrophotometrically measured.

The laboratory rotating discs contactor (RDC) used in kinetic studies is schematically shown in Fig. 1. The bottom part of the main body is divided into three compartments by two separating walls (2). In each of the three compartments there is a rotating disc (3), covered by hydrophilic coating. In the experiments, the two outer compartments were occupied by the feed solution and the middle one by the receiving

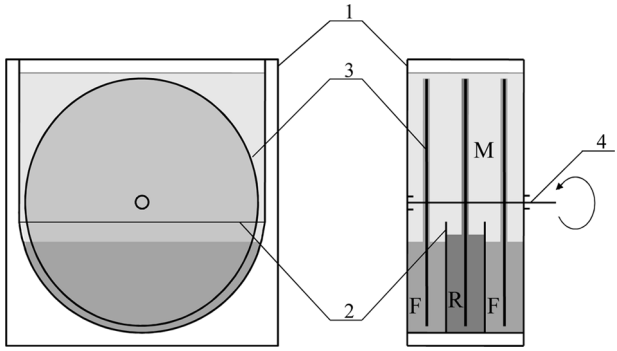

Figure 1. Schematic diagram of the laboratory pertraction contactor, used in this study: 1-body; 2-separating walls; 3-rotating discs; 4-common shaft.

solution. The upper part of the pertraction device was filled with the organic liquid, which covered both aqueous solutions. Hence, the lower part of each disc was immersed in the corresponding aqueous solution and the upper part in the organic liquid. While rotating, the aqueous solutions formed mobile liquid films on the disc surfaces, in this way increasing the contact surface between organic and aqueous solutions. Discs rotation provided a continuous agitation of the three phases and renewal of the aqueous films on the discs. For additional homogeneity of F and R solutions, as well as for handy sample takings, both liquids were recirculated using peristaltic pumps.

The effect of agitation on alkaloid pertraction kinetics was studied using as feed phase 290 cm^3 model glaucine aqueous solutions with $pH_F = 9.0$, buffered by potassium phosphate buffer. Selective recovery of aporphine alkaloids was studied using as feed solution 290 cm^3 native aqueous extract from yellow horn poppy grass, buffered at $pH_F = 9.0$. In all studies 1050 cm^3 *n*-heptane as membrane liquid and 145 cm^3 aqueous solution of H_3PO_4 ($pH_R = 2.0$) as receiving solution were used.

Solid-liquid extraction of alkaloids from *Glaucium flavum Crantz* was coupled with a simultaneous pertraction in an integrated process. The schematic diagram of the experimental set-up is shown in Fig. 2. A slightly basic aqueous solution, buffered at $pH \sim 9.0$ (290 cm^3) and an aqueous solution of H_3PO_4 ($pH = 2.0$, 145 cm^3) were poured into the RDC-compartments for the feed and the receiving phases, respectively, and both solutions were then covered with 1050 cm^3 *n*-heptane (1). The grounded dry herb (solid phase S) was put in the extractor (2) and approximately 360 cm^3 of basic aqueous solution with $pH \sim 9.0$ (the same as in the pertraction device) were added. The obtained suspension was

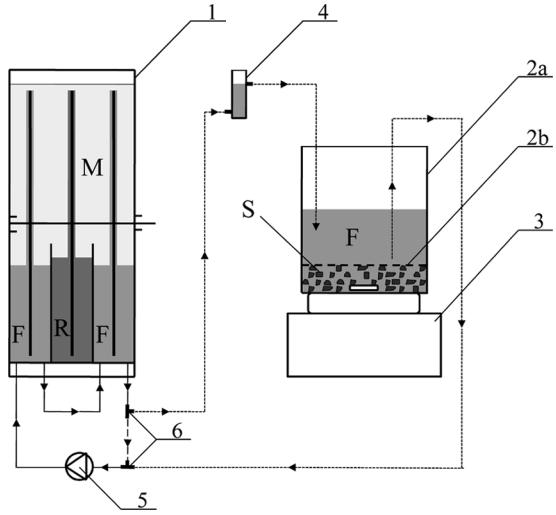

Figure 2. Diagram of the experimental set-up: 1-rotating discs contactor; 2-extractor (2a-extraction vessel; 2b-fine grid); 3-magnetic stirrer; 4-overflow drain; 5-peristaltic pump; 6-three way valves. Dashed lines correspond to the F-phase recirculation in the case of batch pertraction; dotted lines correspond to the F-phase recirculation in the case of integrated process; solid lines–for both cases.

homogenized by a magnetic stirrer (3). The extractor was coupled with the feed solution compartments of the pertraction contactor and the obtained native liquid extract recirculated by means of a pump (5) in a closed loop throughout both devices. Solid particles were kept in the lower part of the extractor by a fine grid (2b), thus preventing them to come out of the vessel and clog the system.

All studies were carried out at a temperature of 293 ± 1 K.

RESULTS AND DISCUSSION

Solid-Liquid Extraction

The solid-liquid extraction of aporphine alkaloids from *Glaucium flavum Crantz* was carried out at different acidities of the aqueous solution. The results are presented in Table 1. The extracts obtained by distilled water ($pH_{end} = 5.7$) and by slightly acid solution ($pH_{end} = 3.6$) were quite similar, while the extract obtained by slightly basic solution ($pH_{end} = 8.6$) was less rich in alkaloids. In all native extracts the presence of other co-extracted species was very high and the content of alkaloids was less than 3.8%.

Table 1. Alkaloids content in the extract obtained after 4 h solid-liquid extraction from *Glaucium flavum Crantz* (2.0 g dry vegetal material/50 cm^3 aqueous solution)

pH of the aqueous solution			Total aporphine alkaloids (%)	
Initial pH	Final pH	Total extract (%)	In the extract	In the plant
3.1	3.6	30.25	3.74	$\geq$1.13
5.5	5.7	32.63	3.59	$\geq$1.17
9.1	8.6	35.30	1.98	$\geq$0.70

In order to determine the total alkaloids content in the vegetal source multifold solid-liquid extraction was carried out. As extracting agent slightly acid aqueous solution (pH = 3.1) was used, because of its better selectivity compared to distilled water. The percentage of total aporphine alkaloids in the dried aerial part of *Glaucium flavum Crantz* used was found to be 1.75% and the content of glaucine in the plant was 1.55%.

Liquid-Liquid Extraction

To apply the liquid membrane process for glaucine recovery from aqueous media, including native extracts of *Glaucium flavum Crantz*, it is necessary to find conditions favorable for its extraction by an organic solvent, but also conditions suitable for its back extraction into another aqueous solution and, therefore, regeneration of the organic liquid. The behavior of various organic solvents (*n*-heptane, chloroform and diisopropyl ether) on glaucine extraction from its model aqueous solutions was studied. The effect of the equilibrium pH on glaucine extraction degree is presented in Fig. 3. The obtained results show that all studied solvents are suitable for glaucine recovery from aqueous solutions. The degree of alkaloid extraction into the organic solvents was found to be strongly dependant on the acidity of the aqueous solutions. In the cases of *n*-heptane and diisopropyl ether, at low pH (pH < 3.0), glaucine remained entirely in the aqueous solution. However, the increase of pH improved strongly glaucine recovery and, at pH > 9.0 more than 90% of the alkaloid was extracted by both *n*-heptane and diisopropyl ether. Among the studied organic solvents, chloroform demonstrated the best extraction ability. Glaucine was almost completely extracted even at pH $\sim$ 4.0. However, the high affinity of glaucine towards chloroform, shown in Fig. 3, presumes that very strong acid aqueous solutions for

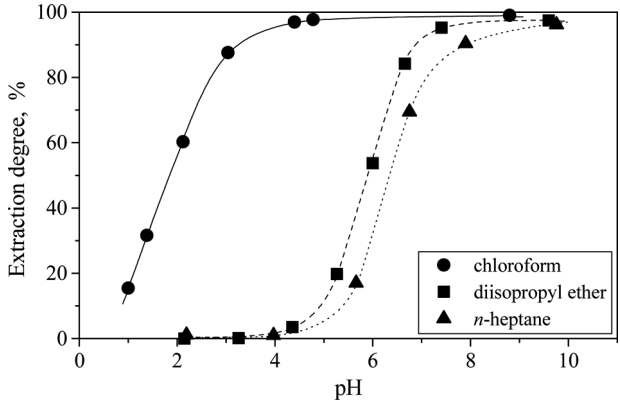

Figure 3. Effect of equilibrium pH on degree of glaucine extraction by various organic solvents.

stripping of the loaded chloroform should be used. Other inconveniences for chloroform use are its toxicity and its aggressiveness to plastic materials, often used in pertraction devices. Both other solvents, *n*-heptane and diisopropyl ether, offer conditions favorable for glaucine extraction (at $pH \sim 9.0$), as well as conditions suitable for its back extraction (at $pH < 3.0$). For the kinetic studies on glaucine pertraction and for the studies on selective alkaloids recovery by liquid membrane permeation, *n*-heptane was selected as organic solvent because of the slight tendency of diisopropyl ether to form explosive peroxides. Since in the equilibrium studies, some variations in pH of the aqueous phase before and after extraction were observed, feed solutions in kinetic studies were buffered in order to keep constant the required pH value ($pH \sim 9.0$). Such buffering of the F phase in the case of native extracts from *Glaucium flavum Crantz* was necessary also because of the large amount of various extracted species in these solutions.

Pertraction in a Rotating Discs Contactor

Glaucine Pertraction using Model Feed Solutions

The kinetics of glaucine transport in three-liquid-phase system was studied using as feed phase model aqueous solutions containing $0.18\,g.dm^{-3}$ glaucine with $pH_F = 9.0$. Figure 4 shows the alkaloid repartition between the three phases versus time during batch pertraction process at constant discs rotation velocity of $10\,min^{-1}$. Obviously, the

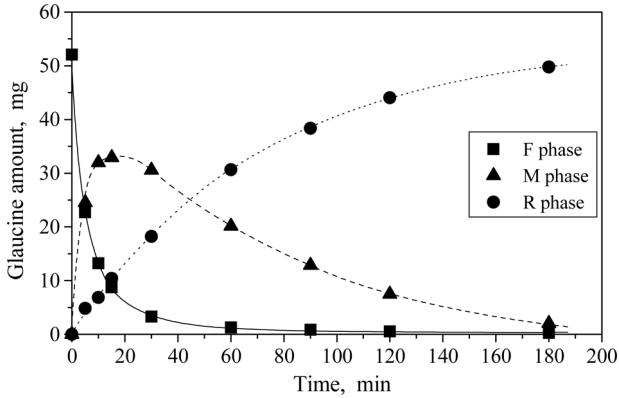

Figure 4. Evolution of glaucine amount in feed, membrane and receiving solutions versus time at $10\,min^{-1}$ ($G_{tot} = 52.1\,mg$, $pH_F = 9.0$, $pH_R = 2.0$).

conditions at the two interfaces F/M and M/R were favorable for glaucine permeation through the organic liquid membrane. Alkaloid was rapidly extracted from the feed solution: more than 90% in 30 min only. The kinetic profile of glaucine amount in the organic liquid is typical for an intermediate phase. After a short initial accumulation in the liquid membrane, the amount of alkaloid in this phase decreased progressively, because of the continuous stripping. Consequently, glaucine was transferred into the receiving solution. The pertraction process was very efficient: at the end of the experimental run (after 3 h) glaucine was completely recovered from the feed solution and about 95% already accumulated in the receiving solution.

The rate of discs liquid film renewal and the intensity of membrane stirring depend on the discs rotation velocity. The effect of agitation on the kinetics of glaucine permeation through the liquid membrane was studied at three constant rotation velocities: 10, 15, and $20\,min^{-1}$. Figure 5 represents the evolution of the dimensionless concentrations of glaucine in both aqueous solutions versus time. The results show that the increase of rotation velocity favors alkaloid extraction from the feed, but the positive effect of the agitation on glaucine accumulation in the receiving solution is more evident. At $20\,min^{-1}$, the alkaloid was almost completely recovered into the R-phase (99.5%). Figure 5 shows also that glaucine was concentrated twice in the receiving solution due to the difference between the volumes of the two aqueous phases.

Glaucine dimensionless concentration profiles in the organic liquid membrane are shown in Fig. 6. Higher rotation velocities provoked a small decrease of the concentration maximum values. It is also evident that the increase of agitation velocity results in faster concentration

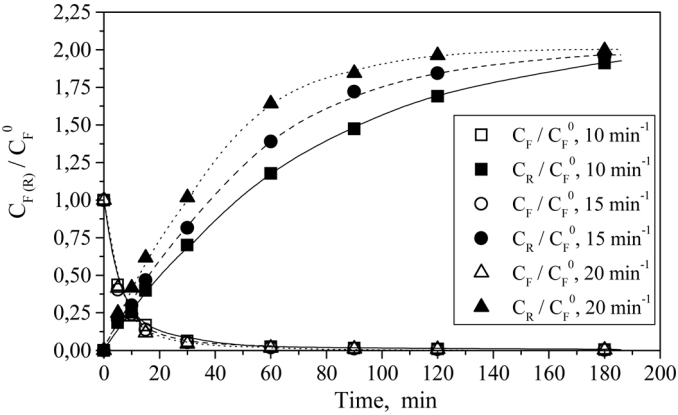

Figure 5. Effect of agitation on glaucine extraction from the feed and its accumulation in the receiving solution ($C_F^0 = 182.0\,g.m^{-3}$, $pH_F = 9.0$, $pH_R = 2.0$).

decrease in the organic liquid membrane, explained by more efficient stripping at these conditions.

Alkaloids Pertraction from Native Aqueous Extracts of *Glaucium flavum Crantz*

Selective recovery of aporphine alkaloids from *Glaucium flavum Crantz* was studied, too. The native aqueous extract from yellow horn poppy

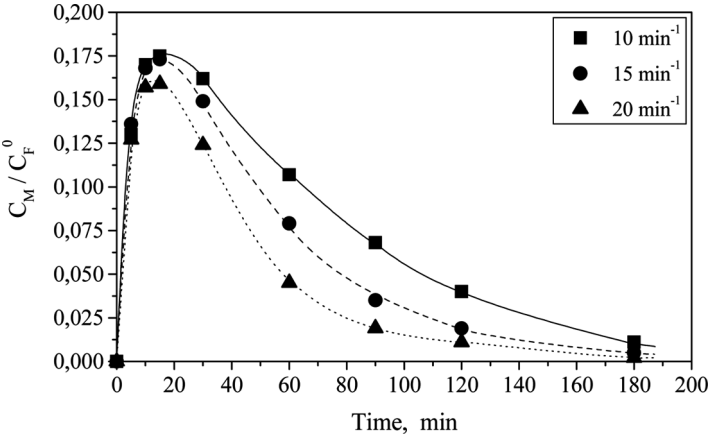

Figure 6. Effect of agitation on the glaucine concentration in the organic liquid membrane ($C_F^0 = 182.0\,g.m^{-3}$, $pH_F = 9.0$, $pH_R = 2.0$).

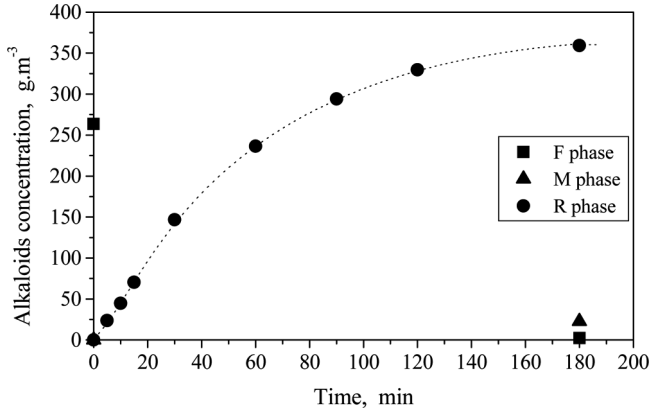

Figure 7. Evolution of alkaloids concentration when native extracts from *Glaucium flavum Crantz* were used at $20\,min^{-1}$ ($C_F^0 = 263.7\,g.m^{-3}$, $pH_F = 9.0$, $pH_R = 2.0$).

grass obtained by solid-liquid extraction and alkalized with phosphate buffer up to $pH_F = 9.0$ was used as feed phase in batch pertraction process. The initial concentration of aporphine alkaloids in this F-solution ($0.26\,g.dm^{-3}$) was similar to the concentration of glaucine in the model feed aqueous solutions. However, the native extract contained also a large amount of co-extracted species and the alkaloids content in the extract was 3.6% only. Pertraction process was carried out at a discs rotation velocity of $20\,min^{-1}$. The results presented in Fig. 7 show a satisfactory recovery of alkaloids. At the end of the experimental run (3 h), about 99% alkaloids were extracted from the feed solution, but their accumulation in the receiving solution was not complete (about 70%). Comparing these results with the ones obtained with model glaucine solutions one can conclude that the presence of other species in the native extract slows down slightly the alkaloids permeation through the *n*-heptane liquid membrane. Due to the selectivity of the liquid membrane used, the major part of co-extracted species remained in the feed solution. The total alkaloids content in the solid residue after drying of the receiving solution was found to be about 25-fold higher than in the feed solution (native extract) dry residue.

The percentage of glaucine with respect to all aporphine alkaloids in the receiving solution was found to be 88.6%.

Integrated Process Extraction-Pertraction

For integrated process implementation, the glaucine extraction from yellow horn poppy was coupled with alkaloids pertraction in the rotating

discs contactor. For this purpose 6.5 g of grounded dry plant material were suspended into an aqueous solution with $pH_F = 9.0$ and the obtained native liquid extract was led into the pertraction device as a feed solution. This solution circulated constantly and consecutively through the extraction and pertraction devices and, therefore, both extraction and pertraction were carried out simultaneously (see Fig. 2). This continuous integrated process provided purification and concentration of the alkaloids extracted from *Glaucium flavum Crantz*. Due to appropriate chosen conditions at the three interfaces S/F, F/M and M/R the alkaloids were transferred from the solid phase into the receiving solution (via F and M solutions). The incessant stripping of the liquid membrane M kept low the concentration of the alkaloids in this phase and provided their recovery from the feed solution (native extract). Feed solution was also stripped continuously by the organic phase M and therefore alkaloids concentration in the feed phase was also very low throughout the process. Hence, the alkaloids were constantly extracted from the solid material practically by almost fresh solvent. In this way, the coupling of solid-liquid extraction with pertraction continuously provided the necessary process driving force until an almost complete exhaustion of the source material.

Figure 8 shows the kinetics of alkaloids accumulation in the receiving solution during the integrated process. A negligible delay of the rate of alkaloids accumulation was observed at the process beginning. It could be attributed to the relatively low alkaloids concentration in the feed

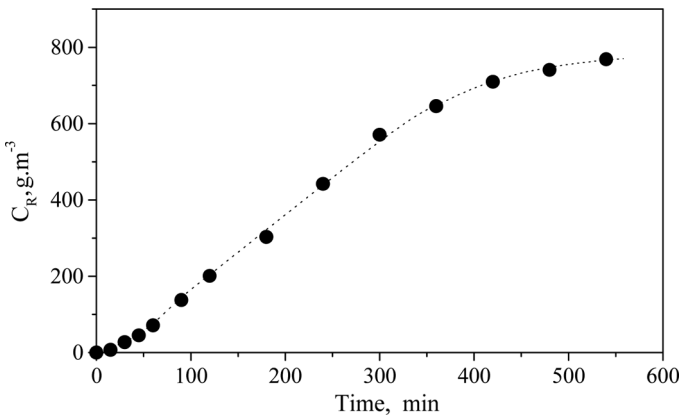

Figure 8. Alkaloids accumulation in the receiving solution in the case of integrated process of solid-liquid extraction from *Glaucium flavum Crantz* and pertraction at 20 min^{-1} ($G_{tot} = 113.8$ mg, $pH_F = 9.0$, $pH_R = 2.0$).

Table 2. Repartition of the alkaloids in all four phases at the end of the integrated extraction-pertraction process (after 9 h)

Phase	Alkaloids concentration $g.m^{-3}$	Alkaloids content	
		mg	%
Solid phase (S)	–	0.24	0.21
Feed solution (F)	2.24	1.51	1.33
Membrane liquid (M)	2.08	2.18	1.91
Receiving solution (R)	768.34	109.87	96.55

solution compartments of RDC, due to still incomplete extraction from the solid phase at the pertraction process beginning, as well as to the delay of the feed solution transport from the extractor to the pertraction contactor. After this initial period (first 30 min), the rate of alkaloids accumulation in the receiving solution was relatively constant until the 5th hour, when the rate of accumulation started to decrease because of the exhaustion of the vegetal source.

Table 2 regroups the alkaloids repartition in all four phases at the end of the integrated extraction-pertraction process. At the end of the experimental run (after 9 h) more than 95% of alkaloids were extracted and transferred into the receiving solution, while the plant material was practically exhausted.

As in the case of batch pertraction from native extracts from *Glaucium flavum Crantz*, the integrated process extraction-pertraction provided a very good purification of the extracted alkaloids. The content of glaucine in the receiving solution with respect to all species present in the R-phase was found to be 78.6% and the percentage of total aporphine alkaloids was 88.7%.

CONCLUSION

Aporphine alkaloids could be selectively recovered from their aqueous solutions, including native extracts from *Glaucium flavum Crantz*, applying a liquid membrane technique. The process using *n*-heptane as a liquid membrane provides a significant purification of the alkaloids, because most of the other co-extracted species do not permeate the liquid membrane. The pertraction process, carried out in a rotating discs contactor, is very efficient, especially when model glaucine aqueous solutions are used. The presence of other co-extracted species in the feed solution slows down slightly the transport of alkaloids through the liquid membrane.

The increase of agitation velocity has a positive effect on the rate of alkaloids pertraction.

The coupling of solid-liquid extraction of aporphine alkaloids from *Glaucium flavum Crantz* with simultaneous purification of the obtained native extracts by pertraction in a single integrated process provides a simple and practical way to obtain products of high purity. After 9 h of integrated extraction-pertraction process, 99.8% of the alkaloids were extracted from the plant material and 96.5% already transferred into the receiving solution. The content of total aporphine alkaloids in the receiving solution with respect to all dissolved species was 88.7%, while in the native aqueous extracts from *Glaucium flavum Crantz* was 3.6% only.

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