Selective Recovery of Tropane Alkaloids Applying Liquid Membrane Technique

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Atropine recovery from its solutions applying a liquid membrane technique was studied. Among several studied organic solvents, chloroform showed best extraction ability towards atropine. Pertraction studies were carried out in a laboratory bulk liquid membrane contactor with agitation of all three phases. Both, aqueous solutions of pure atropine and extract of *Atropa Belladonna L.* roots were used as feed solutions, as well as chloroform and diluted sulphuric acid as a liquid membrane and a stripping liquor, respectively. The effect of phase agitation on alkaloid pertraction was studied for the cases when pure atropine solutions were used as feed phase. A pertraction process carried out with native liquid extracts from *A. Belladonna* provided selective alkaloid recovery and its concentration in the acceptor solution.

Key words:
Liquid membranes, atropine, pertraction, tropane alkaloids, purification

Introduction

Recently, a marked interest in the recovery of various alkaloids from natural plants is observed. Among these valuable substances, due to their medicinal properties, tropane alkaloids are at the focus of special attention.[1–4] Atropine, the racemic of hyosciamine, is the chief alkaloid in *Atropa Belladonna L.* roots and one of the most useful tropane alkaloids from medical point of view. Usually, the alkaloids are extracted in basic solutions in their free base forms or in acidic solutions as salts.[5–8] The obtained extracts contain many undesirable co-extracted substances and the amount of alkaloids in the extracts is rather low. Purification of the native extracts is a subject of long and vexatious repetitive extraction – stripping operations.[9,10]

Liquid membrane processes, called also pertraction processes, are an attractive alternative of conventional extraction, offering possibilities for selective recovery of various species from their solutions.[11–15] Solute transport across a liquid membrane is a combination of extraction and stripping operations performed simultaneously in one apparatus. Two aqueous solutions, the feed solution F, and the acceptor solution A, are separated by a third, organic liquid M, representing the "liquid membrane", which is insoluble in the other two liquids. The solute is transferred from the feed to the acceptor solution under the effect of appropriately chosen equilibrium conditions at the two interfaces F/M and M/A. The main advantage of this process over conventional solvent extraction is the possibility to remove the equilibrium limitations due to continuous membrane stripping and to recover solutes even in the cases of low distribution coefficients.[11]

The aim of the present study was to study the process of atropine recovery from its solutions using a liquid membrane technique and to apply this procedure for selective recovery of the alkaloids from native aqueous extracts of *A. Belladonna* roots.

Experimental

Reagents and analytical methods used

Both, equilibrium and pertraction kinetic studies were carried out using model atropine aqueous solutions. For this purpose atropine (>97 %, Fluka) was used as received. Chloroform (purrum, POCh, Gliwice, Poland), *n*-heptane (Fluka), diethyl ether and diisopropyl ether (>98.5 %, Merck) were used as solvents. Ammonia solution (p.a. grade, Mendeleev, St. Zagora, Bulgaria) and sulphuric acid (Merck) were used to adjust the acidity of the aqueous solutions.

When model solutions of atropine were used, its concentration in the aqueous solutions was measured directly or after appropriate dilution by UV spectroscopy, using UNICAM Helios β apparatus at λ = 257 nm. All measured concentrations correspond to the linear part of the absorbance/concentration curve. A shift of the maximum absorbance wavelength upon solution pH value variation was

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not observed. The atropine amount in the organic solutions was obtained from the mass balance.

Tropane alkaloids were extracted from dry, ground roots of *A. Belladonna* (collected in 2002, region of Gotze Deltchev, Bulgaria) by solid-liquid extraction. The obtained native liquid extract, after being filtered, was used as a feed phase when the selectivity of the pertraction process with respect to the tropane alkaloids was studied. In this case the total amount of tropane alkaloids in the aqueous phases was obtained using a method described in the European Pharmacopoeia,[10] based on a preliminary purification of the samples, applying repeatedly liquid-liquid extraction and a subsequent titration. According to this procedure, since the atropine is the predominant alkaloid in the *A. Belladonna* roots, the total amount of tropane alkaloids was calculated as atropine. In fact, besides the atropine (hyosciamine), the alkaloids scopolamine and apoatropine are also present in the *A. Belladonna* roots, respectively in the native extracts, but their amount is very low.[2,6] As far as their structures, properties, and molecular mass are very similar to these of atropine and their presence in the extracts was very low, the total amount of alkaloids was calculated as atropine in all analyses. For analytical uses chloroform, diethyl ether and ammonia, as well as ethanol, sodium sulphate and sulphuric acid (all p.a. reagents from Merck) and sodium hydroxide (p.a. grade, Himsnab, Dimitrovgrad, Bulgaria) were used. Since, for the alkaloids analyses in the feed solution large sample volumes were required, the initial and final alkaloids concentrations in this phase were determined only. The high alkaloids purity in the acceptor phase allowed a direct UV-measurement of their concentration in this solution throughout the pertraction process. The final alkaloids concentration in the acceptor solution was determined by both methods and a disagreement was not observed.

The amount of all species dissolved in the initial and final aqueous solutions (including tropane alkaloids, but also co-extracted substances) was measured after solvent evaporation and the alkaloids percentage in these extracts was obtained by the above-mentioned method recommended by the European Pharmacopoeia.

The pH values of the aqueous solutions were measured by means of laboratory pH meter (Radelkis type OP-264/1) with precision ± 0.01 pH unit.

Experimental equipment and procedures

Atropine distribution data at equilibrium between the aqueous solutions and the studied organic solvents were obtained using 0.1 dm^3 separating funnels. In each experiment equal volumes of atropine aqueous solution (nearly 1.0 kg m^{-3}) and organic solvent were shaken for 15 min.

Kinetics of tropane alkaloids recovery was studied in a laboratory bulk liquid membrane contactor with agitation of all three phases, shown schematically in Fig. 1. This pertraction device consists of two coaxial cylindrical glass vessels and baffles. The heavier organic membrane liquid M is disposed in the common, bottom part of both cylinders. The upper liquid layer in the outer cylinder (1) contains the feed solution F, while the upper liquid layer in the inner cylinder (2) is the acceptor (strip) solution A. Four glass baffles (3) are placed in the outer cylinder and another immobile baffle (4), fixed on the inner cylinder axis. The outer cylinder and the baffles are immobile. The inner cylinder rotates with a constant velocity, driven by the electric motor (5). In this way all liquids are stirred without allowing stagnant zone formation in the vessels.

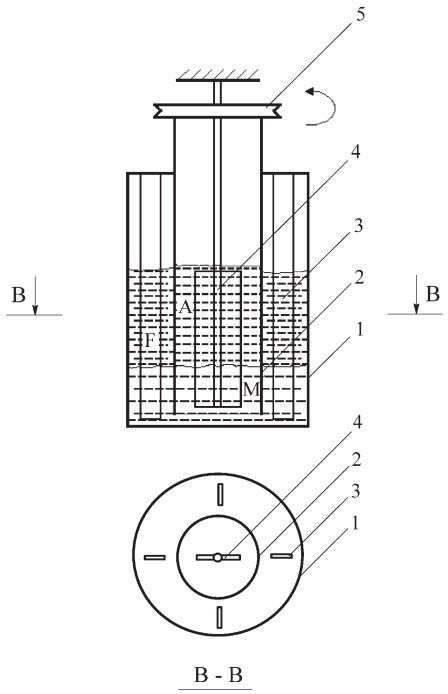


Fig. 1 – *Principal diagram of laboratory pertraction contactor, used in this study: (1) outer vessel, (2) internal, open-bottomed rotating cylinder, (3) feed solution and membrane solution baffles, (4) immobile central baffle for acceptor solution and membrane solution, (5) driven grooved pulley*

When the effect of agitation on atropine recovery was studied, as a feed phase 0.23 dm^3 solution of atropine (0.5 kg m^{-3} atropine content), buffered with ammonia/ammonium sulphate buffer solution to pH 9.5, was used. Chloroform (0.23 dm^3) was used as a liquid membrane and 0.1 mol dm^{-3} solution of sulphuric acid (0.06 dm^3) as an acceptor phase.

When the selectivity of alkaloids recovery was studied, a solid-liquid extraction was previously carried out. For this purpose ground dry roots of *A. Belladonna* were fractionated by size. In this study the fraction of 0.4–2.0 mm was used. Alkaloids were extracted from the plant with a slightly acidic aqueous solution (5.0 mmol dm^{-3} sulphuric acid). Obtained native extract (0.45 kg m^{-3} alkaloids), alkalised with ammonia buffer solution to pH 9.5, was used as a feed phase in the pertraction process. The liquid membrane and the acceptor solution were identical to these used in the other pertraction studies.

Both, equilibrium and pertraction studies were carried out at 293 K. In order to obtain representative values, each experiment was repeated at least three times. All the data deviated more than 3.0 %, with respect to the average values, were rejected.

Results and discussion

Equilibrium studies

The behaviour of various organic solvents (*n*-heptane, chloroform, diethyl ether, diisopropyl ether), on atropine extraction from ammonia solutions, was studied and the obtained distribution coefficients are shown in Table 1. Best results (D = 32.5 at pH 9.43) were obtained when chloroform was used. The polarity of atropine molecule determines its better solubility, respectively higher distribution coefficient, in the case of polar solvents, such as chloroform, than in non- or less polar solvents, such as *n*-heptane and ethers, according to the "like dissolves like" rule. Therefore, the following studies were carried out with chloroform as organic solvent. The equilibrium data show that the *n*-heptane is obviously not appropriate for atropine recovery from ammonia aqueous solutions, while the diethyl ether and the diisopropyl ether could be used for this purpose. It should be mentioned that for all studied organic solvents the stripping of loaded organic solutions by 0.1 mol dm^{-3} sulphuric acid was complete.

Table 1 – *Distribution factor ($D = \gamma_{org}^*/\gamma_{aq}^*$) of atropine between various organic solvents and ammonia aqueous solutions*

	Chloroform	Diethyl ether	Diisopropyl ether	*n*-Heptane
Distribution factor (D)	32.50	1.85	1.22	< 0.01
Aqueous solution pH	9.43	9.40	9.42	9.45

Atropine equilibrium distribution between chloroform and dilute aqueous solutions of sulphuric acid or ammonia was evaluated as a function of the pH-value of the aqueous solution. The obtained results are presented in Fig. 2. In the cases of sulphuric acid solutions, atropine was practically not extracted into the chloroform and remained entirely in the aqueous phase as atropine sulphate. However, the extraction of atropine from ammonia solutions is significant and increasing with the increase of pH-value. In ammonia media, the alkaloid is in free base form and much more soluble in the organic solvents than in water.

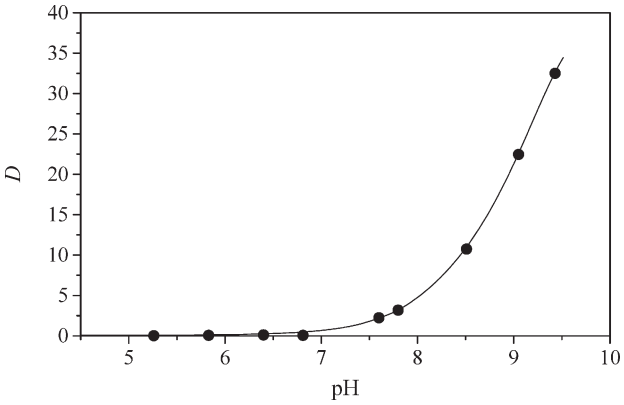


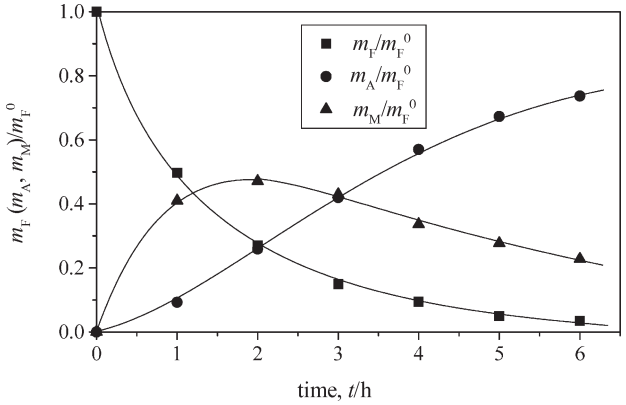
Fig. 2 – *Effect of aqueous phase pH-value on atropine distribution factor D at 293 K*

In our studies, feed aqueous solutions of moderate ammonia content (solutions of pH ≈ 9.5) were used, because the excessive use of alkali increases the risk of alkaloids hydrolyse.[1]

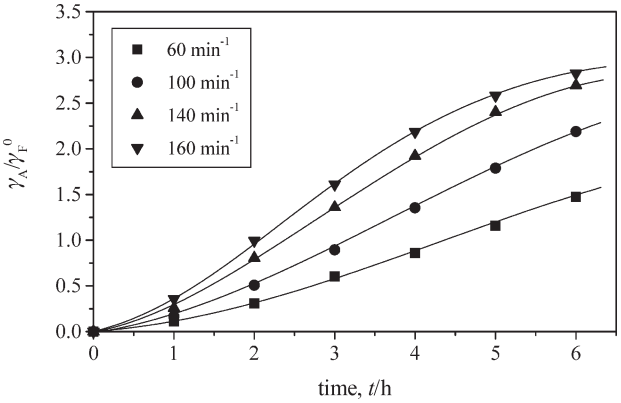
Pertraction studies

Kinetics of atropine transfer through a chloroform liquid membrane was studied in the above-described laboratory pertraction device. The atropine in the feed phase was in free base form, which favoured its extraction into the organic membrane. The presence of sulphuric acid in the acceptor phase provided continuous membrane stripping and atropine accumulation in the acceptor solution as sulphate.

Fig. 3 illustrates the typical changes of atropine content in all three phases versus time during batch pertraction process. As a result of atropine extraction into the chloroform liquid membrane, its amount in the feed solution decreases continuously. Simultaneously, the removed alkaloid is back extracted from the membrane by the sulphuric acid and accumulated in the acceptor phase. It should be noted, that in this case the extraction and stripping rates were rather low because of the small contact areas between the phases, and the relatively low



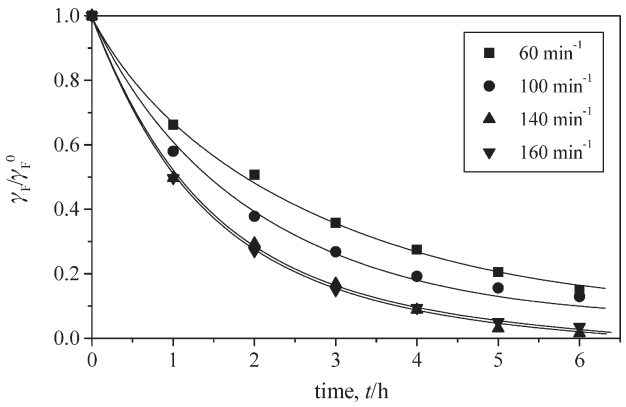
F i g . 3 – *Evolution of atropine content m in feed (F), membrane (M) and acceptor (A) solutions versus time at 160 min^{-1} (m_F^0 = 115 mg, pH$_F$ 9.5, T = 293 K)*



F i g . 5 – *Effect of agitation on atropine accumulation in the acceptor solution (γ_F^0 = 0.5 kg m^{-3}, pH$_F$ = 9.5, T = 293 K)*

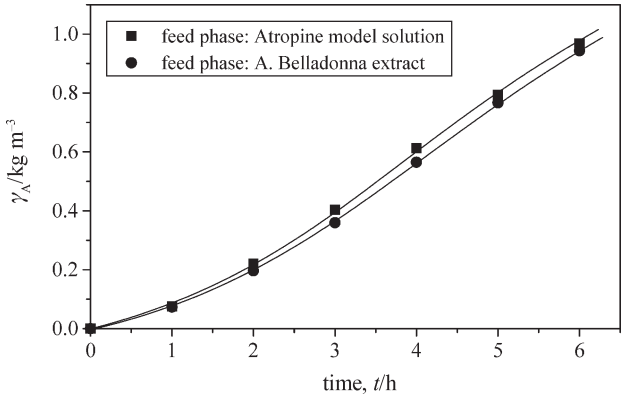
mass transfer coefficients provided by the laboratory device used. The latter was demonstrated by variation of the agitation speed, carrying out atropine pertraction runs at rotation velocities of the inner cylinder of n = 60, 100, 140 and 160 min^{-1}, respectively. Higher rotation speed was not applied, because of the increased risk of pertraction process deterioration due to eventual droplet formation and unacceptable intermixing between phases F and A.

The evolution of atropine dimensionless concentrations in the feed and in the acceptor solutions versus time at various stirring intensities is shown in Fig. 4 and Fig. 5, respectively. The obtained results show that the agitation improves, both, extraction and stripping processes. However, at higher velocities, the effect of agitation on atropine extraction is less pronounced. The considerable variation of stripping efficiency upon the agitation velocity allows concluding that this second transfer step is diffusion-controlled. As for the first step, the extraction process, the same conclusion could be made for the experimental runs at low and moderated agitation velocities. The insignificant improvement of the extraction process with the agitation above 140 min^{-1} gives ground to assume that, at these conditions, the process is limited by the kinetics of atropine extraction. Therefore, both, diffusion and kinetics of atropine extraction into chloroform control the extraction process, while the process of stripping is predominantly diffusion-controlled. The observed difference in the effects of agitation on transfer efficiency for the extraction and stripping steps could be explained by the deference between the volumes and organization of the aqueous solutions in the laboratory contactor used. Fig. 5 shows as well that during the pertraction process the atropine was concentrated in the acceptor solution: the atropine concentration in the acceptor phase attained three times its initial concentration in the feed solution.



F i g . 4 – *Effect of agitation on atropine extraction from the feed (γ_F^0 = 0.5 kg.m^{-3}, pH$_F$ 9.5, T = 293 K)*

As mentioned already, the selectivity of alkaloids recovery was a subject of this study, also. For this purpose, the native aqueous extract obtained after solid-liquid extraction of *A. Belladonna* roots, alkalised with ammonia buffer solution to pH 9.5, was used as a feed phase in the pertraction process. Obtained native extract contained many undesirable co-extracted substances: the content of tropane alkaloids, with respect to the mass of all extracted species after solvent removal, was less than 2.0 percent. Taking into account the presence in the feed solution of many other substances, including ingredients with surface active behaviour and therefore the increased risk of droplet formation and process deterioration, the study was carried out at moderate agitation velocity of 100 min^{-1}. The obtained results demonstrate almost the same rhythm of alkaloids accumulation in the acceptor solution as in the case of pertraction, using pure atropine solution at 100 min^{-1} (Fig. 6). Hence, the presence of accompanying substances in the feed phase has not a significant effect on the alkaloids, transfer rate. Pertraction process provided also a good purification of the tropane alkaloids, because the essential



F i g . 6 – *Comparison between the rate of alkaloids accumulation in the acceptor phase A when: a) the feed phase was a model solution of atropine (γ_F^0 = 0.5 kg m^{-3}, pH$_F$ 9.5, T = 293 K); b) the feed was native aqueous extract of A. Belladonna roots (γ_F^0 = 0.45 kg.m^{-3}, pH$_F$ 9.5, T = 293 K)*

part of co-extracted from the plant species remained in the feed or membrane solution. Pertraction process guarantees more than 30 times refinement of the extracted alkaloids: the content of tropane alkaloids in the residue after acceptor solution drying was found to be about 62.0 %, while in the dried native extract it was less than 2.0 %.

Conclusions

The obtained results show that atropine can be successfully recovered from its solutions applying a liquid membrane (pertraction) processes. As expected, phase agitation has positive effect on transfer rate, however, it was observed that these effects are not identical for extraction and stripping steps. Hence, one could suggest that, in the pertraction device used, both, diffusion and kinetics of atropine control the first, extraction step, while the solute stripping is predominantly diffusion-controlled process. Tropane alkaloids can be selectively recovered from native extract solutions containing a large amount of co-extracted species, as well. The presence of other extractable species in the feed solution has not a significant effect on alkaloids recovery. Pertraction process provides selective recovery, concentration and purification of the tropane alkaloids, because the majority of accompanying substances remains in the feed phase or membrane solution.

ACKNOWLEDGMENT

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List of symbols

c – concentration, mol dm^{-3}

D – distribution factor (distribution Nernst's law), –

m – mass, mg

n – rotation speed, m^{-1}

t – time, h, min

T – temperature, K

γ – mass concentration, kg m^{-3}

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