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Extraction of Pelletierine from *Punica granatum L.*by Liquid Membrane Technique and Modelling

Adel A. Al-Hemiri[*]**, Khalid M. Abed** [**]**, Ayaad W. Al-Shahwany**†
[*] Environmental Engineering Department-College of Engineering-University of Baghdad-Iraq
[**] Chemical Engineering Department-College of Engineering-University of Baghdad-Iraq
† Biology Department-College of Sciences -University of Baghdad-Iraq

Abstract

This work was conducted to study the extraction of pelletierine sulphate from *Punica granatum* L. roots by liquid membrane techniques. Pelletierine sulphate is used widely in medicine. The general behavior of extraction process indicates that pelletierine conversion increased with increasing the number of stages and the discs rotation speed but high rotation speed was not favored because of the increased risk of droplet formation during the operation. The pH of feed and acceptor solution was also important. The results exhibit that the highest pelletierine conversion was obtained when using two stages, (10 rpm) discs speed of stainless steel discs, (pH=9.5) of feed solution and (pH=2) of acceptor solution in n-decane. Assuming the existence of two thin reaction layers in the feed and stripping solutions, mathematical model was developed to describe the pelletierine transport. On the basic of the experimental data obtained under various conditions and the model proposed, it was found that the solute transfer into the liquid membrane is mainly diffusion-controlled.

Keywords: Liquid membrane, Extraction, pelletierine, *Punica granatum* L., Modelling.

الخلاصة

يهدف هذا البحث الى دراسة استخلاص دواء الپلترين سلفيت من جذور نبات الرمان بأستخدام تقنيات السائل الغشائي. الپلترين يستخدم بصوره واسعه في الطب ,حيث يستخدم للقضاء على الديدان الشريطيه والديدان المعويه وكعلاج للاسهال و الدازنتري ,وكمضاد بكتيري. وبينت الدراسات الحديثه بأنه يستعمل كمضاد فطري عندما يمزج مع الاملاح الحديديه , وهذه المجموعه نفسها استعملت لاختبار تأثيره على نمو فيروس الايدز.

السلوك العام لعملية الاستخلاص يشير الى ان تحول الپلترين يزداد عند زيادة عدد المراحل وسرعة دوران الاقراص لكن السرع العاليه كانت غير مفضله بسبب تكون قطرات على الاقراص وبالتالي نقصان بالمساحه السطحيه للطبقات المتجدده. ولكن درجة الحامضيه للمحلول الداخل والمستلم كانت محكومه ضمن حد معين.

اظهرت النتائج ان اعلى تحول للپلترين تم الحصول عليه عند استخدام مرحلتين , سرعة دوران الأقراص (10 دوره / دقيقة) و درجة الحامضية للمحلول الداخل =9.5 , درجة الحامضيه للمحلول المستلم = 2 وبأستخدام الديكان كسائل غشائي .

تم تطوير موديل رياضي لوصف عملية انتقال الپلترين على افتراض وجود طبقتي تفاعل رقيقه في المحلول الداخل والمستلم . على اساس البيانات التجريبيه التي تم الحصول عليها والنموذج الرياضي المقترح وجد ان انتقال المذيب الى الغشاء السائل مسيطر عليه بشكل اساسي من قبل الانتشار.

Introduction

Pelletierine $C_8H_{15}NO$ is a liquid alkaloid obtained from the root bark of *Punica granatum* Linn. It is anthelmintic and amoeboid. Pelletierine triggers, like strychnine, a raised stimulant reflex, which can escalate to tetanus and is effective against diverse tapeworms, ring worms and nematodes [1]. More recent studies showed that the simple extract have efficacy against the virulent intestinal bacteria *salmonella typhi* [2] and vibrio cholera, and most recently, viruses including *Herpes simplex* [3] and human immunodeficiency virus (HIV) and tumors [4].

Because of the structural diversity of alkaloids, there is no single method of their extraction from natural raw materials [5]. Most methods exploit the property of most alkaloids to be soluble in organic solvents but not in water, and the opposite tendency of their salts. Most plants contain several alkaloids. Their mixture is extracted first and then individual alkaloids are separated. Plants are thoroughly grounded before extraction. Most alkaloids are present in the raw plants in the form of salts of organic acids.

Liquid membrane extraction was introduced as an alternative separation technique to the liquid-liquid extraction and to the separation by means of solid polymeric membrane. This property of membranes makes them useful in the textile and food industries, in hydrometallurgy, medicine, biotechnology, environmental protection, in the separation of hydrocarbons and gases, and in the concentration and separation of amino acids, metal ions and other mixtures and suspensions [6, 7].

Liquid membranes have also been used extensively in the extraction of both organic molecules such as pesticides and herbicides in waters and wastewaters and metals in water and wastewater matrices as well as other organic pollutants [8].

The use of liquid membranes presents an attractive approach to produce valuable products of high quality at reduced costs, giving the opportunity to use as liquid membranes less powerful but more selective, less toxic and less expensive solvents than in the case of classical solvent extraction [9] [10] [11][12].

Membrane-based extraction methods have now gained popularity as methods of choice in the extraction of both ionisable and non-ionisable molecules from different samples. The main attractive features for these techniques include the use of minimal organic solvents, high selectivity and clean-up efficiency, with high enrichment factors. In most cases the overall cost involved is low due to the simplicity of the techniques which normally involve relatively fewer steps and handling procedures as compared to many other sample-preparation techniques [8].

A bulk liquid membrane process for recovery of medicinal compounds from dilute ammoniacal leach solutions was used in this work. Applying pertraction in a rotating film contactor (RFC) the alkaloid was successfully recovered from model solution of pelletierine, as well as from native aqueous extracts obtained from the *Punica granatum* L. roots.

The aim of this work was to study the process of pelletierine recovery from its solution using a liquid membrane technique and to apply this procedure for selective recovery of alkaloids from native aqueous extraction of *Punica granatum* L. roots to produce pelletierine sulphate medicine.

Experimental Work

Reagent and Analytical Methods Used

Various reagents were used in this work as liquid membrane, n-decane and n-nonane (99% BDH), n-hexane (95% ALDRICH) and methyl cyclohexane (95% HOPKIN & WILLIAMS). Ammonia (25% CHEM-SUPPLY) and sulfuric acid (98% GCC) were used to adjust the acidity of the aqueous solutions. The concentration of pelletierine in the strip solution was measured by UV-spectrophotometer SP-3000 (OPTIMA INC) at wave length λ=254 nm. The pH values of the aqueous solutions were measured by means of the laboratory pH meter (CRISON, MM40).

Experimental Equipment

The studies of pelletierine pertraction were carried out in a laboratory rotating film contactor (RFC) made from *Perspex* (poly methyl methacrylate). The lower part of contactor is divided, as shown in Fig.1, into four compartments: two for the feed and two for the acceptor solution. Compartments containing the same aqueous solution are interconnected. The organic membrane liquid occupies the common upper part of the contactor.

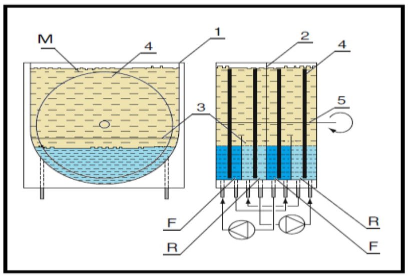

Fig.1, Experimental apparatus contactor: (1) body of rotating film contactor, (2) stage wall, (3) feed/stripping solution separating walls, (4) rotating disks, and (5) common shaft.

Four discs, 1 mm thick and 18 cm in diameter, mounted vertically on a common shaft, rotated in each compartment, providing continuous renewal of the aqueous films, covering the discs, as well as the stirring of all three liquids. The lower part of each disc (up to one-third of the disc diameter) is immersed in the corresponding aqueous solution and the larger, upper part is immersed in the organic membrane liquid, as shown in Fig.1. The aqueous solutions from mobile liquid films on the corresponding disc surfaces, which contact with the organic membrane .The two stages could be connected in a way permitting co-, counter or batch operation modes. To homogenise the aqueous solutions and to provide samples from each solution, both liquids were re-circulated by means of two peristaltic pumps Watson-Marlow Limited (Falmouth Cornwall England). For constant shaft rotating in small rpms: DC-motor (50 rpm) and variable DC power supply (Smart Power System, EMA series).

For the pertraction studies, the following three-liquid-phase system was used:

- Feed (donor) solution (F): 250 ml aqueous solution of ammonia, containing 1.13 mmol/liter of Pelletierine $C_8H_{15}NO$;
- Membrane solution (M): 500 ml : n-decane, n-nonane, n-hexane and methyl cyclohexane;
- Stripping solution (S): 250 ml aqueous solution of sulfuric acid.

Experimental Procedure

5.0 grams of *Punica granatum* L. roots were milled to fine powder and leached by 250 ml of buffer solution of (NH_3-$(NH_4)_2SO_4$) adjusted to appropriate pH. This solution was shaken for half an hour and filtered to obtain the feed solution.

The solution from the above step was poured into the RFC using glass funnel, the acceptor solution was adjusted to appropriate pH using few drops of sulfuric acid and placed into the second compartment, and the remainder volume was filled with the liquid membrane.

Mathematical Model

Overall mass transfer

The overall mass transfer under steady-state condition consists of three mass transfer processes: the mass transfer in feed phase, in the membrane phase and in strip phase.

This analysis starts with the basic mass transfer equation:

$J=K\,(C-C^*)$ ………….. (1)

Where J is the mass flux (mole/m^2.s), K is the mass transfer coefficient (m/s), C and C* is concentration and equilibrium concentration (mole/m^3) respectively.

Assuming a batch process and according to the model accepted, it is assumed that pelletierine is transported from the bulk of feed solution (F) to the reaction zone, representing a thin film of the feed solution adjacent to the F/M interface (Fig. 2). In general, the reaction zones are thinner than the diffusional layers [13]. The diffusional flux of pelletierine from the bulk of the donor phase to the organic membrane is given by the expression:

$J= K_{FM}.\,(C_F-C_F^*)$, or

$J= K_{FM}.\,(C_F-m_1C_M)$ …………… (2)

Where K_{FM} is the individual mass transfer coefficient between the feed and membrane solution, C_F and C_F^* are the concentration and equilibrium concentration of the feed solution respectively, m_1 is the equilibrium constant and C_M is the concentration in the organic membrane.

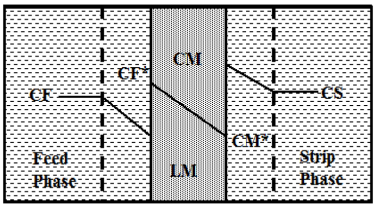

Fig.2, Mechanism of pelletierine transport across liquid membrane (LM)

By analogy the diffusional flux from membrane to the strip solution is given by the expression:

$J= K_{MS}.\,(C_M-C_M^*)$, or

$J= K_{MS}.\,(C_M-m_2C_S)$ ………….. (3)

Where K_{MS} is the individual mass transfer coefficient between the membrane and stripping solution, m_2 is equilibrium constant; C_S is the concentration in the stripping solution.

The overall diffusional flux from feed to strip solution is given by the following expression:

$J= K_{FS}.\,(C_F-C_S)$ ………….. (4)

Rewriting Eq. (2) & (3):

$$C_M=\frac{C_F}{m_1}-\frac{J}{K_{FM}.m_1} \qquad ……….. (2)^*$$

$$C_M=\frac{J}{K_{MS}}+m_2.C_S \qquad ………. (3)^*$$

By equating $(2)^*$& $(3)^*$

$$\frac{C_F}{m_1}-\frac{J}{K_{FM}.m_1}=\frac{J}{K_{MS}}+m_2.C_S$$

$$\frac{C_F}{m_1}-\ m_2.C_S\ =\ J\ (\frac{1}{m_1.K_{FM}}+\frac{1}{K_{MS}})$$

$$\frac{C_F}{m_1}-\ m_2.C_S\ =\ J\ (\frac{1}{K_{FS}}) \qquad ………. (5)$$

Where K_{FS} is the overall mass transfer coefficient.

Total resistance to mass transfer = sum of individual resistances; i.e.,

$$\frac{1}{K_{FS}}=\frac{1}{K_{FM}}+\frac{1}{K_{MS}} \qquad ……………. (6)$$

By substituting Eq. (2), (3) and (4) in to Eq. (6) and rearranging of equation (6) we get:

$$C_M = C_S\left(\frac{m_2-1}{1-m_1}\right) \qquad \text{..............} (7)$$

Material balance on solute

$$V_F\, C^o_F = V_F\, C_F + V_M\, C_M + V_S\, C\text{........} (8)$$

Where V_F, V_M and V_S are the feed, membrane and striping solution volume; C^o_F is the initial concentration of feed solution.

By substituting Eq. (7), into Eq. (8) and rearranging it we get:

$$C_F = C^o_F - C_S\left[\frac{V_S}{V_F} + \frac{V_M}{V_F}\left(\frac{m_2-1}{1-m_1}\right)\right]\text{......} (9)$$

Mass balance equations

With the stagnant feed and strip phase, the mass balance equation in the feed and strip phase using Eq. (5) is:

$$-V_F\frac{dC_F}{dt} = +V_S\frac{dC_S}{dt} = K_{FS}.\ A\ \left(\frac{C_F}{m_1} - m_2C_S\right) \qquad \text{..................}(10)$$

Where A is the interfacial area (represented by the face of the liquid membrane body facing the disc).

The equations are subjected to the following initial conditions:

$$\left.\begin{array}{l} C_F=C^o_F \\ C_S=0 \\ C_M=0 \end{array}\right\} \qquad \text{...............}(11)$$

At $V_F=V_S=250$ ml $=250$ cm^3,
$V_M=500$ ml $=500$ cm^3,
$A= 160$ cm^2
$C^o_F= 1.13$ mmol/liter
$m_1= 300$, $m_2= 0.003$

Where m_1 and m_2 is measured experimentally.

The mathematical model, based on the rate equation (Eq. (10) and (11), mass balance Eq. (9) and the earlier mentioned initial conditions describes the batch process of pelletierine pertraction in a rotating film contactor.

By substitute the boundary conditions and integrating the general Eq. (10)

$$\int_{C^o_F}^{C_F}\frac{dC_F}{C_F-0.54668} = -0.416K_{FS}\int_0^t dt\text{.....} \qquad \text{...}(12)$$

$$\ln\frac{C^o_F-0.54668}{C_F-0.54668} = 0.416\ K_{FS}.t \qquad \text{...}\ (13)$$

A plot of $\ln\frac{C^o_F-0.54668}{C_F-0.54668}$ against time (t) gives gradient of (0.416 K_{FS}), from which K_{FS} is calculated.

Results and Discussion

Selection of organic membrane

Four organic non-polar solvent were used as liquid membrane.

Low molecular weight solvents failed to give reasonable extraction efficiency while n-decane (with the highest molecular weight, 142.29) gave a good extracting ability and mass transfer coefficient as shown in Table 1and Fig. 3.

The increase in molecular weight of the organic compounds results in the increase of its organic properties, and since pelletierine is an organic compounds thus its solubility increases with the increase of the molecular weight of the solvent (likes dissolve like). These results are in agreement with the results which were obtained by Dimitrov et al [14].

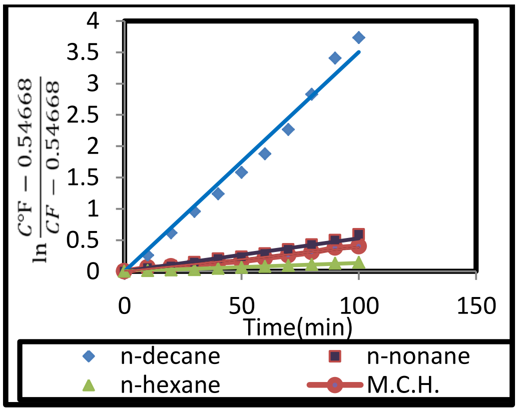

Fig. 3,Plotting equation (13) showing effect of type of solvent

Table 1, Values of mass transfer coefficient evaluated from Fig. (3), showing the effect of type of solvent

No.	Run	K_{FS} value (m/s)
1.	n-decane	$1.4*10^{-3}$
2.	n-nonane	$2.1*10^{-4}$
3.	n-hexane	$4.0*10^{-5}$
4.	M.C.H	$1.5*10^{-4}$

Effect of pH of Feed (donor) Solution on the Extraction Percentage and Mass Transfer Coefficient

In order to study the effect of pH in the feed phase(pH_F) on the mass transfer performance of BLM process, pH in the feed phase is adjusted with ammonia buffer solution. Experimental studies were carried out at various pH values (7-11). The overall mass transfer coefficient increases with increasing pH; and maximum mass transfer coefficient is observed at pH of 9.5; then it decreases as shown in Fig. 4 and Table 2.

It is to be concluded that the best pH for the feed solution that gave good extraction percentage is around 9.5. Such results are in agreement with that obtained by Dimitrov et al (2005 and 2006) [15] [16].

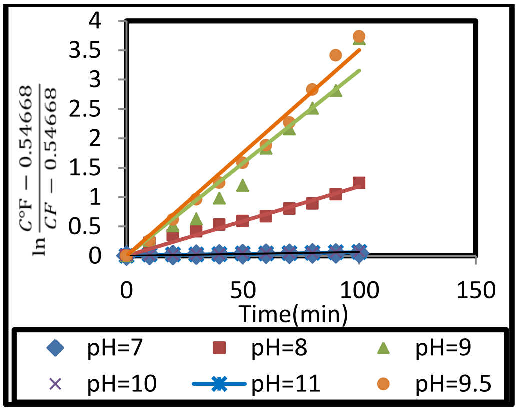

Fig. 4, Plotting equation (13) showing the effect of pH of feed solution

Table 2, Values of mass transfer coefficient evaluated from Fig. (4), showing the effect of pH of feed solution

No.	Run	K_{FS} value (m/s)
1.	pH_F=7.0	$2.8*10^{-5}$
2.	pH_F=8.0	$4.7*10^{-4}$
3.	pH_F=9.0	$1.3*10^{-3}$
4.	pH_F=9.5	$1.4*10^{-3}$
5.	pH_F=10	$2.8*10^{-5}$
6.	pH_F=11	$2.8*10^{-5}$

Effect of pH of Stripping (acceptor) Solution on the Extraction Percentage and Mass Transfer Coefficient

In order to study the effect of pH in the stripping phase(pHs) on the mass transfer performance of BLM process, pH in the feed phase is adjusted with few drops of sulfuric acid. Experimental studies were carried out at various pH values (1.5-3). The overall mass transfer coefficient increases with increasing pH; and maximum mass transfer coefficient is observed at pH of 2; then it decreases as shown in Fig. 5 and Table 3.

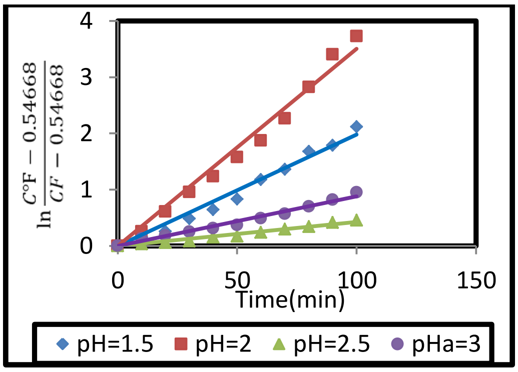

Fig. 5, Plotting equation (13) showing the effect of pH of stripping solution

It is observed that the pH of the aqueous acceptor phase played an important role on the extraction of pelletierine values when all the experimental conditions were kept constant except for the pH value of acceptor solution.

These results are in agreement with Dimitrov et al (2005 and 2004) [15, 17].

Table 3, Values of mass transfer coefficient evaluated from Fig. 5, showing the effect of pH of acceptor solution

No.	Run	K_{FS} value (m/s)
1.	pH_S=1.5	$1.2*10^{-5}$
2.	pH_S=2.0	$1.4*10^{-3}$
3.	pH_S=2.5	$3.9*10^{-6}$
4.	pH_S=3.0	$7.9*10^{-6}$

Effect of Discs Rotation Speed

Pertraction efficiency grows with the increase of discs rotation speed, due to the better agitation of all three phases and the faster renewal of the aqueous films. The overall mass transfer coefficient increases with increasing discs rotation speed as shown in Fig. 6 and table 4. This is similar to the results obtained by Dimitrov et al (2002) [13].

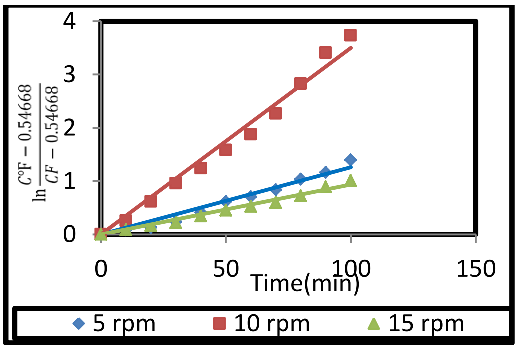

Fig. 6, Plotting equation (13) showing the effect of discs speed

On the other hand when higher rotation speed (higher than 10 rpm) is used also gives smaller amount of pelletierine sulphate and small value of mass transfer coefficient. Higher rotation speed was not favored because of the increased risk of droplet formation and process deterioration, where these agree with the results obtained by Dimitrov et al(2005) [18]and (2008) [14].

Table 4, Values of mass transfer coefficient evaluated from Fig. 6, showing the effect of disk speed

No.	Run	K_{FS} value (m/s)
1.	5 rpm	$5.0*10^{-4}$
2.	10 rpm	$1.4*10^{-3}$
3.	15 rpm	$3.7*10^{-4}$

Effect of Number of Stages

Increasing number of stages in RFC design play an important role to increase the yield or extraction efficiency and overall mass transfer coefficient. If two stages are used, this mean four hydrophilic disks that lead to an increase in surface area which will be in contact with the feed solution; therefore, pelletierine exhausting from roots increase and the same increase occurs in membrane. The overall mass transfer coefficient increases with increasing the number of stages as shown in Fig. 7 and Table 5 [19].

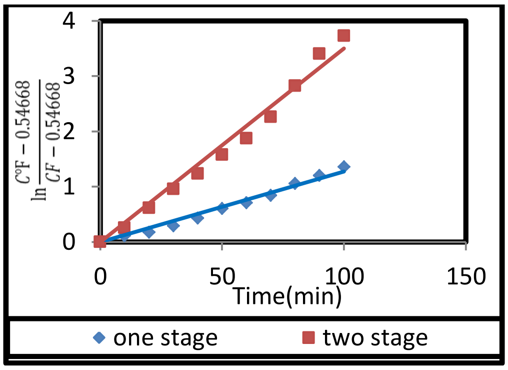

Fig. 7, Plotting equation (13) showing the effect of number of stages[19]

Table 5, Values of mass transfer coefficient evaluated from Fig. (6), showing the effect number of stages [19]

No.	Run	K_{FS} value (m/s)
1.	One stage	$5.0*10^{-4}$
2.	Two stage	$1.4*10^{-3}$

On the other hand increasing the number of stages leads to an increase in surface area which is in contact with the acceptor solution, thus increasing H_2SO_4 molecules in contact with the membrane. These results are in higher conversion than those from one stage.

Conclusions

A bulk liquid membrane technique for simultaneous extraction and stripping process based on surface renewal theory is presented. The pertraction in rotating film contactor is a suitable technique for pelletierine extraction from its solutions. The following results are found from BLM pertraction of pelletierine from *Punica granatum* L. roots:

- The non-polar n-decane was found to be the most suitable for pelletierine recovery by pertraction process.
- The best stripper (accepter) solution was H_2SO_4 with pH=2.
- The best pH value of feed (donor) solution was pH=9.5
- Three speeds were used, viz, 5, 10 and 15 rpm. The best speed was 10 rpm where high rotation speed was not favored because of the increased risk of droplet formation during the operation.
- Increasing the number of stages caused increased pelletierine extraction.
- Assuming the existence of two boundary layer reactions zones in the aqueous solution mathematical model of the process was proposed. On the basis of the experimental results and the model the overall mass transfer coefficients were evaluated.

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