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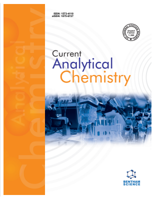
RESEARCH ARTICLE

Separation of Indole-3-acetic Acid from Tryptophan by Bulk Liquid Membrane



Ioana Diaconu[1], Hassan Y. Aboul-Enein[2,*], Cristina Orbeci[1], Andrei A. Bunaciu[3], Ecaterina A. Serban[4] and Elena Ruse[1]

[1]University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, 1-7 Polizu Street, Bucharest - 1, 011061, Romania; [2]Pharmaceutical and Medicinal Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Center, Dokki, Cairo 12622, Egypt; [3]S.C AAB_IR Research SRL, 7 Modoran Ene Street, Bucharest - 5, 051832, Romania; [4]National Research and Development Institute for Industrial Ecology ECOIND, 71-73 Drumul Podul Dambovitei, Bucharest - 6, 060652, Romania

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Abstract: ***Background***: Indole-3-acetic acid (IAA) is an important growth hormone for plants obtained by biosynthesis from tryptophan.

Aim: In this paper was studied the competitive transport of two biologically active compounds, indole-3-acetic acid (IAA) and tryptophan (TRP), through a liquid membrane.

Methods: The separation of the two compounds was obtained using a hybrid liquid membrane system having trioctylphosphine oxide (TOPO) as a carrier.

Results: The most important operational parameters of the system, and pH influence on the efficiency of the transport process, in correlation with the speciation diagrams of the two compounds: TRP and IAA, were studied. The evaluation of the transport process was performed by calculating the composition of the phases at the end of the transport process and the organic substrate flow at the membranes exit.

Conclusion: Due to the transport efficiency of over 90% in the case of IAA and the high selectivity at the transport between IAA and TRP, the procedure can be applied for the preparation of a sample containing these analytes.

Keywords: Bulk liquid membranes, indole-3-acetic acid, tryptophan, membrane separations, biosynthesis, trioctylphosphine oxide.

1. INTRODUCTION

Phytohormones are molecules that play a very important role in the regulatory processes of plants, having a well-defined role in the process of germination, growth and development, reproduction, as well as in the protective response of plants against biotic and abiotic stress [1-10].

Phytohormones are found in very low concentrations in plants [11], and therefore, it is necessary to separate or isolate them in order to purify and subsequently determine the optimal level of plant phytohormones [12].

The main classes of phytohormones are auxins, abscisic acid, brassinosteroids, cytokinins, ethylene, gibberellins, jasmonates, salicylic acid, and strigolactones.

Among the most studied and of particular importance in the development and growth of plants is indole-3-acetic acid (IAA). IAA is one of the most common phytohormones found in plants that can be synthesized through tryptophan (TRP)-dependent or independent pathways [13]. The IAA concentration in plants is adjusted through different modalities, including biosynthesis, with the help of bacteria, thus increasing the growth yields of the respective plants [14, 15].

Although multiple modalities of IAA synthesis have been proposed [13, 16-20] through both TRP-dependent and independent pathways, none of the proposed pathways are completely determined [21]. The path to obtain IAA from TRP is usually through some enzymes [17, 22, 23].

There are many discussed paths of synthesis of IAA starting from TRP. Some of the most important are: the indole 3-pyruvic acid (IPyA) pathway, starting from TRP, passing through IPyA and then indole 3-acetaldehyde (IAAld) and finally to IAA; the pathway of tryptamines (Tam), when from TRP synthesizes Tam then IAAld and finally IAA; the

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[*] Address correspondence to this author at the Pharmaceutical and Medicinal Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Center, Dokki, Cairo 12622, Egypt;
E-mail: haboulenein@yahoo.com

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pathway of indole acetic amide (IAAm), in these cases, starting from TRP, IAAm is synthesized, and finally, IAA is obtained [19, 24-26].

The liquid membranes have received special attention in the field of separations [27-35]. Liquid membranes (LM) are distinguished for their high yields, high selectivity, low energy costs, and they can be used in sample concentration processes and are easy to scale [34, 35]. These remarkable characteristics [34, 35] make them a good tool with multiple applications in the separation and recovery of metal ions [36], removal of organic compounds paints [37], and pharmaceutical compounds such as salicylic acid [38], or precursors thereof [39]. This method of sample preparation can be integrated in a tandem system that can allow selective analyses at low concentrations. Recent research suggests these systems as a new strategy in analytical chemistry. [40]

The paper presents a new method of separation and preconcentration of two compounds that can be found together in the process of biosynthesis, *i.e*., indole-3-acetic acid and tryptophan. The proposed method is based on the use of bulk liquid membranes because it represents a viable alternative to classical separation methods such as extraction, ionic exchange, and precipitation by eliminating their disadvantages. Liquid membranes represent an easy to use laboratory technique, an efficient technique that can be applied at low analyte concentration with high reproducibility and selectivity.

2. MATERIALS AND METHODS

2.1. Reagents and Chemicals

The reagents used were of analytical grade and without further purification. TRP and IAA used as transport substrate were purchased from Merck (Darmstadt, Germany). HCl, NaOH used for preparing the feed and receiving phase were purchased from Merck (Darmstadt, Germany). Trioctylphosphinoxide (TOPO), used as a carrier, was dissolved in chloroform and was also purchased from Merck (Darmstadt, Germany).

2.2. Equipment

Determination of IAA and TRP content in the aqueous phases of the membrane system was performed spectrophotometrically using a LAMBDA 750 spectrophotometer (Perkin Elmer Co).

The two compounds having characteristic absorption bands in the ultraviolet domain are as follows: indole-3-acetic acid at wavelength, λ_{max} = 280 nm and tryptophan at wavelength, λ_{max} = 216 nm [34]. The pH determination of the aqueous phases of the membrane system was performed with a pH-meter SevenMulti Mettler Toledo with a combined glass / AgCl electrode / Ag.

2.3. Procedure

The transport experiments were carried out in a tube transport cell presented in several previous papers [32, 41]. This study investigates the influence of pH in the feed phase upon the efficiency and selectivity of the mixture of IAA and TRP transport, because, in other previous studies [34, 35], there were optimized operational parameters such as the influence of organic substrate concentration in feed phase, a stripping agent in receiving phase, influence of the carrier type in the membrane.

Thus, during the experiments, the membrane system consisted of: feed phase (FP): TRP solution and / or IAA each in concentration of 10^{-4}mol / L [34, 35], in the pH range = 2-5 obtained with HCl and NaOH, or TRP and IAA at different molar ratios (TRP/IAA = 1-10) and pH=2; V_{FP} = 20 cm^3; membrane (M): TOPO, 10^{-2}mol / L in chloroform; V_M = 50cm^3 [35]; receiving phase (RP): NaOH solution, 10^{-2}mol / L, V_{RP} = 7cm^3 [34, 35].

For the preparation of the aqueous phases of the membrane system (feed and receiving phase), distilled water, saturated in chloroform, was used. For the preparation of the membrane, chloroform saturated in water was used.

In order to obtain the pH of the feed phase, the procedure is as follows: a solution of organic substrate (IAA/ TRP) of concentration 10^{-4}mol/L in the presence of HCl, 10^{-2} mol/L is titrated with a solution of the same substrate (the same concentration) in the presence of NaOH , 10^{-2} mol/L in order to obtain the desired pH=2-5.

The transport experiments were performed at room temperature of 20 ± 1ºC. The stirring speed of the phases was 180-200 rotations per minute and the transport time was 4 hours.

In order to choose the pH range in the experimental studies, the distribution diagrams of the chemical species in aqueous solution, as a function, of pH were used. The degree of formation, α_c, of $H_cA^{-(n-c)}$ species resulting in aqueous solution upon dissolving the H_nA polyacid is defined by the Equation (1):

$$\alpha_c = \frac{[H_c A^{(n-c)-}]_{\text{equilibrium}}}{[H_n A]_{total}} \tag{1}$$

Particularizing Equation (1) for compounds that form the aim of this study, there were obtained the relations with which the degree of formation of chemical species in aqueous solution based on pH is calculated.

Thus, for calculating the distribution diagrams in the case of IAA, the relations used were (Equations 2 and 3):

$$\alpha_0 = \frac{1}{1 + 10^{pKa - pH}} \tag{2}$$

$$\alpha_1 = \frac{1}{1 + 10^{pH - pKa}} \tag{3}$$

Where, α_0 represents the degree of formation of the R-COO$^-$ (A$^-$) anion and α_1 the degree of formation of the undissociated acid R-COOH (HA).

In the case of the TRP for calculating the distribution diagrams of the chemical species, the relations used were (Equations 4-6):

$$\alpha_0 = \frac{1}{1+10^{pK_{a_2}-pH}+10^{pK_{a_1}+pK_{a_2}-2pH}} \quad (4)$$

$$\alpha_1 = \frac{1}{1+10^{pK_{a_1}-pH}+10^{pH-pK_{a_2}}} \quad (5)$$

$$\alpha_2 = \frac{1}{1+10^{pH-pK_{a_1}}+10^{2pH-pK_{a_1}-pK_{a_2}}} \quad (6)$$

where: α_0 - the degree of formation of the species A^{2-}; α_1 - the degree of formation of the species HA^-; α_2 - the degree of formation of the species H_2A; K_{a1}, K_{a2} are the acidity constants.

The composition of the phases was calculated with the relationship (Equations 7):

$$\%mol = \frac{V_w \cdot C_w}{V_{FP_0} \cdot C_{FP_0}} \cdot 100 \quad (7)$$

where: V_w = volume of aqueous phase (feed or receiving), L; C_w = concentration of organic substrate in the aqueous phase at the end of the process, mol/L; V_{FP0} = volume of feed phase, L; C_{FP0} = initial concentration in the organic substrate of the feed phase, mol/L.

In order to assess the transport flux, the following relationship was used (Equations 2):

$$J = \frac{V_{RP} \times C_{RP}}{A \cdot t} \quad (8)$$

Where: V_{RP} - volume of the receiving phase, L; C_{RP} - concentration of the organic substrate in the receiving phase, mol/L; A - area of the interface membrane / receiving phase, cm^2; t - time, hour.

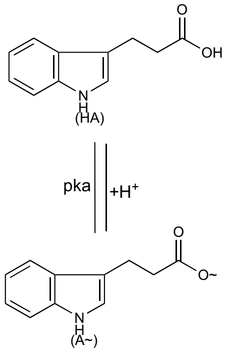
The selectivity (α) was calculates using Relation 9:

$$\alpha = \frac{J_{IAA}}{J_{TRP}} = \frac{D_{m.,IAA}}{D_{m,TRP}} \cdot \frac{K_{a,IAA}}{K_{a,TRP}} \cdot \frac{R_{IAA}}{R_{TRP}} \cdot \frac{\left[S_{IAA}\right]_w^0}{\left[S_{TRP}\right]_w^0} \quad (9)$$

3. RESULTS AND DISCUSSIONS

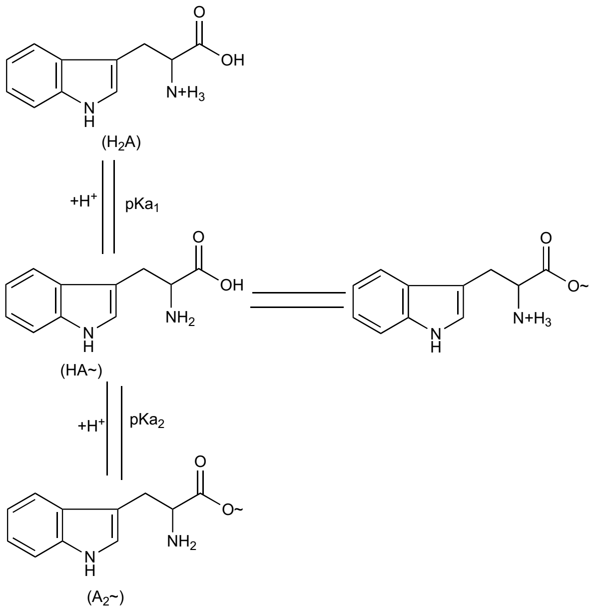
In aqueous solutions of acid, basic or ampholytic acid-base compounds are involved in proton exchange equilibriums controlled by pH.

Thus, for IAA Equilibrium, (**1**) is presented:



Equilibrium (**1**). Equilibria with transfer of protons in aqueous solution containing IAA.

While for the TRP, the following chemical Equilibrium (**2**) is considered :



Equilibrium (**2**). Equilibria with transfer of protons in aqueous solution containing TRP.

As a result of these equilibria in an aqueous solution, different chemical species are formed with different activity when transported through an organic liquid membrane. In general, the active forms in the process of transport through an organic liquid membrane are the undissociated forms that have a higher solubility in organic membranes [42]. Ionic forms are less active when transported through an organic liquid membrane, especially when they contain the Cl- anion [42]. In the case of the two studied compounds, IAA and TRP, the degree of formation of different chemical species in aqueous solution depending on pH was assessed with the help of speciation diagrams. The speciation diagrams obtained using relations (2-6) are presented in Figs. (**1** and **2**).

Using the pH as an operational parameter, the behavior of a membrane system containing the IAA and / or TRP as a feed phase depends on the pH of the feed phase studied. In some previous studies [34, 35], it was demonstrated that the

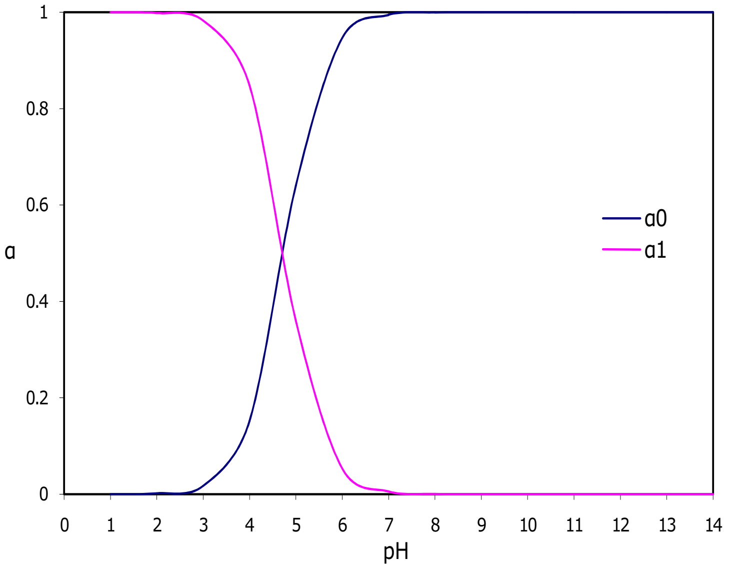


Fig. (1). Speciation diagram of IAA depending on the pH. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

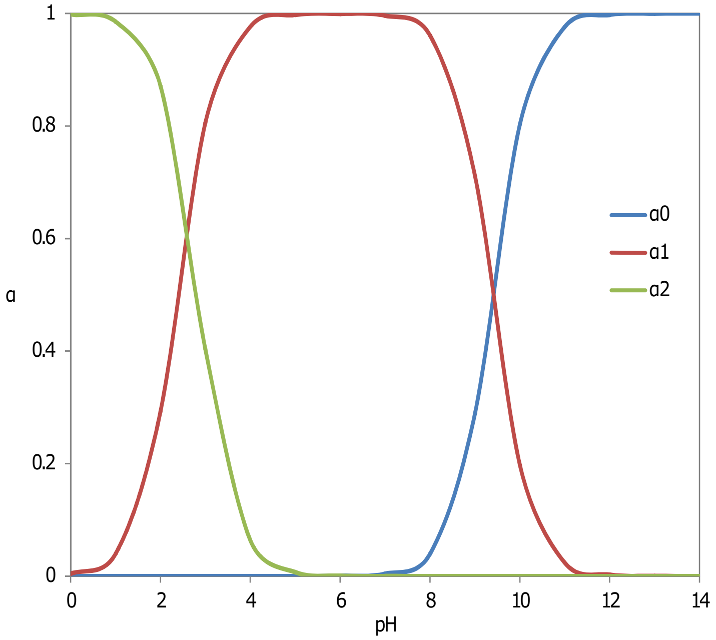


Fig. (2). Speciation diagram of TRP depending on the pH. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

best results are obtained at concentrations of an organic substrate of 10^{-4}mol / L in the feed phase and at a concentration of 10^{-2} mol / L NaOH in the receiving phase. Maintaining these parameters at constant values, the pH of the feed phase in the range 2-5 was varied. The pH values at which the transport experiments were performed were obtained with the help of HCl and NaOH.

The experimental study aimed to obtain experimental data on the efficiency of the transport process in a pH range of the source phase in which one compound is active in transport and the other is not. From the analysis of the speciation diagrams presented in Figs. (**1** and **2**), it is found that the pH range to be investigated is acidic. Therefore, in the pH range = 2-5, the transport of the two compounds through a liquid chloroform membrane containing TOPO transporter was studied. The carrier would act as an interphase catalyst. [43] It complexes the organic substrate at the M │FP interface, and through the membrane, it transports it to the M │RP interface where the decomplexation and release of the organic substrate take place. Following the reaction of the organic compound with the RP stripping agent (NaOH), a compound inactive during transport is obtained. This explains the transport of the organic compound against the concentration gradient (uphill transport).

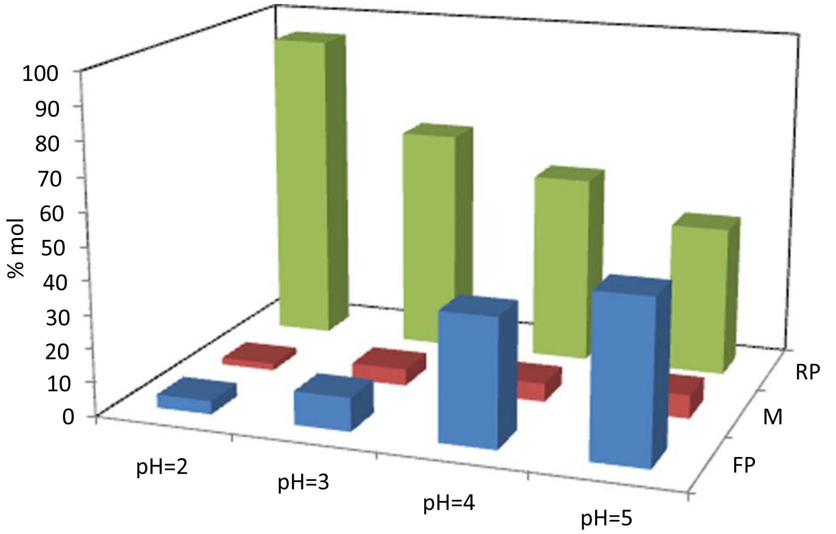


Fig. (3). The content of IAA and TRP in RP at the end of the transport process depending on the pH of the feed phase Experimental conditions: Feed phase –IAA or TRP solution each in concentration 10^{-4}mol/L at different pH values; Membrane – carrier TOPO, 10^{-2}mol/L in chloroform; Receiving phase - NaOH solution 10^{-2}mol/L. Transport time 4 hours. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

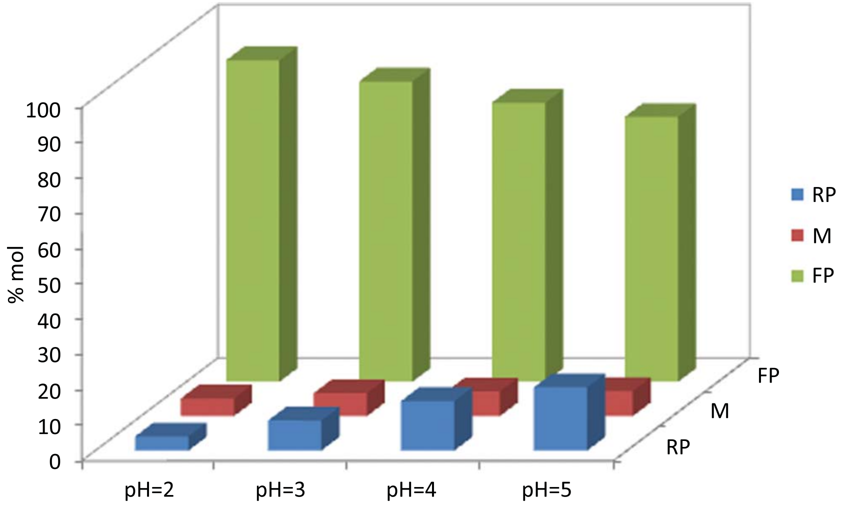


Fig. (4). The transport flows of the two compounds depending on the pH of the feed phase. Experimental conditions: Feed phase –IAA or TRP solution each in concentration 10^{-4}mol/L at different of pH values; Membrane – carrier TOPO, 10^{-2}mol/L in chloroform; Receiving phase - NaOH solution 10^{-2}mol/L. Transport time 4 hours. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

In order to assess the transport process, the composition of the phases, %mol, as well as the transport flow at the exit from the membrane was monitored as a function of pH.

The results obtained at the transport of the two compounds are presented in Figs. (**3** and **4**).

The best transport flows and yields are obtained for IAA at pH = 2. The efficiency of the process is more than 90%.

These results can be easily explained with the help of speciation diagrams. They demonstrate that the ionic forms that exist in the feed phase at different pH values are inactive at the transport through the liquid membrane. At pH = 2, with an efficiency of 99%, the IAA is active in the molecular form while being transported to the TRP, whereas, at the same pH, the ionic form (ammonium cation) is inactive.

On the basis of these studies, a membrane separation system of these two compounds was developed, which consisted of:

- Feed phase – IAA and TRP Solution, each at a concentration of 10^{-4} mol/L at pH = 2 (obtained with HCl);
- Membrane phase– carrier TOPO, 10^{-2}mol/L in chloroform;
- Receiving phase - NaOH solution 10^{-2}mol/L. The transport experiment time was 4 hours.

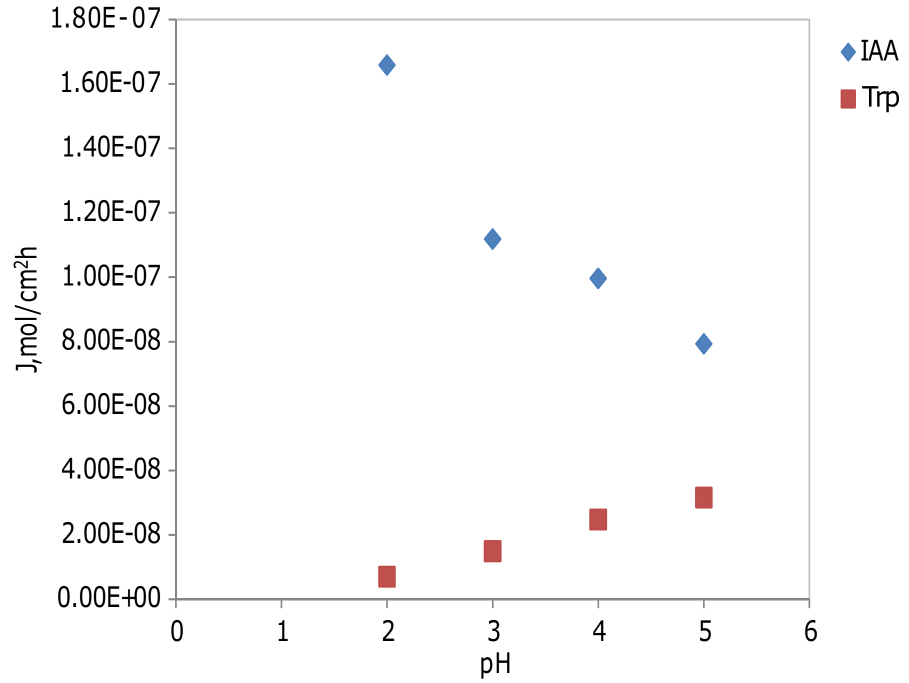


Fig. (5). Separation of IAA from TRP. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The values of the transport flow in the competitive transport process of IAA and TRP under the operating conditions (pH = 2) are presented in Fig. (**5**).

The analysis of the receiving phase indicates an IAA content of approximately 90%, while TRP is present in only 4%.

The large differences between the transport flows of these two compounds lead to the possibility of their separation.

Knowing the values of the transport flows of the two compounds (J_{IAA}, J_{TRP}) one can assess the selectivity (α) in the competitive transport process of IAA and TRP using relation 9:

$$\alpha = \frac{J_{IAA}}{J_{TRP}} = 22{,}5$$

This value corresponds to selective separations in the case of these membrane separation procedures [44]. The selectivity of these processes depends on many factors, as seen in relation 9.

It was found that the selectivity of the competitive transport processes depends on the diffusion coefficients of the complexes that the respective compounds form with the transporter ligand ($D_{m,IAA}$, $D_{m,TRP}$), the association constants between the organic substrate and the carrier ($K_{a,IAA}$, $K_{a,TRP}$), the distribution coefficients of the respective compounds in the membrane system phases (R_{IAA}, R_{TRP}), as well as the initial concentrations of these compounds in the aqueous phase ($[S_{IAA}]_w^0$, $[S_{TRP}]_w^0$).

The selectivity of the process is not significantly influenced by the excess of TRP from the feed phase, as it results from Fig. (**6**). In this figure, the efficiency of the process was assessed as a function of the molar ration TRP: IAA within the range 1:1 - 10:1.

Based on the results, it was concluded that the transport through bulk liquid membranes could be considered a viable technique for preparation of samples that contain TRP and IAA.

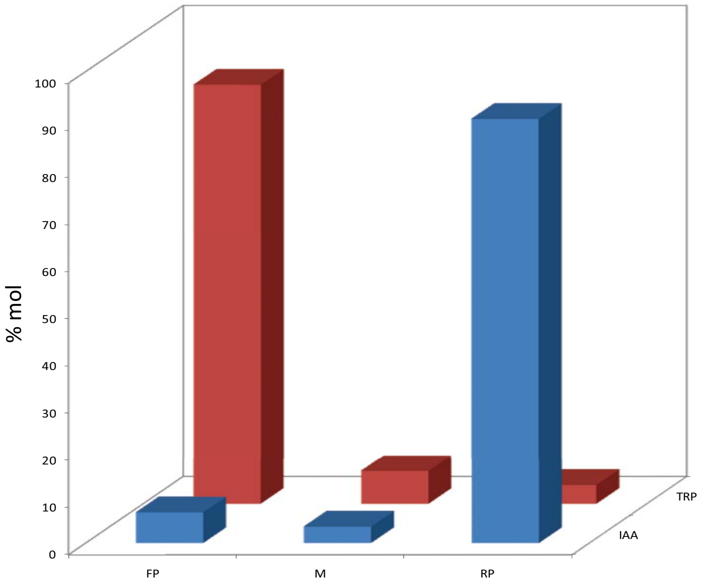
CONCLUSION

In this paper, the possibility of separating two compounds present in a biochemical synthesis process, namely the IAA of the TRP, was studied. For this purpose, the pH was used as an operational parameter. Based on the speciation diagrams, it was established that at pH = 2, IAA is mainly in the active molecular form for transport, while the TRP is in the inactive ionic form for transport.

The carrier TOPO acts as an interphase catalyst. For phosphoryl groups, the carriers' complexes the organic substrate, increasing its solubility in membranes. This action of the carrier does not occur in the case of TRP. The solubility of TRP in membranes cannot be increased due to Cl-pair anion.

Based on the results obtained, a membrane separation procedure of IAA from TRP was proposed, which consisted of:

- Feed phase: IAA and TRP each in concentration of 10^{-4} mol / L at pH = 2 (obtained with HCl),
- Membrane phase: TOPO 10^{-2} mol / L in chloroform,
- Receiving phase: NaOH, 10^{-2} mol / L.



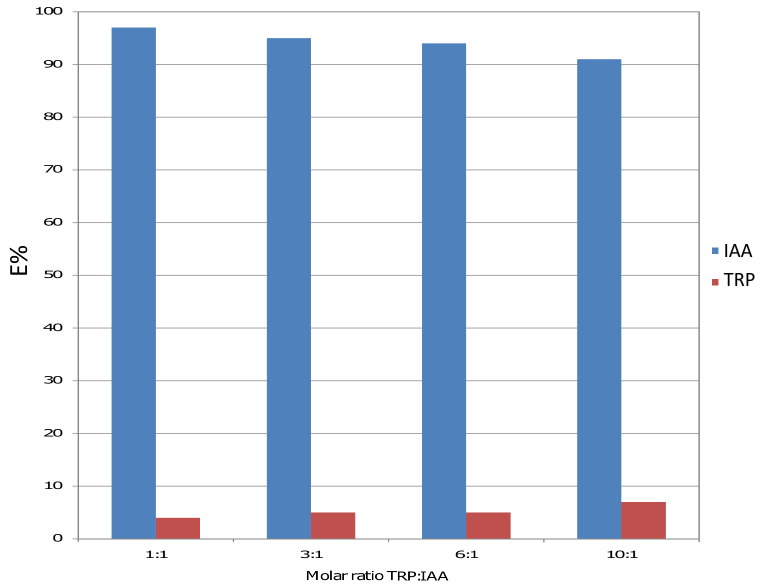


Fig. (6). The influence of the molar ratio TRP: IAA upon the efficiency (E%) of the transport of the two compounds. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Transport time was found to be 4 hours. Stirring speed of the phases was 180 rpm / min.

Following this procedure, a receiving phase containing more than 90% IAA and only 4% TRP is obtained.

Due to the transport efficiency of over 90% in the case of IAA and the high selectivity at the transport between IAA and TRP, the procedure can be applied for the preparation of a sample containing these analytes.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used in this study.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of this study are available within the article.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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