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Determination of Benzyl-hexadecyldimethylammonium 1,4-Bis(2-ethylhexyl)sulfosuccinate Vesicle Permeability by Using Square Wave Voltammetry and an Enzymatic Reaction

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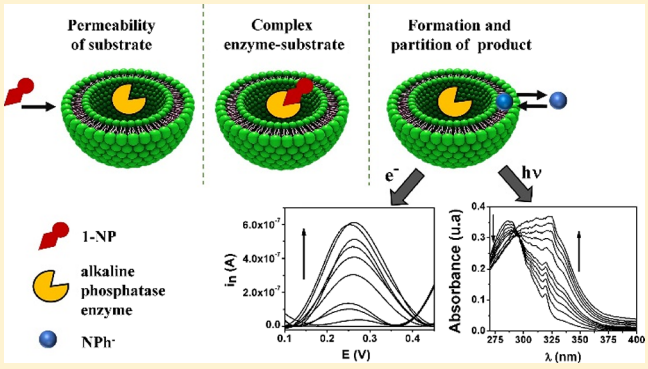
Supporting Information

ABSTRACT: This report describes the studies performed to determine the permeability coefficient value (P) of 1-naphthyl phosphate (1-NP) through the benzyl-hexadecyldimethylammonium 1,4-bis(2-ethylhexyl)sulfosuccinate (AOT−BHD) vesicle bilayer. 1-NP was added in the external phase and must cross the bilayer of the vesicle to react with the encapsulated enzyme (alkaline phosphatase) to yield 1-naphtholate (NPh^-), the product of the enzymatic hydrolysis. This product is electrochemically detected, at basic pH value, by a square wave voltammetry technique, which can be a good alternative over the spectroscopic one, to measure the vesicle solutions because scattering (due to its turbidity) does not make any influence in the electrochemical signal. The experimental data allow us to propose a mathematical model, and a value of $P = (1.00 \pm 0.15) \times 10^{-9}$ cm s^{-1} was obtained. Also, a value of $P = (2.0 \pm 0.5) \times 10^{-9}$ cm s^{-1} was found by using an independent technique, ultraviolet−visible spectroscopy, for comparison. It is evident that the P values obtained from both the techniques are comparable (within the experimental error of both techniques) under the same experimental conditions. This study constitutes the first report of the 1-NP permeability determination in this new vesicle. We want to highlight the importance of the introduction of a new method and the electrochemical response of the product generated through an enzymatic reaction that occurs in the inner aqueous phase of the vesicle, where the enzyme is placed.



1. INTRODUCTION

Recently, it has been proven that when two traditional ionic surfactants [anionic sodium 1,4-bis(2-ethylhexyl)sulfosuccinate (NaAOT) and cationic benzyl-*n*-hexadecyldimethylammonium chloride (BHDC)] are mixed in a 1:1 ratio and the inorganic salt is totally removed, a new catanionic surfactant benzyl-*n*-hexadecyldimethylammonium 1,4-bis(2-ethylhexyl)-sulfosuccinate (AOT−BHD) is synthesized.[1]

The interaction of ionic pairs from cationic and anionic surfactants induces the formation of molecular bilayers and large unilamellar vesicles (LUVs) spontaneously.[2−5] The spontaneous formation and shape of the AOT−BHD vesicles were characterized by using dynamic light scattering and small-angle X-ray scattering techniques.[1] One of the advantages of this system when spontaneously forming vesicles is that, in general, the LUVs are prepared by the use of different techniques (such as ultrasound or extrusion) which involve a long and laborious preparation.[6]

One of the major physicochemical studies of interest in vesicles is related to the permeability of different substrates through their bilayer structures.[7−10] These studies are important because the vesicles can be used as systems in controlled drug administration,[11,12] in the supply of anti-inflammatories for the skin,[13] or in diagnostic imaging[14] to mention some applications. The permeability can be measured in vesicles by different methods: the measures of electrical resistance or electrical capacitance of ions,[15] photochemical,[16,17] radioactivity counting using radioactive tracers,[18] spectrophotometry,[19−22] or electrochemistry.[23]

One interesting study for the determination of permeability was proposed by Sato et al.[16] In this work, the authors propose an experimental method with its corresponding mathematical model. In this method, the authors encapsulate the solute inside the unilamellar vesicles and continue in time the concentration of the solute in the external aqueous region of the vesicles and they do not separate the solute of the vesicle solution.

Faure et al.[24] propose a modification of the Sato's experimental method, which consists of determining the permeability of a solute through an enzymatic reaction. The idea is to study the reaction inside the vesicles because the confined enzymes are known to keep their activity upon confinement.[25] To accomplish this, the authors encapsulated an

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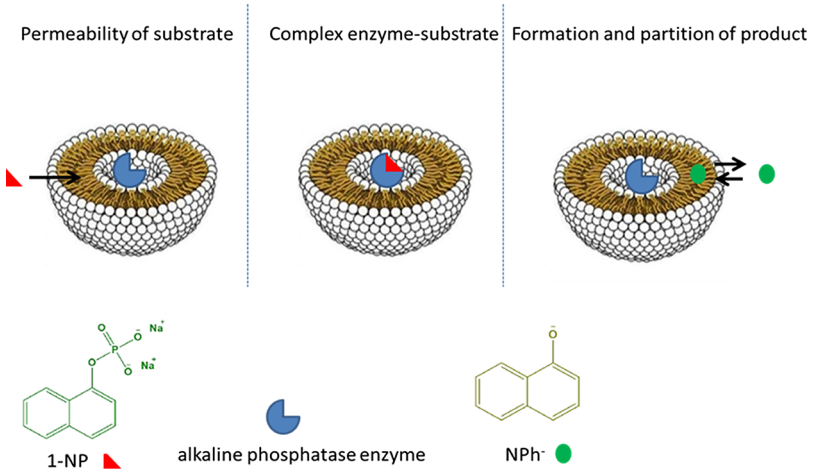
Scheme 1. Representation of the Processes Occurring in the Enzymatic Hydrolysis in the AOT−BHD Vesicles



enzyme inside the vesicles and their corresponding enzymatic substrate was added in the outer aqueous region of the vesicles. With this experimental design, it is intended to generate two concentration gradients, with which the permeability of both the substrate into the vesicles and the reaction product toward the external aqueous region is favored. According to the above, permeability determination could be followed for both the enzyme substrate and the reaction product concentrations, according to their corresponding physicochemical properties. In general, monitoring the solute concentration over time in the presence of vesicles is carried out by the use of spectroscopic techniques, which has a disadvantage when making the measurements in vesicle solutions because they are opalescent and make their determination difficult by these techniques.

It has been demonstrated that electrochemical techniques, such as cyclic voltammetry and square wave voltammetry (SWV), can be a good alternative to measure different drugs confined in vesicles.[26,27] Because scattering of the vesicle solutions makes the investigation of the concentrated solutions (due to its turbidity) almost unfeasible by using spectroscopic techniques, the electrochemical techniques present the advantages to study those molecules because scattering does not make any influence in the electrochemical signal. We have investigated 1-naphthol (NPh) in LUVs made with AOT−BHD, by electrochemical and spectroscopic techniques. The electrochemical results show that NPh experiences a partition process between the water and LUV bilayer phases, which are corroborated by absorption spectroscopic studies at pH 6.40 and 10.75.[28] The NPh electrochemical responses allowed us to propose a model to explain the electrochemical experimental results and, in conjunction with our measurements, to calculate the NPh partition constant (K_p) value between the phases.

In addition, at pH 10, ionized NPh (NPh^-) has the advantage that it is optical, hydrophilic, and electroactive and is the product of the enzymatic reaction of 1-naphthyl phosphate (1-NP) in the presence of the alkaline phosphatase (AP) enzyme, which allows us to follow its concentration by electrochemical and spectroscopic techniques.[27]

The aim of this study is to determine the 1-NP permeability coefficient across the bilayer of these new vesicles through an enzymatic reaction, at basic pH value, by using SWV. The enzyme is encapsulated inside the AOT−BHD vesicles, and the substrate must cross the bilayer to react and to yield the ionized NPh (NPh^-) species (see Scheme 1) which is detected electrochemically. The experimental data allow us to propose a mathematical model based on the Faure model[24] to obtain the 1-NP permeability coefficient. Also, this value was obtained by using the spectroscopic technique for comparison. The values obtained by these two methods are in very good agreement.

2. EXPERIMENTAL SECTION

2.1. Materials. NaAOT, from Sigma (>99% purity), was used as received. BHDC, from Sigma (>99% purity), was recrystallized twice from ethyl acetate.[29−31] NPh, AP enzyme, and the substrate sodium 1-NP, (>99% purity) from Sigma-Aldrich, were used without further purification.

2.2. Methods. The catanionic surfactant used, AOT−BHD, was synthesized by using a method reported in ref 1. Briefly, equimolar quantities of NaAOT/dichloromethane and BHDC/dichloromethane solutions were mixed and stirred at room temperature for 72 h.

A white precipitate appears because of the NaCl formation during stirring, from the original surfactant counterions. All NaCl salt were discarded from the dichloromethane solution by centrifugation. This solution was washed with small amounts of water until confirmed by the $AgNO_3$ test that the aqueous fraction is free of chloride ions. Dichloromethane was removed by vacuum evaporation. Once isolating AOT−BHD, it was thereafter purified by mixing with activated charcoal, then filtered through a plug of neutral alumina into a round-bottom flask, dried under vacuum, and stored under nitrogen. The surfactant obtained, AOT−BHD, was a colorless highly viscous liquid.

LUVs were obtained by hydrating the surfactant using a 0.05 M $LiClO_4$ solution, and the pH 10.75 value was adjusted using a Na(OH) 6.00 M solution. The pH was monitored before and after each spectroscopic and electrochemical measurement.

To encapsulate the enzyme inside the AOT−BHD LUV, the surfactant was hydrated with [AP] = 1×10^{-7} M in a 0.05 M $LiClO_4$ solution at pH 10.75. Then, the LUVs with the enzyme incorporated were separated from the free enzyme by gel chromatography as described below; the [AP] encapsulated was 1×10^{-8} M.

To prepare the gel filtration column, 2 or 3 g of Sephadex G-50 (Sigma-Aldrich) was added to 30 mL of $LiClO_4$ (0.05 M) solution at pH 10.75. The gel suspension was placed in a column (1 cm × 30 cm). A solution of $LiClO_4$ (0.05 M) at pH 10.75 was used as the mobile phase. To perform the separation, 2.0 mL of the vesicular solution was incorporated in the column and the fractions were immediately collected in vials of 2.0 mL, and the turbidity for each of the fractions was measured by using UV spectroscopy, between the wavelengths of 200 and 300 nm. From the results obtained by using UV, it was determined that fraction number 4 presented higher concentration of vesicles (greater turbidity), and this fraction was used for the studies.

2.3. Measurements. The absorption spectra were measured by using a Hewlett-Packard, ultraviolet−visible (UV−vis) 8453 spec-

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trophotometer equipment at (25.0 ± 0.1) °C. The path length used in the absorption experiment was 1 cm. To subtract the background contribution from the absorption spectra, the samples were measured in the absence of NPh at the same concentrations of AOT−BHD.

An Autolab PGSTAT 30 potentiostat, controlled by the GPES 4.8 software, was employed for SWV measurements. The characteristic parameters used to obtain square wave voltammograms were the following: 0.025 V for the square wave amplitude (ΔE_{SW}); 0.005 V for the staircase step height (ΔE_s); and 20 Hz for the frequency (f). The working electrode was a Pt disk (area = 0.126 cm^2). It was polished, sonicated, and copiously rinsed with distilled water. With the purpose of obtaining good response, the electrode was cycled several times in the blank solutions (0.05 M $LiClO_4$) prior to use until reproducible response in current was obtained. The counter electrode was a Pt foil of large area (2 cm^2). A freshly prepared Ag/AgCl quasireference electrode was used. All of the experiments were performed under a purified nitrogen atmosphere at 25.0 ± 0.1 °C. The pH measurements were performed by using an Orion 720A pH meter that was calibrated with commercial buffers. A pH = 10.75 was obtained for the different solutions by adjusting this pH with a solution of Na(OH) 6.00 M. OriginPro 8.0 was used for analysis and calculations.

3. MODEL USED

Faure et al.[24] propose an experimental method to describe the permeation process, in which it uses a modification of the differential equations proposed by Hamilton and Kaler.[32] For this, Faure considers a solute present simultaneously inside and outside of a unilamellar vesicle.

This modification is represented in eq 1

$$V_{out}\delta C_{out}(t) = \frac{SP}{2}[C_{in}(t) - C_{out}(t)]\delta t \quad (1)$$

where $C_{in}(t)$ and $C_{out}(t)$ correspond to the concentrations of the solute inside and outside the vesicle, respectively, as a function of time, and V_{out} is the volume of the external aqueous region of the vesicle. S denotes the sum of the surface area inside and outside the bilayer of the unilamellar vesicle, and P is the permeability of the solute through the bilayer expressed in cm s^{-1}. $\delta C_{out}(t)$ is considered as the variation of concentration of the solute in the external aqueous region in time; this concentration changes due to the passage of the solute through the thickness of the bilayer of the vesicle and is described in eq 1. The mass balance of the solute, and thus the total number of solute molecules, inside or outside of the vesicle, does not change with the time and is expressed in eq 2.

$$C_{tot}V_{tot} = C_{in}V_{in}(t) + C_{out}V_{out}(t) \quad (2)$$

Considering that the volume encapsulated in the vesicles (V_{in}) is much smaller than the volume of the external aqueous region ($V_{in} \ll V_{out}$), the total volume (V_{tot}) will be equal to the volume of the external aqueous region of the vesicles (V_{out}). Considering the above, eq 2 is expressed as a function of $C_{in}(t)$ according to eq 3

$$C_{in}(t) = \frac{V_{out}}{V_{in}}[C_{tot} - C_{out}(t)] \quad (3)$$

Replacing eq 3 in eq 1, simplifying the terms and expressing the resulting equation as a differential equation, eq 4 is obtained

$$\frac{d[C_{out}(t)/C_{tot}]}{dt} = \frac{SP}{2V_{in}}\left[1 - \frac{C_{out}(t)}{C_{tot}}\right] \quad (4)$$

The solution of the differential eq 4 is an exponential function, as shown in eq 5

$$C_{out}(t) = C_{tot} + [C_{out}(0) - C_{tot}]\exp[-t/\tau] \quad (5)$$

where $C_{out}(0)$ is the concentration of the solute in the external aqueous region at time zero, and the term τ is considered as the time taken by the solute to cross the bilayer, which is described in the following equation[24]

$$\tau = \frac{2V_{in}}{SP} \quad (6)$$

the term τ contains the term P (eq 6), which can be experimentally determined by measuring the variation of the solute concentration over time according to eq 5.

The ratio between the internal volume V_{in} and the surface area of the vesicle (S) can be described as eq 7, assuming a spherical geometry, where R is the vesicle radius, and L is the thickness of the bilayer[24]

$$\frac{V_{in}}{S} = \frac{1}{3}\frac{[R - L]^3}{R^2 + [R - L]^2} \quad (7)$$

Assuming that $L \ll R$, the following expression is obtained

$$\frac{V_{in}}{S} = \frac{R}{6} \quad (8)$$

Replacing eq 8 in eq 6, eq 9 is obtained. It must be noted that the term τ in this equation is valid only for a spherical unilamellar vesicle

$$\tau = \frac{R}{3P} \quad (9)$$

According to Scheme 1, in our experimental conditions, the substrate for the enzyme, 1-NP which is a nonelectroactive molecule, must cross the bilayer to react with the enzyme and yield NPh^- species whose concentration in time will be followed electrochemically. At $t = 0$, $C_{out}(0) = 0$ because there is no NPh^- in the outer aqueous phase; therefore, from eq 5, it can be deduced that the concentration of NPh^- over time is

$$C_{out}(t) = C_{tot}(1 - \exp(-t/\tau)) \quad (10)$$

It is important to highlight that, as will be explained later, under the assumption that the NPh^- species generated inside the vesicle immediately undergoes a partition process between the bilayer and the aqueous external phase, the delay in the appearance of the NPh^- signal is due to the time that takes 1-NP to cross the bilayer and react with the enzyme. Therefore, the experimental τ value was determined from eq 10 which contains the term P that can be considered as the permeability of 1-NP.

4. RESULTS AND DISCUSSION

4.1. Electrochemical Studies in Water. In Figure 1A, the net current of the SW voltammogram corresponding to NPh^- in water with $LiClO_4$ at pH 10.75 is shown. This result agrees with that obtained in the previous study[27] where an oxidation signal can be observed. In this SW voltammogram, the signal presents a peak potential (E_p) near 0.25 V which corresponds to the oxidation of NPh^-.

The obtained total current peak (i_T) is proportional to the concentration of NPh^- according to the following equation deduced for SWV[33]

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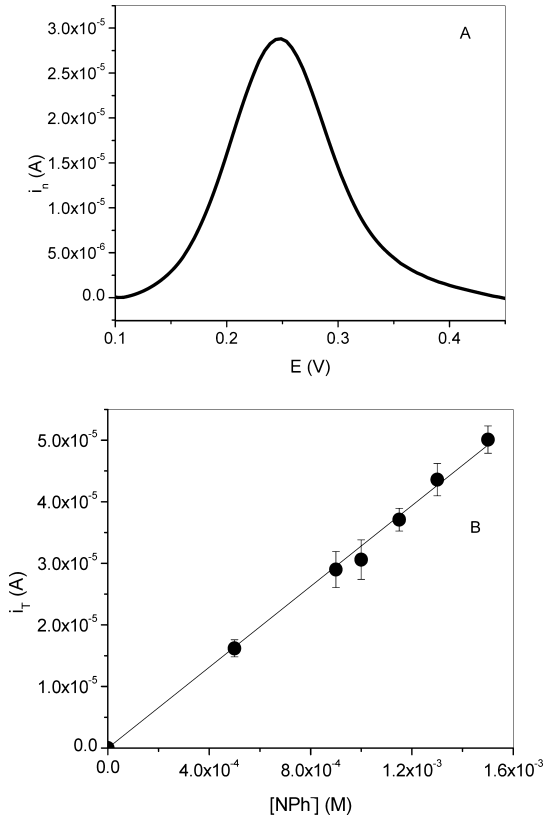

Figure 1. (A) Square wave voltammogram of NPh^- in $[LiClO_4]$ = 0.05 M, pH 10.75. $[NPh^-] = 9.00 \times 10^{-4}$ M, ΔE_s = 5 mV, ΔE_{SW} = 25 mV, and f = 20 Hz. (B) Dependence of the peak total current on the NPh^- concentration in $[LiClO_4]$ = 0.05 M, pH 10.75. Experimental data (•) and the solid line (—) are the linear fitting of the experimental data. Slope = 0.0328 ± 0.0001 A M^{-1} and R = 0.998.

$$i_T = \frac{nFA}{(\pi t_p)^{1/2}}\psi \times D_{NPh^-}{}^{1/2} \times C^*_{NPh^-} = B \times D_{NPh^-}{}^{1/2} \times C^*_{NPh^-} \quad (11)$$

where n is the electron number transferred per molecule, A is the electrode area, F is the Faraday constant, D_{NPh^-} is the electroactive species diffusion coefficient in water, ψ is the current function of SWV, and t_p is the pulse time of SWV and $t_p = 1/2f$, where f is the frequency of SWV. $C^*_{NPh^-}$ is the analytical concentration of the electroactive species.

From a plot of i_T as a function of $[NPh^-]$, a linear response is obtained, as shown in Figure 1B. Taking a value of $D_{NPh^-} = 2.17 \times 10^{-5}$ cm^2/s obtained from ref 27, from the slope a value of 7075.98 A cm^2 $s^{1/2}$ mol^{-1} is obtained for the constant $B = \frac{nFA}{(\pi t_p)^{1/2}}\psi$ (eq 11). Assuming that the oxidation mechanism in both media (without and with vesicles) does not change, the value of B will be used later (section 4.2.1) in the calculation of the concentration of NPh^- enzymatically generated.

Figure 2 shows SW voltammograms recorded over time when 1-NP is added to a solution that initially contains only the AP enzyme. As can be observed, NPh^- is generated which was detected electrochemically. The voltammograms change with time until a value of 30 min when the reaction is totally completed. It is noteworthy that in solution neither 1-NP nor the AP enzyme is the electroactive species; so, the signal only corresponds to the formation of NPh^-.

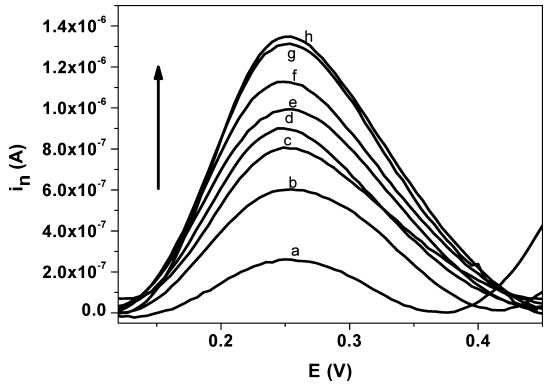

Figure 2. Square wave voltammograms of NPh^- formed enzymatically in the homogeneous medium at different reaction times: (a) 0.2, (b) 6, (c) 10, (d) 14, (e) 20, (f) 25, (g) 30, and (h) 35 min. [1-NP] = 1.95×10^{-3} M, [AP] = 1×10^{-7} M, ΔE_s = 5 mV, ΔE_{SW} = 25 mV, and f = 20 Hz in $[LiClO_4]$ = 0.05 M, pH 10.75.

4.2. Electrochemical in AOT−BHD Vesicles. Figure 3 shows the SW voltammograms as a function of time, for [1-NP] = 1.00×10^{-3} M; [AP] = 1×10^{-8} M; and [AOT−BHD] = 2 mg mL^{-1}. In this experience, the enzyme is only present in the inner aqueous phase of the vesicle. As can be observed, after 6 min, the NPh^- oxidation signal starts to appear. According to Scheme 1, the delay time could indicate that the NPh^- formation is retarded because 1-NP must cross the bilayer to be in contact with the enzyme to begin the reaction. The same feature was observed for different 1-NP concentrations, namely, 7.45×10^{-4}, 1.95×10^{-3}, and 4.95×10^{-3} M (results are shown in Figure S1 in the Supporting Information section).

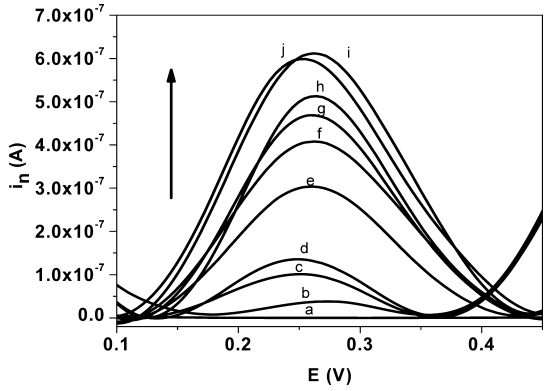

Figure 3. Square wave voltammograms of NPh^- formed enzymatically in the AOT−BHD medium at different reaction times: (a) 0.2, (b) 6, (c) 9, (d) 15, (e) 24, (f) 39, (g) 42, (h) 45, (i) 51, and (j) 60 min. [AOT−BHD] = 2 mg mL^{-1}, [1-NP] = 1.00×10^{-3} M, [AP] = 1×10^{-8} M, ΔE_s = 5 mV, ΔE_{SW} = 25 mV, and f = 20 Hz in $[LiClO_4]$ = 0.05 M, pH 10.75.

A question may arise here about the origin of the delay time observed. Is it because of the 1-NP or NPh^- permeation across the bilayer? To get an answer, we performed the following experiment. Vesicles with only NPh^- species present in the inner water phase were prepared, and immediately after preparation, the electrochemical signal is detected. This led us to think that the NPh^- species permeates the membrane much faster than the 1-NP one. Furthermore, previous work has shown that NPh^- undergoes a partition process between water and the vesicle bilayer.[28] Thus, for our enzymatic reaction

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performed in the AOT−BHD vesicles, we can conclude the following: (i) the electrochemical signal is due to NPh^- generated inside the vesicles; (ii) the delay time is because the 1-NP species must cross the bilayer to meet the enzyme; and (iii) the net current observed comes from the NPh^- species in water and in the vesicle bilayer (Scheme 1).

Figure 4A shows the dependence of i_T generated for NPh^- formed enzymatically with time, at different 1-NP concentrations. It is important to mention that i_T is not only proportional to the concentration of the electroactive species but also depends on other factors such as the diffusion coefficient of NPh^- and the parameter B determined previously (section 4.1, eq 11). In addition, once NPh^- is generated inside the vesicle, it diffuses toward the outside phase and a partition process is present, as discussed above. Therefore, i_T values will have the contribution of NPh^- species in the vesicle bilayer (NPh_v^-) and in the external aqueous phase (NPh_f^-). Thus, to determine the total concentration of NPh^- species at every time, we will have to use a mathematical model.

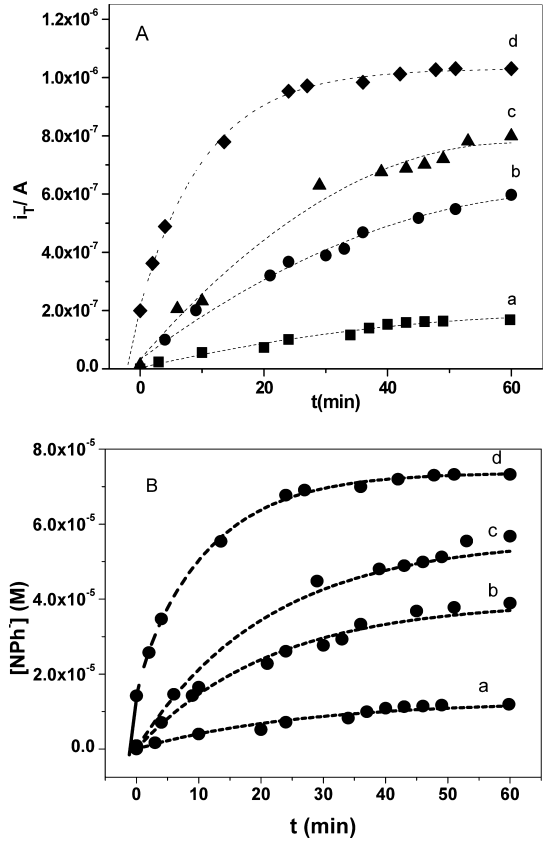

Figure 4. Dependence of i_T (A) and $[NPh^-]$ (B) from SWV formed enzymatically over time from different [1-NP]: (a) 7.45×10^{-4}, (b) 1.00×10^{-3}, (c) 1.95×10^{-3}, and (d) 4.95×10^{-3} M. ΔE_s = 5 mV, ΔE_{SW} = 25 mV, and f = 20 Hz in $[LiClO_4]$ = 0.05 M, [AOT−BHD] = 2 mg mL^{-1}, and [AP] = 1×10^{-8} M, pH 10.75.

4.2.1. Determination of $[NPh^-]$ from i_T Values. As discussed previously, in the presence of AOT−BHD LUVs, the NPh^- species exists in two possible phases: the external aqueous and the vesicle bilayer. Assuming that both species are discharged at the same potential (ref 28), i_T can be expressed as eq 12

$$i_T = B(D_{NPh_f^-}{}^{1/2} \times [NPh_f^-] + D_{NPh_v^-}{}^{1/2} \times [NPh_v^-]) \quad (12)$$

where $D_{NPh_f^-}$ and $D_{NPh_v^-}$ are the diffusion coefficients of NPh^- species free and at the vesicle bilayer, respectively. Both values were already determined in refs 27 and 28. As the measurements were made in the same medium and under the same conditions and assuming that the oxidation mechanism is the same inside and outside of the vesicle, B is the constant that was determined in Figure 1B.

The NPh^- distribution process can be expressed by the following equations, and the values were determined in ref 28

$$AOT{-}BHD + NPh_f^- \overset{K_p}{\rightleftarrows} NPh_v^- \quad (13)$$

$$K_p = \frac{[NPh_v^-]}{[NPh_f^-][AOT{-}BHD]} \quad (14)$$

By replacing $[NPh_v^-]$ in eq 12, we obtain i_T as a function of $[NPh_f^-]$ as shown in eq 15

$$i_T = B \times (D_{NPh_f^-}{}^{1/2} \times [NPh_f^-] + D_{NPh_v^-}{}^{1/2} \times [NPh_f^-] \times [AOT{-}BHD] \times K_p) \quad (15)$$

By replacing the values of $D_{NPh_f^-}$ (2.17×10^{-5} cm^2/s),[27] $D_{NPh_v^-}$ (0.6×10^{-7} cm^2/s),[28] K_p (0.84 mg/mL),[28] and the constant B (7075.98 A cm^2 $s^{1/2}$ mol^{-1}), the value of i_T is obtained as a function of $[NPh_f^-]$ (eq 16)

$$i_T = 38.02[NPh_f^-] \quad (16)$$

According to the mass balance

$$[NPh^-]_T = [NPh_v^-] + [NPh_f^-] \quad (17)$$

where $[NPh^-]_T$ is the NPh^- total concentration. Combining eqs 14, 16, and 17, we obtain eq 18 that shows $[NPh^-]_T$ as a function of i_T

$$[NPh^-]_T = 71.08 i_T \quad (18)$$

This equation allows us to determine the $[NPh^-]$ at different times from the i_T values shown in Figure 4A. Figure 4B shows the dependence of $[NPh^-]$ over time obtained for different [1-NP]. From this plot, we will obtain the 1-NP permeability value as shown in the next section.

4.3. Determination of the Permeability of 1-NP. *4.3.1. Calculating the Permeability Value by Using SWV.* From Figure 4B, it can be observed that for t > 50 min, the enzymatic reaction has been completed. From the plateau, the maximum $[NPh^-]$ values were obtained; C_{tot} of NPh^- formed from each [1-NP].

Equation 10 in our conditions can be expressed as eq 19

$$\frac{C_{out}(t)}{C_{tot}} = (1 - \exp(-t/\tau)) \quad (19)$$

where $C_{out}(t) = [NPh^-]$ was obtained for each time.

Figure 5 shows the variation of experimental values of the relation $\frac{C_{out}(t)}{C_{tot}}$ as a function of time for the different [1-NP] used. As expected, all data converge in a single curve as predicted by eq 19 if τ is independent on the substrate concentration (1-NP). The data were fitted to eq 19 by using a nonlinear regression method to find the value of τ = 19.21 ± 0.39 min. From this τ value and using eq 9 for R = 40 nm,[1] a P value of $(1.00 \pm 0.15) \times 10^{-9}$ cm s^{-1} is obtained.

Because no data of P are known for 1-NP, we decided to use an independent technique to compare the P values obtained.

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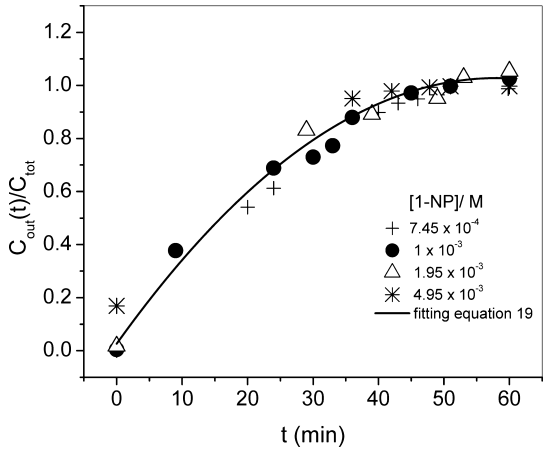

Figure 5. Dependence of the relation $\frac{C_{out}(t)}{C_{tot}}$ of NPh^- formed over time for different [1-NP] from SWV. The different symbols are experimental data obtained from different [1-NP] over time, and the solid line (—) is the fitting of the experimental data to eq 19.

The next section shows the studies carried out by using UV−vis spectroscopy.

4.3.2. Calculating the Permeability by Using UV−Vis Spectroscopy. Figure 6A shows typical absorption spectra for the 1-NP hydrolysis at different reaction times in the AOT−BHD vesicles for [1-NP] = 2.5 × 10^{-5} M. We follow the product of the reaction (NPh^-) at λ_{max} = 332 nm spectrophotometrically. For the determination of the concentration of NPh^- in the enzymatic hydrolysis, we used its coefficient of molar absorptivity (ε) obtained in these experimental conditions. The ε values found in the AOT−BHD vesicles at pH 10.75 are 9485 ± 193 cm^{-1} M^{-1}. From the absorbance values shown in Figure 6A and the ε value, the NPh^- concentrations as a function of time ($C_{out}(t)$) were determined for the enzymatic hydrolysis. Figure 6B shows the variation of experimental values of the relation $\frac{C_{out}(t)}{C_{tot}}$ as a function of time for [1-NP] = 2.5 × 10^{-5} M, where C_{tot} is [NPh^-] when the reaction has been completed. These data were fitted to eq 19 by using a nonlinear regression method to find the value of τ = 13.2 ± 0.9 min. From this value of τ and by using eq 9 with R = 40 nm,[1] a value of P = (2.0 ± 0.5) × 10^{-9} cm s^{-1} was found. It is evident that the P value obtained by using UV−visible spectroscopy is comparable (within the experimental error of both techniques) to that obtained by means of electrochemical techniques under the same experimental conditions.

5. CONCLUSIONS

In this study, we determined the 1-NP permeability coefficient in AOT−BHD vesicles at basic pH value through an enzymatic reaction by using SWV. The enzyme was encapsulated inside AOT−BHD vesicles, and 1-NPh^-, the product of the reaction, was detected electrochemically. Here, we demonstrated that, using a simple mathematical model, it is possible to determine the value of the permeability of 1-NP through the vesicle bilayer, which gives a value of (1.00 ± 0.15) × 10^{-9} cm s^{-1}. This value was also obtained by using other independent spectroscopic techniques for comparison; the permeability value obtained was (2.0 ± 0.5) × 10^{-9} cm s^{-1}, which shows a very good agreement of these two techniques. We highlight that

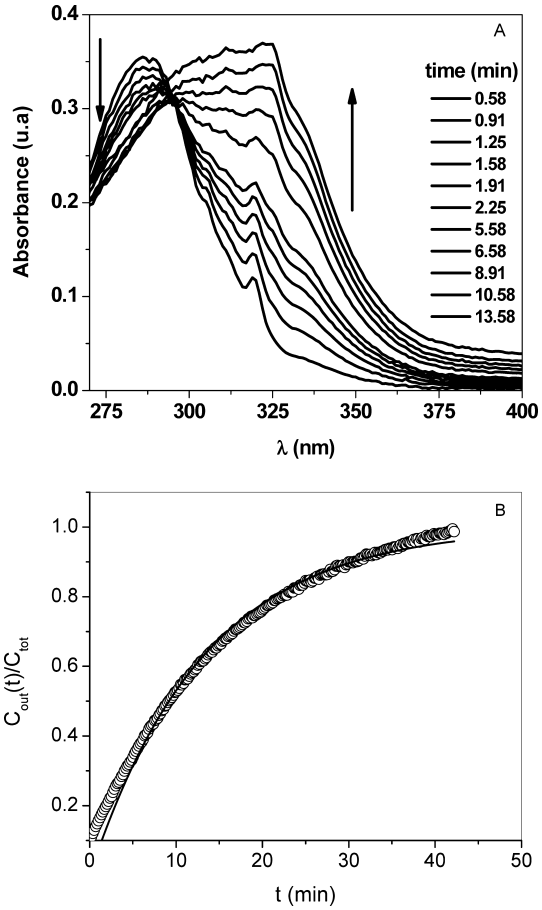

Figure 6. (A) Absorption spectra of the hydrolysis of 1-NP catalyzed by AP over time in [$LiClO_4$] = 0.05 M; [AOT−BHD] = 2 mg mL^{-1}; [1-NP] = 2.5.10 × 10^{-5} M; and [AP] = 1.0 × 10^{-8} M, pH 10.75. (B) Dependence of the relation $\frac{C_{out}(t)}{C_{tot}}$ of NPh^- formed over time for [1-NP] = 2.5 × 10^{-5} M from UV spectroscopy at λ_{abs} = 332 nm. The solid line (—) is the fitting of the experimental data to eq 19.

using an enzymatic hydrolysis reaction inside the AOT−BHD vesicles and a simple mathematical model, it is possible to determine the permeability of the bilayer to 1-NP by using a new method which can be a good alternative over the spectroscopic one and to measure the vesicle solutions because scattering (due to its turbidity) does not make any influence in the electrochemical signal.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.7b03001.

Square wave voltammograms of NPh^- formed enzymatically in the AOT−BHD medium over time for different [1-NP] (PDF)

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Notes

The authors declare no competing financial interest.

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