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How the external solvent in biocompatible reverse micelles can improve the alkaline phosphatase behavior†

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In the last decade, the nature of the nonpolar solvents that can be part of reverse micelles (RMs) has been the topic of several investigations to improve their applications. In this sense, the hydrolysis of 1-naphthyl phosphate catalyzed by the enzyme alkaline phosphatase (AP) was used as a probe to investigate the effect of the change of the external solvent on RMs formulated with the anionic surfactant sodium diethylhexyl sulfosuccinate (AOT). As external nonpolar solvents, two biocompatible lipophilic esters, isopropyl myristate and methyl laurate, and the traditional nonpolar solvents, *n*-heptane and benzene, were used. The results were compared among the RMs investigated and with the reaction in homogeneous media. Thus, the effect of the nanoconfinement as well as the impact of the replacement of a conventional external nonpolar solvent by biocompatible solvents were analyzed. The results indicate that the catalytic efficiency in the AOT RMs is larger than that in homogeneous media, denoting a different hydration level over the AP enzyme, which is directly related to the different degrees of nonpolar solvent penetration to the RM interface. Our findings demonstrated that toxic solvents such as *n*-heptane and benzene can be replaced by nontoxic ones (isopropyl myristate or methyl laurate) in AOT RMs without affecting the performance of micellar systems as nanoreactors, making them a green and promising alternative toward efficient and sustainable chemistry.

1. Introduction

Reverse Micelles (RMs) are a class of organized media with nanometric sizes, composed of at least three components: a polar solvent (generally water), a surfactant, and some nonpolar solvent. The solution formed is optically transparent and thermodynamically stable.[1] In the polar core of the RMs, besides water, polar molecules can be dissolved provided that they are non-soluble in the external nonpolar organic solvent.[1–5] This nanosized aqueous core is very useful for a wide range of applications such as nanoparticle and polymer synthesis, the enhancement of chemical reaction rates, and even to model water in biological confinement since the water properties are deeply affected when is restrained to a nanoscopic scale.[6–11] Thus, properties such as microviscosity, micropolarity, hydrogen bond abilities, and electron donor or acceptor properties are deeply modified.[1,12] Consequently, RMs have diverse applications in the field of science and technology.[13–20] One of the most important applications is their use as nanoreactors, where a chemical reaction occurs inside the micelle.[21,22]

To prepare RMs different surfactants are used but the majority of the investigations utilize the anionic surfactant sodium diethylhexyl sulfosuccinate (AOT, Scheme 1) since it can form RMs in a wide range of nonpolar solvents. The properties of the AOT RMs can be altered by changing different variables with the most important ones being temperature and nonpolar solvent, and mainly by varying the water content in the solution. Thus, the water content is usually defined as W_0 = [H_2O]/[surfactant].[1]

Due to the diverse applications of RMs,[1,23] in the last decade, there has been much interest in assessing the nature of nonpolar solvents that can be part of these organized

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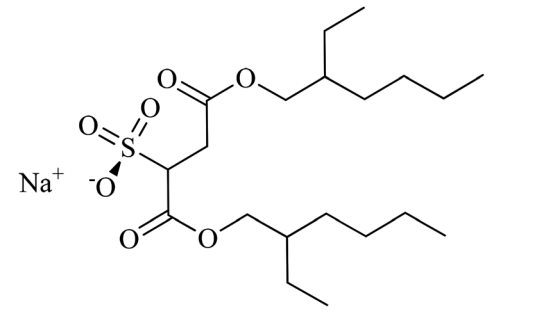
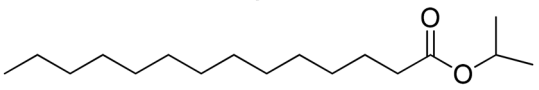
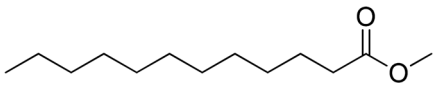
†Electronic supplementary information (ESI) available: Fully experimental procedures, the molar absorptivity values (ε) for the hydrolysis product of 1-naphthyl phosphate (1-naphtholate) in the different systems (Table 1). Also contains the following figures: Fig. S1. Changes in absorbance value at 336 nm as a function of time for the hydrolysis of 1-naphthyl phosphate catalyzed by AP in water. Fig. S2. Variation of the concentration of the product 1-naphtholate in buffer solution at different reaction times. Fig. S3–S5. Effect of 1-naphthyl phosphate concentration on the initial rate (v_0) of 1-naphthyl phosphate hydrolysis catalyzed by AP in methyl laurate/AOT/water, benzene/AOT/water, and *n*-heptane/AOT/water RMs. See DOI: 10.1039/d0ob02371j



AOT



Isopropyl Myristate



Methyl Laurate

Scheme 1 Chemical structure of AOT and the biocompatible nonpolar solvents used.

systems.[24–26] The traditional RMs cause environmental problems, particularly considering their application to an industrial scale, in food science or drug delivery. The majority of traditional nonpolar solvents used are toxic or at least nonenvironmentally friendly. In this sense, to generate RMs, more biocompatible alternative solvents have been tested.[16,23,24,27–38] For example, lipophilic esters such as isopropyl myristate (isopropyl myristate, Scheme 1) have been widely used in biologically resembling systems, pharmaceutics, and drug delivery.[16,24,25,32,34,39–42]

In particular, in our group, AOT RMs have been formulated with isopropyl myristate and another lipophilic ester (methyl laurate, Scheme 1) with interesting results.[24,25,41] Girardi *et al.*[24] showed that methyl laurate and isopropyl myristate can be used as a nonpolar phase in AOT RMs without any cosurfactant. Dynamic light scattering experiments revealed a linear increase of size with the water content as expected for a spherical non-interacting droplet. However, at the same W_0, methyl laurate-based RMs are larger than the isopropyl myristate ones. Also, the aggregation number is larger for the methyl laurate RMs. This fact was explained considering that it is possible that isopropyl myristate due to its structural properties can better penetrate the interface leading to smaller RMs. An interesting feature that should be mentioned is the strong similarity observed when comparing droplet sizes, the maximum amount of water solubilized, and the aggregation number values between *n*-heptane and methyl laurate AOT RMs and benzene and isopropyl myristate AOT RMs. More recently, the micropolarity and the hydrogen-bond ability of the interfaces of these RMs, methyl laurate/AOT/water, and isopropyl myristate/AOT/water, were monitored through the use of the solvatochromism of a molecular probe (1-methyl-8-oxyquinolinium betaine) and Fourier transform infrared spectroscopy (FT-IR).[25] The studies confirm that due to the dissimilar penetration of these oils into the interfacial region, the micropolarity and the encapsulated water structure are different. Hence methyl laurate-based RMs have a more polar interface than the isopropyl myristate ones. Moreover, water molecules form stronger hydrogen bond interactions with the polar head of AOT in methyl laurate compared to that in isopropyl myristate. These results evidence the importance of the nonpolar phase penetration in RMs which allows modulating the interface characteristics and could give an alternative when classical toxic solvents need to be replaced by a non-toxic solvent for biological applications.

In this sense, RMs allow the solubilization of hydrophilic biological molecules such as enzymes in organic solvents, so they are also of great interest in enzymatic catalysis.[36,43–49] In many cases, it has been found that the application of RMs produces favorable effects compared to that of the homogeneous medium (bulk water), such as superactivity[50,51] or greater stability of the enzymes.[52,53] Furthermore, RMs are versatile nanoreactors in the study of enzymatic catalysis since they allow controlling the activity and stability of biomolecules through various factors, such as water content or the type of surfactant.[43,50]

There are numerous reports on enzymatic reactions in RMs. These studies include various enzymes such as α-chymotrypsin,[46–48,52,54] trypsin,[55] lipase,[45,56] peroxidase,[57] alcohol dehydrogenase,[58] pyruvate kinase,[59] cutinase,[60] lysozyme,[61] and alkaline phosphatase,[62–68] among others.

Alkaline phosphatases (APs) are a family of plasma membrane-bound glycoproteins that catalyze the hydrolysis of a wide variety of phosphate esters in an alkaline medium (pH values between 8 and 11).[69–73] They are found in many human tissues, including bones, intestines, kidneys, liver, placenta, and white blood cells.[71,72] In the first step of the reaction mechanism, the substrate binds to the AP enzyme forming an E–S complex, leading to the phosphorylation of the serine enzyme residue in the active site with the product's release. This is followed by the hydrolysis of the phosphoenzyme, forming an enzyme–phosphate complex. Finally, the dissociation of the phosphate bound to the enzyme occurs. This last step is the limiting one for the reaction in alkaline aqueous solution.[74] However, the reaction mechanism is different in RMs, changing the enzymatic hydrolysis's limiting step from phosphate release to phosphorylation or dephosphorylation.[63,68]

AP has been widely studied, although, compared to other enzymes, such as α-chymotrypsin, its catalytic behavior in RMs has been less explored. Ohshima *et al.*[62] were pioneers in the study of AP in RMs. They used micelles made up of *n*-heptane and different surfactants AOT, phosphatidylcholine, phosphatidylethanolamine, and phosphatidic acid. The studies revealed that the enzymatic reaction is highly dependent on

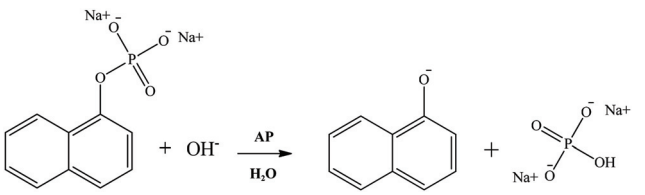
the water content and the surfactant used. Furthermore, the authors analyzed the effect of the external nonpolar solvent on the enzymatic activity of AP entrapped in AOT RMs. They used four *n*-alkanes (carbon number: 7–10), finding very similar results in all cases.

More recently, various studies continue to evaluate the enzymatic activity of AP in RMs, and the dependence of their activity on the properties of the system. AP was studied in isooctane/AOT,[63,64,66–68] *n*-octane/AOT,[75] and *n*-decane/AOT[65] RMs among other systems. In these reports, the effects of pH, water content, temperature, and surfactant concentration have been determined, finding that there are optimal working conditions for each system in which AP presents its maximum activity. Although all the parameters evaluated affect the enzymatic activity, studies have shown that the most relevant parameter in all cases is W_0 since AP's kinetic properties in RMs, such as the initial velocity of the reaction, strongly depend on the water content. This indicates that the AP enzyme inside the RM is very sensitive to the amount of water around it and that the optimal W_0 depends on several factors.

As mentioned before, there are various reports on the effects of pH, water content, temperature, and surfactant concentration on the catalytic behavior of AP in RMs. However, studies of the effect of the external nonpolar solvent are scarce. Furthermore, most of the AP studies in RMs have been carried out using traditional nonpolar organic solvents, such as isooctane,[63,64,66–68] *n*-octane,[75] and *n*-decane.[65]

In the present work, the study of the hydrolysis of sodium 1-naphthyl phosphate catalyzed by the enzyme AP (Scheme 2) in AOT RMs is reported, using as nonpolar solvents *n*-heptane, benzene, isopropyl myristate, and methyl laurate. In this way, the effect of the change of the external solvent on the enzyme activity is analyzed. It is important to note that isopropyl myristate and methyl laurate were chosen as they are considered nontoxic lipophilic solvents.[24,38,42,76–81] Hence, their use makes them a promising alternative to traditional solvents used in biocatalysis.

Finally, it is important to note that enzymatic catalysis studies using biocompatible solvents such as isopropyl myristate and methyl laurate as external solvents in RMs are practically not found in the literature. Therefore, our findings provide valuable information on the use of biocompatible organized media as nanoreactors. It is shown that toxic solvents such as *n*-heptane and benzene can be replaced by isopropyl myristate or methyl laurate in AOT RMs without affecting the performance of micellar systems as nanoreactors, making them a promising green alternative towards efficient and sustainable chemistry. Interestingly, the enzyme seems to work more efficiently because of the confinement effect, as we will show.



Scheme 2 Hydrolysis of sodium 1-naphthyl phosphate catalyzed by the enzyme alkaline phosphatase (AP).

2. Results and discussion

To evaluate the effect of the type of external nonpolar solvent in AOT RMs on the enzymatic behavior of AP, first, we monitored the kinetic reaction in homogeneous media (buffer solution), and second, inside the different RMs. Thus, we worked with the RMs formed by isopropyl myristate/AOT/water, methyl laurate/AOT/water, benzene/AOT/water, and *n*-heptane/AOT/water analyzing the effect of the external solvent's change on the kinetic parameters (k_{cat} and K_M) of the reaction catalyzed by AP.

2.1. Enzymatic reaction in water

Fig. 1 shows the typical UV-vis absorption spectra of the progress of the hydrolysis reaction of 1-naphthyl phosphate in buffer solution (pH = 10) at different times. A decrease in the intensity of the absorption band of the substrate 1-naphthyl phosphate at 285 nm, an increase in the absorbance of the product (1-naphtholate) at 336 nm with time, and a clear isosbestic point at λ = 301 nm were observed. These facts indicate the formation of the 1-naphtholate without the presence of intermediates and/or product decomposition.

To obtain the kinetic parameters of the hydrolysis of 1-naphthyl phosphate catalyzed by AP (k_{cat} and K_M), the initial reaction rates were determined at different concentrations of 1-naphthyl phosphate, keeping the enzyme concentration constant (see the ESI† for the detailed procedure). Fig. 2 shows

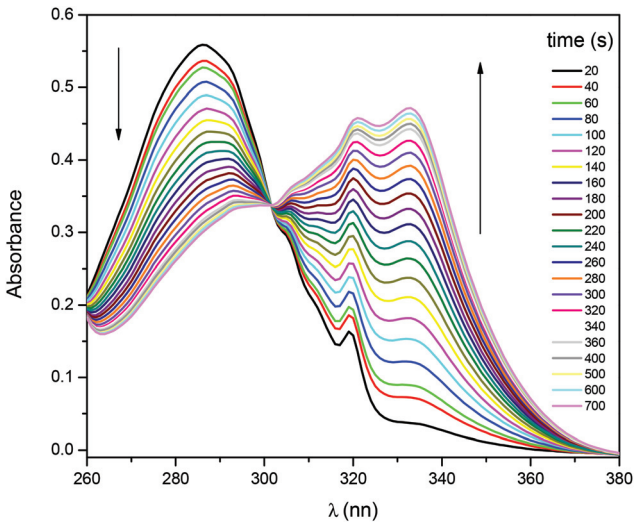


Fig. 1 UV-vis spectra for 1-naphthyl phosphate hydrolysis catalyzed by AP in $Na_2CO_3/NaHCO_3$ buffer solution, at 35 °C. [1-Naphthyl phosphate] = 2×10^{-4} M. [AP] = 1×10^{-7} M. [Buffer] = 0.01 M, pH = 10.

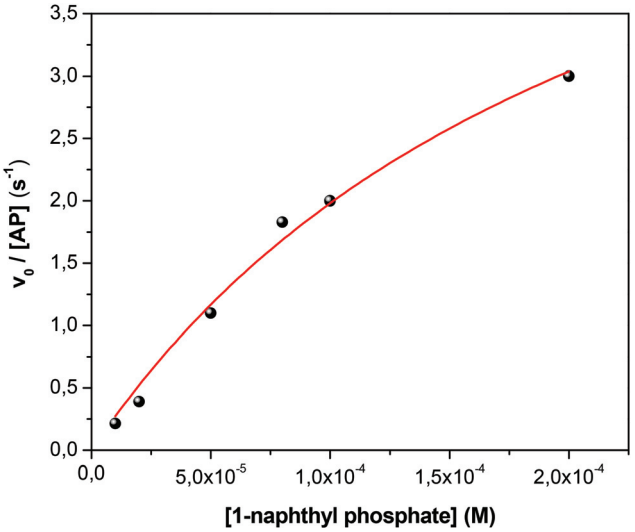


Fig. 2 Effect of 1-naphthyl phosphate concentration on the initial reaction rate (v_0) of 1-naphthyl phosphate hydrolysis catalyzed by AP in buffer solution at 35 °C. [AP] = 1 × 10^{-7} M. [buffer] = 0.01 M, pH = 10. The solid line represents the fit according to eqn (1).

the experimental data evaluated in homogeneous solution and the corresponding fitting according to the Michaelis–Menten mechanism by using the well-known eqn (1).

$$\frac{v_0}{[\mathrm{AP}]} = \frac{k_{\mathrm{cat}}[1-\text{naphthyl phosphate}]}{K_{\mathrm{M}} + [1-\text{naphthyl phosphate}]} \quad (1)$$

From the fitting of Fig. 2, the k_{cat} and K_{M} values were determined and the catalytic efficiency ($k_{\mathrm{cat}}/K_{\mathrm{M}}$) was calculated.

As can be observed from Fig. 2, the hydrolysis of 1-naphthyl phosphate catalyzed by AP follows perfectly the reaction model proposed by Michaelis and Menten.[82,83] The nonlinear fit of the graph using eqn (1) allowed us to determine values of k_{cat} = 6.5 ± 0.1 s^{-1} and K_{M} = (23 ± 1) × 10^{-5} M in homogeneous media. Consequently, the catalytic efficiency ($k_{\mathrm{cat}}/K_{\mathrm{M}}$) of AP is 28 200 ± 1400 M^{-1} s^{-1}.

2.2. Enzymatic reaction in AOT RMs

The enzymatic activity of AP was also evaluated in the RM systems formed by isopropyl myristate/AOT/water, methyl laurate/AOT/water, benzene/AOT/water, and *n*-heptane/AOT/water. The polar solvent used was a 0.01 M Na_2CO_3/$NaHCO_3$ buffer solution at pH 10. Furthermore, all studies were carried out at W_0 = 10 and [AOT] = 0.1 M. The W_0 value was chosen equal to 10 to compare exclusively all the systems with the same amount of water. Although the variation in the water content may affect the kinetics parameters, in this case the focus is on the analysis of the effect of the external solvent in RMs.[47,48,52,68,84]

The aqueous AOT RMs show a particular behavior with regard to their pH.[85–87] Recently, using an electrochemical technique we suggested that the value of the pH of the AOT RM interface cannot be assumed to be equal to that of the original water solution used to prepare the RMs and, in a large range of water content (W_0 = 8–28) it is weakly acidic even when a buffer solution of pH around 12 is used.[87] Similarly, previous studies showed that the nonionized form of phenols is the most stable species when entrapped in the AOT RMs; thus the organized media appears to act as a pH buffer system that prevents ionization.[86] In this sense, to evaluate what happens with the hydrolysis reaction of 1-naphthyl phosphate catalyzed by AP in AOT RMs even incorporating buffer solution at pH = 10, the appearance of a UV-vis absorption band centered at 323 nm, corresponding to 1-naphthol, was tested. Fig. 3 shows the absorption spectra once the hydrolysis reaction of 1-naphthyl phosphate in isopropyl myristate/AOT/water, methyl laurate/AOT/water, benzene/AOT/water, and *n*-heptane/AOT/water RMs is completed (time = 15 minutes). Furthermore, the final UV-vis spectrum of the hydrolysis of 1-naphthyl phosphate in the homogeneous solution is shown, where the absorption band corresponds to 1-naphtholate.

As can be observed, in all the AOT RMs the naphtholate species is not detected and only the formation of 1-naphthol is observed. These results reinforce the evidence observed earlier that the AOT RMs act as buffer solution and the interface is acidic.[87] In this sense, it is important to remark that the concept of pH in the RMs is not straightforward as in the bulk. Several examples of unusual results have been observed before that support this fact. At the interface, processes such as deprotonation of phenols or protonation of amines are phenomena not detected in AOT RMs. These results were explained as consequences of the different interfacial interactions (mainly strong hydrogen bond interactions with the sulfonate group of the anionic surfactant) present when water is entrapped inside RMs. Thus, solutes show unexpected nucleophilicities, strong electrostatic interactions, inhibition of protonation or deprotonation processes, polarity and viscosity parameters among others, and these are examples of evidence found in RMs.[86–89] Interestingly, Fig. 3 also confirms

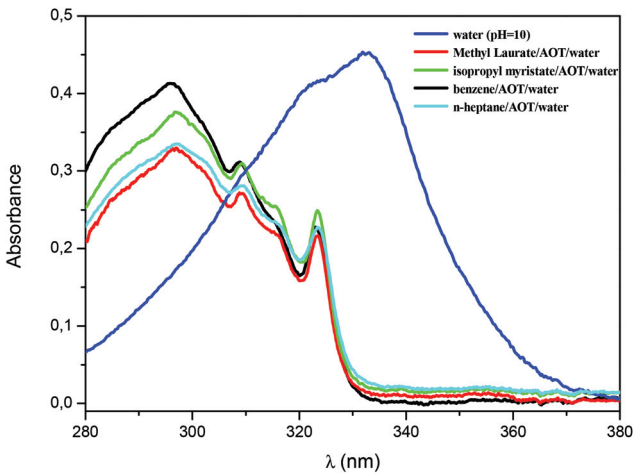


Fig. 3 Absorption spectra for 1-naphthyl phosphate hydrolysis catalyzed by AP in nonpolar solvent/AOT/water RMs, at 35 °C. W_0 = 10, [AOT] = 0.1 M, [1-naphthyl phosphate] = 1 × 10^{-4} M. [AP] = 1 × 10^{-7} M. Reaction time = 15 minutes.

that the enzymatic reaction takes place at the interface and not in the pool. This is because if the reaction is performed in the pool,[85] the naphtholate formation should be detected. Moreover, the enzymatic reaction under the same experimental conditions was investigated but with the addition of water without controlling the pH value. The same kinetic parameter values were obtained reinforcing that the reaction takes place at the RM interface.

As in the homogeneous media, the initial velocity values (v_0) were determined as a function of the substrate concentration in each micellar system at constant W_0 = 10 and [AOT] = 0.1 M. It is important to mention that as both AP and 1-naphthyl phosphate are insoluble in the nonpolar external solvent, no partition process occur and no variation of the surfactant concentration was needed. Additionally, to compare the different RMs and not extend the amount of data collected we decided only to test a single W_0. Fig. 4 shows typical data obtained for the isopropyl myristate/AOT/water system. The fit obtained is consistent with the validation of the Michaelis–Menten mechanism in the RMs. Using eqn (1), the values of the catalytic constants k_{cat} = 5.1 ± 0.2 s^{-1}, K_M = (11.9 ± 0.6) × 10^{-5} M and k_{cat}/K_M = 43 000 ± 2000 were obtained. The plots of v_0 *versus* substrate concentration for the enzymatic reaction in the systems formed by methyl laurate/AOT/water, benzene/AOT/water, and *n*-heptane/AOT/water are shown in Fig. S3–S5 in the ESI† and the kinetics parameters are summarized in Table 1.

The results (Table 1) show that the catalytic efficiency values for the hydrolysis of 1-naphthyl phosphate catalyzed by AP in all the AOT RMs evaluated are higher than those in water. Interestingly, even when the pH is not the appropriated value by AP, the reaction is enhanced in comparison with bulk water. These results are believed to be due to the confinement effect that the enzyme experiences when it is entrapped in AOT RMs. The confinement effect refers to the change in the properties of a compound due to entrapment within a limited space (cavity, chamber, cell, *etc.*) of nanometric size (1–100 nm).[90,91] In this way, the water encapsulated in reverse micellar systems that have dimensions of a few nanometers presents characteristics that are well differentiated from those found for bulk water, mainly due to the interaction of water with the surfactant molecules.[92] Confinement severely affects the water structure and dynamics such as dipole orientation, tetrahedral order parameter, and H-bonded populations. Interfacial water molecules were found to be the most severely affected.[91,93] Thus, in AOT RMs the water molecules change their properties as a consequence of the strong interactions with the surfactant at the interface, which would produce a suitable interface to solvate the enzyme.

Table 1 Experimental kinetic parameters of the hydrolysis of 1-naphthyl phosphate catalyzed by AP in homogeneous solution and, in different AOT RMs. W_0 = 10. [AOT] = 0.1 M. T = 35 °C

System	k_{cat} (s^{-1})	K_M (M) × 10^{-5}	k_{cat}/K_M (M^{-1} s^{-1})
Water	6.5 ± 0.1	23 ± 1	28 200 ± 1400
Isopropyl myristate/AOT/water	5.1 ± 0.2	11.9 ± 0.6	43 000 ± 2000
Methyl laurate/AOT/water	2.4 ± 0.1	2.5 ± 0.1	96 500 ± 4800
Benzene/AOT/water	1.3 ± 0.6	3.3 ± 0.1	37 092 ± 1800
n-Heptane/AOT/water	15.1 ± 0.7	23.7 ± 0.5	65 000 ± 3800

Furthermore, when comparing the RM systems, differences are observed. When analyzing the RMs formed with biocompatible solvents, it is found that, when using methyl laurate as the external compound, k_{cat}/K_M is higher than that when using isopropyl myristate. Similarly, when comparing the systems formed by traditional nonpolar solvents, it is observed that in *n*-heptane/AOT/water, the catalytic efficiency is higher than that in benzene/AOT/water.

At this point, it is important to mention that the change of the external solvent in organized media such as RMs is not trivial and several surprising results were obtained.[24,25,32,42,81,94–98] For example, we demonstrated that the polarity and viscosity parameters have a strong impact on the penetration phenomena. Thus, more polar and viscous solvents penetrate deeper the interface in AOT RMs. Moreover, a concept such as the molar volume or the chemical structure does not explain properly the observed results.[24,95]

The characteristics of AOT RMs formulated in the biocompatible solvents isopropyl myristate and methyl laurate have been previously investigated through different techniques, such as dynamic light scattering and static light scattering, and FT-IR, ^{1}H NMR, and UV-vis absorption spectroscopy using the 1-methyl-8-oxyquinolinium betaine molecular probe.[24,42,80,99] The obtained results showed a different degree of penetration of the external solvent into the micellar interface; in particular, isopropyl myristate penetrates more than methyl laurate. The

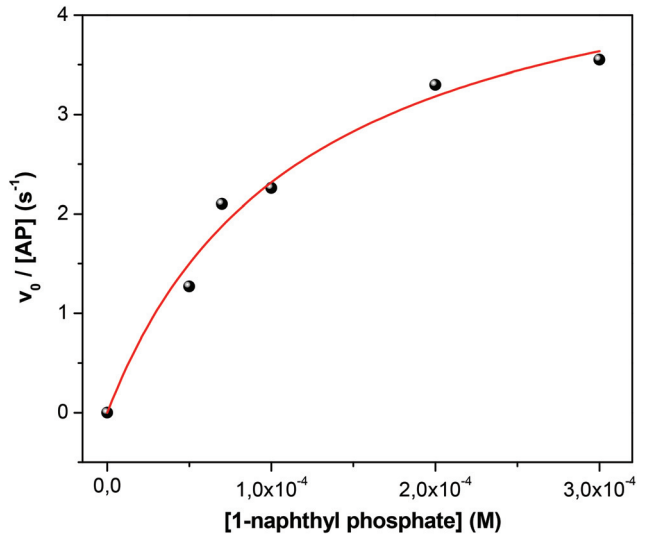
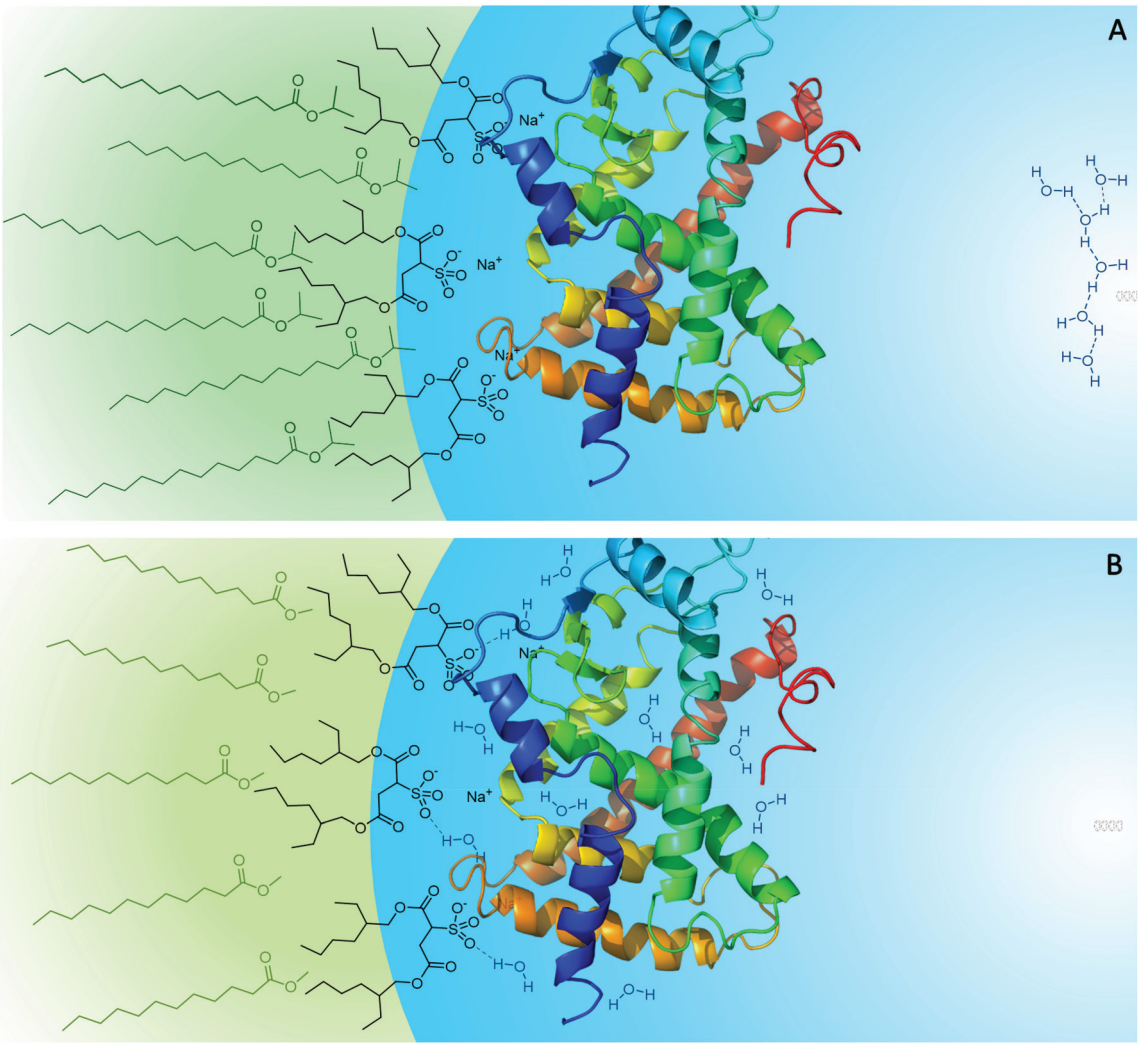


Fig. 4 Effect of 1-naphthyl phosphate concentration on the initial reaction rate (v_0) of 1-naphthyl phosphate hydrolysis catalyzed by AP in isopropyl myristate/AOT/water, at 35 °C. W_0 = 10, [AP] = 1 × 10^{-7} M. [Na_2CO_3/$NaHCO_3$] = 0.01 M, pH = 10. The solid line represents the fit according to eqn (1).

penetration of these external solvents at the interface of the AOT RMs was directly related to the solvent's viscosity and polarity. The more viscous and more polar the external solvent, the greater its ability to penetrate to the micellar interface. Isopropyl myristate is more polar and viscous than methyl laurate, which results in deeper penetration of isopropyl myristate at the interface of micellar systems.[24] Considering that the purpose of the present work is to evaluate the external solvent effect using an enzymatic reaction as a probe, we explored a very sensitive reaction to the water properties. Studies using isopropyl myristate and methyl laurate as external solvents in AOT RMs and AP as the enzyme are not found in the literature. Additionally, a comparison of later systems and the traditional RMs created with *n*-heptane and benzene allows us to understand the effect on the interfacial water when these are employed as reactants in an enzymatic reaction.

As mentioned above, the different penetration levels of the biocompatible solvents lead to the interfacial properties, such as micropolarity and hydrogen bonding donor capacity, being different for isopropyl myristate/AOT/water and methyl laurate/AOT/water.[80] In RMs formed with methyl laurate, the interface is more polar, and there is a higher proportion of water molecules that interact with the micellar interface compared to the isopropyl myristate/AOT system. Then, considering that the 1-naphthyl phosphate hydrolysis reaction occurs at the micellar interface, where the AP enzyme is located, the difference in catalytic efficiency in isopropyl myristate/AOT and methyl laurate/AOT could be explained through the different penetration levels of the external solvent towards the micellar interface, and how this influences the interfacial properties. By increasing the external solvent's penetration in AOT RMs, the proportion of water molecules that interacts with the micellar interface decreases. Consequently, the water molecules around the enzyme's active sites are displaced by the nonpolar solvent, changing the hydration and probably the mobility of the AP, factors essential for its activity; its efficiency decreases.



Scheme 3 Schematic representation of the isopropyl myristate/AOT/water (A) and methyl laurate/AOT/water (B) systems. In both RMs, the AP enzyme is located at the micellar interface. In the micelles formed with isopropyl myristate, the enzyme is less hydrated due to the greater penetration of the external solvent, which displaces the water molecules from the micellar interface.

This effect is more pronounced when using isopropyl myristate as an external solvent since, as mentioned before, it penetrates more into the micellar interface (Scheme 3).

On the other hand, in previous studies, it was observed that isopropyl myristate/AOT and methyl laurate/AOT show similar behavior, in terms of solvent penetration and water–surfactant interactions, to the benzene/AOT and *n*-heptane/AOT systems, respectively. As in the case of biocompatible solvents, when comparing benzene/AOT and *n*-heptane/AOT, it is found that the aromatic compound penetrates the interface of AOT RMs more than the aliphatic solvent because benzene is more viscous and polar than *n*-heptane. Thus, the lower catalytic efficiency of AP in benzene/AOT/water compared to that in *n*-heptane/AOT/water can also be attributed to the lower hydration of the enzyme. In AOT RMs, the aromatic solvent's higher penetration displaces the water molecules from the micellar interface to a greater extent than *n*-heptane.

It is important to note that Table 1 shows that benzene/AOT/water is the micellar system with lower catalytic efficiency. This is consistent with the fact that aromatic solvents, such as benzene, penetrate the micellar interface more than the aliphatic solvents, such as *n*-heptane, isopropyl myristate, and methyl laurate.

The results demonstrate the effect of the different penetration levels of the external solvents on the properties of the micellar interface generated, which is reflected in the AP enzyme's catalytic efficiency. Furthermore, they reinforce the idea that, in AOT RMs, isopropyl myristate and methyl laurate have an analogous behavior to benzene and *n*-heptane, respectively. Just as isopropyl myristate penetrates the micellar interface more than methyl laurate resulting in lower catalytic efficiency, benzene penetrates more than *n*-heptane obtaining lower catalytic efficiencies in benzene/AOT compared to *n*-heptane/AOT.

In summary, these results present a promising field as the unique properties of alkane/AOT/water RMs can be obtained using environmentally friendly and nontoxic lipophilic solvents, such as isopropyl myristate and methyl laurate, as an excellent alternative to be used in a sustainable procedure.

3. Conclusions

In this work we evaluate the external component effect using an enzymatic reaction as a probe, exploring a very sensitive reaction to the properties of water. The unknown effect of isopropyl myristate and methyl laurate as external solvents in AOT RMs and AP as the enzyme was unraveled. Additionally, a comparison of these systems and the traditional RMs created with *n*-heptane and benzene, as well as homogeneous media, allows us to understand the effect on the interfacial water when this is employed as a reactant in an enzymatic reaction. To the best of our knowledge, this is the first report investigating an enzymatic hydrolysis reaction in reverse micellar systems formed in biocompatible organic solvents.

The kinetics data of the hydrolysis reaction of 1-naphthyl phosphate inside AOT RMs indicate that the enzyme is perfectly active in all the RMs explored. Interestingly, in the AOT RMs the product is not detected as naphtholate even in the alkaline aqueous solution used to form the RMs. The detection of 1-naphthol is consistent with an acidic interface where the reaction takes place. Even though, the catalytic efficiency in AOT RMs is larger than that in homogeneous media, denoting a different and favorable hydration level over the AP under confinement.

The obtained results reinforced the different penetration levels of the biocompatible external solvents in AOT RMs and the similarities between *n*-heptane-methyl laurate and benzene-isopropyl myristate. Thus, the use of an enzymatic reaction as a probe is an interesting alternative to explore the interfacial composition and interfacial properties of RMs, since it is very sensitive to the properties of the media.

Finally, our findings provide valuable information on the use of biocompatible organized media as nanoreactors. Most of the antecedents of enzymatic catalysis in reverse micelles involve the use of traditional organic solvents, which represents a problem from the point of view of sustainable chemistry. The present work shows that it is possible to carry out an enzymatic hydrolysis reaction, in particular the hydrolysis of 1-naphthyl phosphate catalyzed by AP, in reverse micellar systems formed in biocompatible organic solvents. Also, the results obtained when using this type of solvent are analogous to those obtained when using traditional solvents. Thus, the toxic solvents such as *n*-heptane and benzene can be replaced by biocompatible solvents such as isopropyl myristate and methyl laurate without affecting the performance of micellar systems as nanoreactors, making them a green and promising alternative toward efficient and sustainable chemistry.

Abbreviations

AOT	Sodium diethylhexyl sulfosuccinate
AP	Alkaline phosphatase
FT-IR	Fourier transform infrared spectroscopy
k_{cat}	Catalytic rate constant
K_M	Michaelis constant
RMs	Reverse micelles
v_0	Initial velocity
W_0	$[H_2O]/[surfactant]$

Conflicts of interest

There are no conflicts to declare.

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