Fully Uncomplexed Cyclodextrin in Mixed Systems of Vesicle−Cyclodextrin: Solvolysis of Benzoyl Chlorides

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In this contribution the influence of β-cyclodextrin (CD) on the behavior of aqueous systems containing vesicles of dipalmitoyl phosphatidyl choline (DPPC) has been studied by determining the kinetics of the solvolysis reaction of substituted benzoyl chlorides whose solvolysis reactivity entails a high sensitivity on media properties. The application of the pseudophase formalism allowed us to obtain the thermodynamic and kinetic coefficients characteristic of the reaction, which are essentially independent of the concentration of CD. We were able to determine the percentages of uncomplexed cyclodextrin in equilibrium with the vesicular system which were in all cases compatible with 100%. The obtained results led us to conclude that the properties of DPPC vesicles are not affected by the presence of CD in the medium and there is no type of interaction between the CD and the vesicular surfactant monomers and, therefore, all cyclodextrin is present in the mixed system as uncomplexed cyclodextrin.

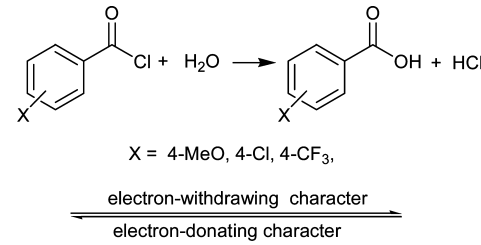
Introduction

There is increasing interest in investigating surfactant aggregates that mimic biological membranes, such as phospholipidic, liposomes or synthetic amphiphile vesicles, because the architecture of these artificial membranes is considerably simpler than that of cell membranes.[1,2] Membrane mimetic agents have been used in reactivity control, photochemical reactions, and provided unique environments for substrates and enzymes. Liposomes are useful as both biomembrane models and potential drug carriers.[3] Since liposomes are closed vesicles consisting of unilamellar or multilamellar membranes, they can encapsulate various molecules in their internal aqueous phase or their phospholipid membranes.[4] In fact, liposomes carrying antibodies or oligosaccharide chains have also been reported as effective and specific reagents in antibacterial, antitumor,[5] and antihuman inmmunodeficiency virus (HIV) therapies.[6] Kinetically, vesicles make a highly appealing reaction medium. Because of their physical and chemical characteristics, they can inhibit chemical reactions[7−9] or they can catalyze reactions acting as microreactors.[7−15]

Cyclodextrins (CD) also have the ability to alter chemical reactivity. The most studied cyclodextrins are α-, β-, and γ-cyclodextrins, which consist in six, seven, and eight glucose units, respectively.[16] Regardless of the finer details of their structure, the most important feature of CDs is their cavity, because this enables them to form inclusion complexes with a great variety of substrates.[16−18] Increasingly, the native CDs now serve as scaffolds on which multiple functional groups can be assembled with controlled geometry opening new areas of supramolecular chemistry.[19−22]

Cyclodextrins as drug complexing agents have been the object of intense interest for both fundamental aspects and practical purposes for a long time.[22−24] Recently, this attention has turned to the problem of biological photosensitization of drugs.[25] Indeed, despite their excellent therapeutic activity, many pharmacologically important chemicals such as antibacterials, antimicotics, and nonsteroidal anti-inflammatory drugs can induce phototoxic, photoallergic, and photomutagenic phenomena strictly related to the drug photochemical reactivity.[26] It has been reported that in some cases such effects can be substantially decreased in the presence of CDs with model cellular systems.[27−29] Application of CDs was, therefore, suggested as a useful strategy to minimize the biological damage induced by drugs and increase drug photostability. However, it should be stressed that drug−CD complexes usually dissociate once introduced into the body, where there is also exposure to a wide range of endogenous species.[29,30]

SCHEME 1



In the work described in this article we studied the influence of β-cyclodextrin (CD) on the behavior of aqueous systems containing vesicles of dipalmitoyl phosphatidyl choline (DPPC) by determining the kinetics, in these media, of the solvolysis reaction of substituted benzoyl chlorides (see Scheme 1). Benzoyl chlorides' geometry and polarity give rise to the formation of an inclusion complex with CD,[31] and their solvolysis reactivity entails a high sensitivity on media properties.[32−34] It is known that the addition of enough amounts of cyclodextrins to micellar systems causes their destruction due to the formation of CD−surfactant monomer complexes. Previ-

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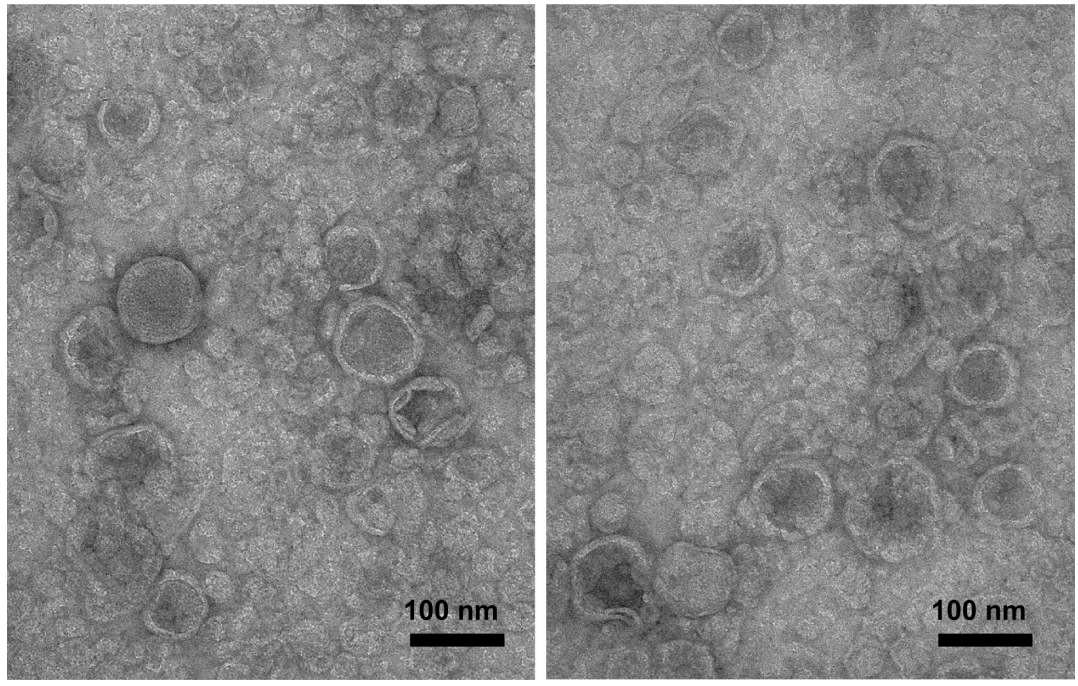


Figure 1. Representative TEM images of DPPC vesicles prepared in the absence (left) and in the presence (right) of 3 mM CD.

ous studies carried out in our group have shown that, at the micellization point, appreciable concentrations of uncomplexed CD exist.[35] Furthermore, the increase of the hydrophobic character of the micellar surfactant monomers leads to an increase in the percentage of uncomplexed CD.[36] The aim of the present work was to study a mixed system composed by cyclodextrins and vesicles (more hydrophobic than micelles and also more realistic models for biological membranes), in which it is possible that the presence of cyclodextrins neither destroys nor alters the properties of the vesicles.

Experimental Section

All the reagents (from Sigma) were of the highest available grade and used without further purification. The stock solutions of benzoyl chlorides were prepared in acetonitrile to prevent them from decomposing too rapidly. CD solutions were made taking into account that commercial CD has a H_2O content of 8 mol mol^{-1}. For aqueous solutions double-distilled and deionized water was used. All experiments were carried out at 25.0 ± 0.1 °C.

Vesicle Preparation. DPPC stock solutions were prepared by weighing the required amount of solute, adding water, and keeping the solution for 30 min in a water bath at 65 °C. Then the solution was sonicated with a tip sonicator (Bandelin UW 2200) for 30 min at 65 °C (in some cases, DPPC solutions were cosonicated in presence of cyclodextrin). After those samples were equilibrated to room temperature and filtered through a 0.45 μm pore size filter twice, the stock solution was diluted to the desired concentrations to prepare samples for kinetic measurements. Although the vesicles were assumed to be stable, we always used dispersions within 3 h after preparation.

Dynamic Light Scattering Measurements. Samples were irradiated with an Ar^+ laser at $\lambda = 514.5$ nm, and data were recorded at three different angles (60°, 90°, and 120°). Scattering data were analyzed by means of a Malvern autosizer 4700 digital correlator. Correlation functions were fitted by using the CONTIN and cumulants methods.

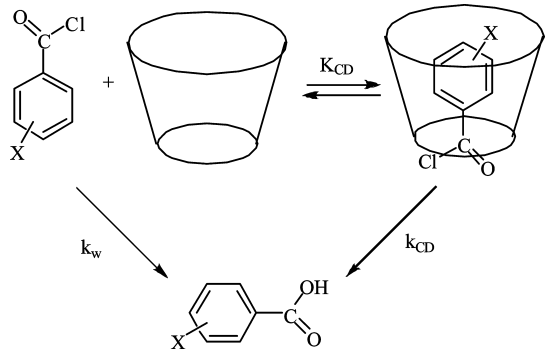
Transmision Electron Microscopy (TEM). Vesicles were imaged with a JEOL JEM-1010 transmission electron microscope using the negative-staining method. A drop of vesicle solution was spread on a 200 mesh copper grid coated with a Formvar film, and the extra droplet was instantly wiped off by filter paper. After being naturally desiccated, a drop of 2% uranyl acetate in ethanol solution was dripped on the copper grid for about 60 s and the extra droplet was also removed. Then the grid was dried naturally for about 3 h before TEM observation.

Kinetic Measurements. Solvolysis reactions were carried out in an Applied Photophysics SX-18MV stopped-flow reaction analyzer thermostatted with a Polyscience water bath. All kinetic experiments were performed using a 1:25 asymmetric mixing kit so that the percentage of acetonitrile in the reaction mixture was always less than 4% by volume. Kinetic profiles were followed by monitoring the decrease in absorbance of benzoyl chlorides. The wavelengths used for the kinetic studies ranged between 250 and 300 nm for 4-MeO, 4-Cl, and 4-CF_3. The concentration range was between 6 and 9×10^{-4} M. Kinetic data were always satisfactorily fitted by the first-order integrated rate equations, and therefore, in what follows, k_{obs} denotes the pseudo-first-order rate constant. Experiments were reproducible to within 5%.

Results

1. Characterization of Vesicles and CD−Vesicle Systems. The size and shape of vesicles depends on the sonication time and temperature at which they are prepared.[37] Sonication above the transition phase temperature (42 °C)[38] leads to larger, multicompartment vesicles that gradually become single-compartment vesicles as the sonication time is extended. We prepared vesicles by sonicating the DPPC dispersions (in some cases in presence of CD) at 65 °C for 30 min. We have characterized our DPPC vesicles at 25 °C in the absence and presence of cyclodextrin using TEM and dynamic light scattering (DLS) techniques. In Figure 1 TEM micrographs are shown for DPPC vesicles in the absence and in the presence of 3 mM CD. The pictures show small unilamellar vesicles with a nearly spherical shape, low polydispersity, and an average diameter of 65 nm. The size and shape of the vesicles is not modified by the presence of cyclodextrin. This result suggests

SCHEME 2



to us the coexistence of vesicles and CD without mutual interactions that lead to destroy the vesicular aggregates. We have also determined the size of the vesicles from DLS measurements. A hydrodynamic diameter of $D_h = 69 \pm 5$ nm was obtained, in good agreement with that determined by TEM. The hydrodynamic diameter of the vesicles prepared cosonicating DPPC in the presence of cyclodextrin ([CD] = 3 mM) was $D_h = 70 \pm 2$ nm (similar to that in the absence of CD), suggesting again no interactions between DPPC monomers and CDs.

2. Influence of CD on the Solvolysis of Substituted Benzoyl Chlorides. Although the hydrolysis of benzoyl chlorides in the presence of CDs has been recently studied,[31] we examined the influence of the CD concentration on the solvolysis reaction of substituted benzoyl chlorides to ensure good consistency in the evaluations of the experimental results. In the presence of CD, the apolar inner cavity of the CD provides a solubilization site for the benzoyl chloride with a reversible formation of a 1:1 inclusion complex, as shown in Scheme 2.

From this kinetic scheme we can obtain the following rate equation:

$$k_{obs} = \frac{k_w + k_{CD}K_{CD}[CD]}{1 + K_{CD}[CD]} \quad (1)$$

where k_w and k_{CD} are the rate constants of the solvolysis of the benzoyl chlorides in bulk water and in the inclusion complex with the cyclodextrin. K_{CD} is the equilibrium constant of the cyclodextrin−benzoyl chloride complex.

Depending on the nature of each benzoyl chloride, the kinetic behavior obtained differs (Figure 2), and this is due to the different mechanisms whereby the reaction takes place. Benzoyl chlorides with electron-withdrawing groups (4-CF_3) favor an associative mechanism, and therefore the inclusion complex is reactive (Figure 2A). On the other hand, benzoyl chlorides with electron-donating substituents (4-MeO and 4-Cl) favor a dissociative mechanism that implies the formation of a nonreactive complex (Figure 2B).[31]

Equation 1 can be fitted to experimental data giving values in good agreement with literature.[31] ($K_{CD} = 200 \pm 23$ M^{-1} and $k_{CD} = (9.2 \pm 0.3) \times 10^{-2}$ s^{-1} for 4-CF_3, $K_{CD} = 391 \pm 15$ M^{-1} for 4-MeO, and $K_{CD} = 225 \pm 21$ M^{-1} for 4-Cl.)

3. Influence of DPPC Vesicles on the Solvolysis of Substituted Benzoyl Chlorides. The study of the solvolysis reaction of substituted benzoyl chlorides in the presence of zwitterionic vesicles of DPPC has been recently carried out in our research group.[39]

Figure 3 shows the influence of the vesicular aggregates on the solvolysis reactions for the different benzoyl chlorides. The observed kinetic behavior will depend on the substituent of the aromatic ring. In general, the kinetic effects of the vesicles on the pseudo-first-order rate constant can be analyzed on basis of the pseudophase model,[40,41] assuming a two-pseudophase system in which the reaction is treated as occurring in both a vesicular pseudophase, representing the DPPC bilayer, and an aqueous pseudophase, representing both the bulk medium and the intravesicular compartment (see Scheme 3). The substrate would be distributed between the two regions, and therefore the reaction can take place in either of them.

The overall reaction rate will be the sum of the rates in both pseudophases. This model leads to the following equation:

$$k_{obs} = \frac{k_w + k_{ves}K_V[DPPC]}{1 + K_V[DPPC]} \quad (2)$$

where k_{ves} and k_w are the first-order rate constants for the vesicular and aqueous pseudophases, respectively, and K_V is the association constant or constant of benzoyl chloride distribution between the two pseudophases.

Table 1 shows the values of rate constants in the vesicular interface and in water, as well as the distribution constant for the three substituted benzoyl chlorides studied obtained from the fit of eq 2 to experimental data.

4. Solvolysis of Substituted Benzoyl Chlorides in the Presence of the Mixed CD−Vesicle System. To study the CD−vesicles mixed system we carried out sets of experiments in which the [CD] was kept constant, and we observed the effect of increasing the vesicles concentration.

4.1. Solvolysis of 4-MeO and 4-Cl Benzoyl Chlorides. To study the effect of DPPC vesicles on the solvolysis of 4-MeO benzoyl chloride containing CD, we conducted experiments at constant CD concentration (1.0×10^{-3}, 3.0×10^{-3}, 5.0×10^{-3}, and 9.0×10^{-3} M) and variable DPPC concentrations (see Figure 3A). In all cases, the reaction rate decreased with increasing DPPC vesicles concentration. The k_{obs} values obtaining by extrapolating to a zero DPPC concentration at each CD concentration are consistent with the k_{obs} values obtained in the presence of CD and in the absence of DPPC (see Figure 2B). The decrease in k_{obs} with increasing [CD] is due to the formation of a nonreactive complex between CD and 4-MeO benzoyl chloride, as we mentioned in section 2. Benzoyl chlorides with electron-donating substituents favor a dissociative pathway in which the departure of the leaving group is the slow step of the reaction.[32] Taking into account that the solvation ability of the interior of the cyclodextrin is minimal,[16,17,42] it is expected a negligible or very low reactivity of the inclusion complex formed between these benzoyl chlorides and the CD, then an increase in CD concentration will lead to a decrease in the reaction rate.

At a constant [CD], an inhibitory effect of DPPC vesicles on solvolysis of 4-MeO benzoyl chloride is observed. Vesicular and substituent effects can be explained in terms of a duality of reaction paths (associative and dissociative). The dissociative mechanism is strongly affected by the properties of the medium.[32,43] Vesicles provide a more apolar medium for solvolysis of benzoyl chlorides, with a lower ability to solvate the leaving group, the slow step of the reaction. The inhibition observed in Figure 3A can be attributed to the association of the substrates to the vesicles. The association prevents the access of the substrate to the bulk water reducing the observed reaction rate. Although reaction in the vesicular pseudophase is taking place, the rate constant (k_{ves}) is much smaller than that in water.[39] In Figure 3A also it can be also observed that the inhibitory effect of vesicles is reduced as the concentration of CD increases due to the competitive association of 4-MeO benzoyl chloride

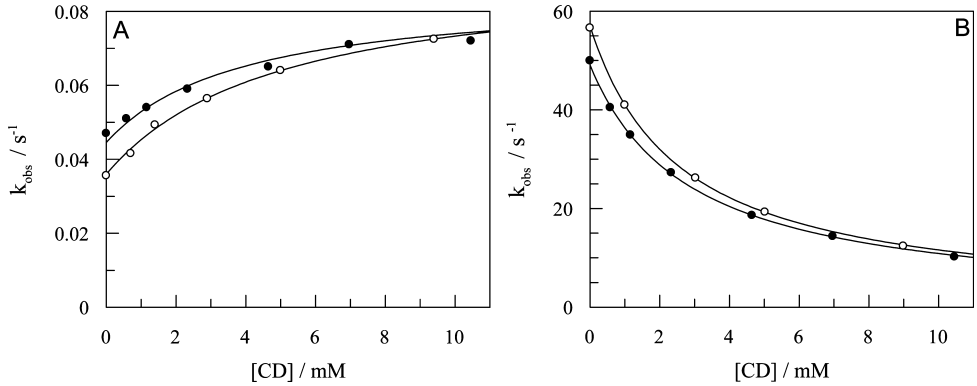


Figure 2. Influence of cyclodextrin concentration on k_{obs} for (A) solvolysis of 4-CF_3 benzoyl chloride and (B) 4-MeO benzoyl chloride, (○) in the absence of DPPC vesicles and (●) in the presence of [DPPC] = 5×10^{-4} M.

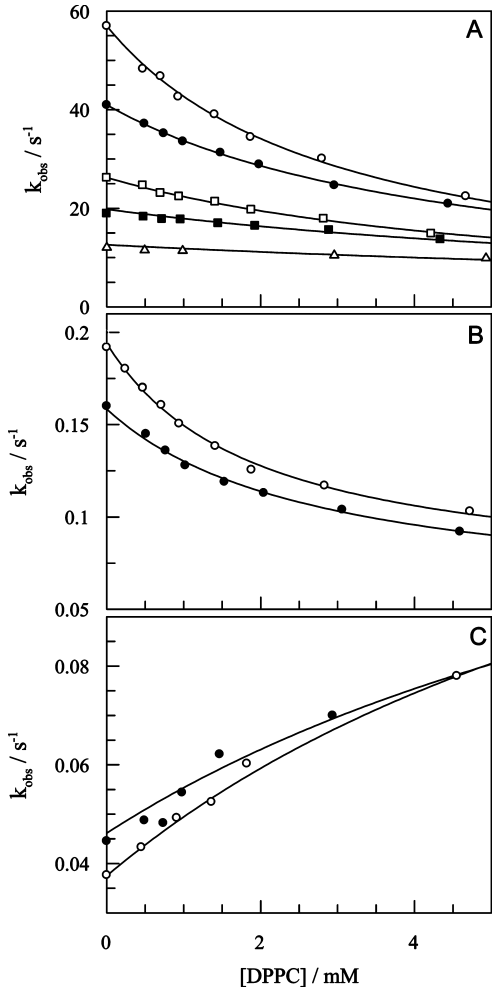


Figure 3. (A) Influence of DPPC concentration on k_{obs} for solvolysis of 4-MeO benzoyl chloride in the presence of different cyclodextrin concentrations: (○) 0.00, (●) 1×10^{-3}, (□) 3×10^{-3}, (■) 5×10^{-3}, and (Δ) 9×10^{-3} M. (B) Influence of DPPC concentration on observed rate constant for solvolysis of 4-Cl benzoyl chloride in (○) absence and (●) [CD] = 1×10^{-3} M. (C) Influence of DPPC concentration on k_{obs} for solvolysis of 4-CF_3 benzoyl chloride in (○) absence and (●) [CD] = 1×10^{-3} M.

to the CD that reduces the amount of substrate available to reacts in the vesicular interface.

Similar results were obtained in the study of the influence of DPPC vesicles on the solvolysis of 4-Cl benzoyl chloride containing CD (see Figure 3B). The observed rate constant decreases as the vesicle concentration increases. As for the 4-MeO substituent, the solvolysis of 4-Cl benzoyl chloride occurs through the dissociative channel, with the departure of the leaving group as rate-limiting step. This behavior, as occurs in the absence of cyclodextrin, is due to the association of the substrate to the vesicles and the smaller reactivity in the vesicular interface than in water, leading to a decrease in the reaction rate. We mentioned in section 2 that 4-Cl benzoyl chloride forms a nonreactive complex with cyclodextrin; therefore, the observed rate constant in presence of the mixed system CD−vesicles is smaller than in presence of DPPC vesicles.

SCHEME 3

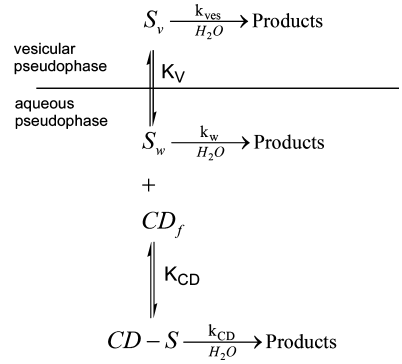


TABLE 1: Percentage of the Free Cyclodextrin and Results of Fitting Eq 3 or 4 to the Experimental Data for the Solvolysis of Benzoyl Chlorides in CD−Vesicle Mixed Systems Keeping Constant the Cyclodextrin Concentration[a]

substrate	[CD]/mM	% $[CD]_f$	k_{ves}/s^{-1b}	K_V/M^{-1b}
4-MeO	0			335 ± 7
4-MeO	1	100 ± 4		300 ± 13
4-MeO	3	99 ± 3		342 ± 13
4-MeO[c]	3	96 ± 3		317 ± 14
4-MeO	5	102 ± 4		304 ± 21
4-MeO	9	109 ± 12		292 ± 51
4-Cl	0		0.064 ± 0.005	518 ± 40
4-Cl	1	105 ± 14	0.053 ± 0.007	445 ± 55
4-CF_3	0		0.16 ± 0.01	107 ± 17
4-CF_3	1	99 ± 38	0.16 ± 0.03	102 ± 34

[a] k_w = 57 s^{-1} (4-MeO), k_w = 0.195 s^{-1} (4-Cl), k_w = 0.037 s^{-1} (4-CF_3), k_{CD} = 0.092 s^{-1} (4-CF_3), K_{CD} = 391 M^{-1} (4-MeO), K_{CD} = 225 M^{-1} (4-Cl), K_{CD} = 200 M^{-1} (4-CF_3). [b] Values obtained from fitting eqs 3 and 4 to the experimental data. [c] DPPC and CD were cosonicated, vide infra.

4.2. Solvolysis of 4-CF_3 Benzoyl Chloride. Figure 3C shows the influence of vesicle concentration on k_{obs} for the solvolysis of 4-CF_3 benzoyl chloride in the presence of a constant concentration of CD, [CD] = 1×10^{-3} M. The observed rate

constant, k_{obs}, increases with increasing DPPC vesicles concentration, as occurs in the absence of cyclodextrin. Solvolysis of benzoyl chlorides with electron-withdrawing groups (as 4-CF_3) goes through an associative channel, with the formation of a tetrahedral intermediate that develops a negative charge as rate-limiting step.[32] The observed catalysis in Figure 3C is because of the enhanced stabilization of the associative intermediate due to favorable interactions of cationic head groups of the DPPC vesicles with the developing negative charge at the reaction center.[39] The presence of CD in the mixed system also leads to an increase in the reaction rate due to the formation of a reactive 1:1 complex between CD and 4-CF_3 benzoyl chloride. The primary hydroxyl groups of the cyclodextrin are nucleophilic and react with benzoyl chlorides with electron-withdrawing groups. It can be observed that the value of the observed rate constant extrapolated to zero DPPC concentration is in agreement with the values obtained in the absence of vesicular surfactant (see Figure 2A).

Discussion

A quantitative interpretation of the experimental behavior observed can be carried out by means of the formalism of the pseudophase model. In the solvolysis of substituted benzoyl chlorides we must consider the existence of three simultaneous reaction paths: the reaction of the free substrate in aqueous medium, the reaction of the complexed substrate with the CD, and the reaction of the substrate in the vesicular surface (see Scheme 3).

On the basis of this mechanism we can obtain the following general expression for the observed rate constant:

$$k_{obs} = \frac{k_w + k_{ves}K_V[DPPC] + k_{CD}K_{CD}[CD]_f}{1 + K_V[DPPC] + K_{CD}[CD]_f} \quad (3)$$

where $[CD]_f$ is the concentration of uncomplexed cyclodextrin that is available to form an inclusion complex with the benzoyl chloride.

In order to apply this kinetic model to our system we need to know the concentration of free cyclodextrin ($[CD]_f$) present in the system. Our group has developed a kinetic model that accounts for reactivity in mixed micellar surfactant−CD systems,[35] allowing us to indicate some characteristics of mixed CD−surfactant systems: (i) for surfactant concentrations lower than the micellization point complexation equilibrium between the surfactant and the cyclodextrin is established. As the surfactant concentration increases we reach a situation in which the concentration of uncomplexed surfactant monomers in equilibrium with the CD is enough for the micellization process to begin. (ii) At the micellization point an appreciable concentration of uncomplexed CD exists.[36] This conclusion was contradictory with the traditional view[44] that considers that only when all the available cavities of the CD are occupied, the monomers can aggregate to form the micelles, and (iii) the results obtained confirm that the percentage of uncomplexed CD increases with the hydrophobic character of the surfactant. Therefore, by modulating the hydrophobicity of cationic surfactants we have found changes in the percentage of uncomplexed CD in equilibrium with the micellar system between 5% and 30%.[45] Moreover, using nonionic surfactant with lower critical micelle concentration (cmc) than cationic ones,[46] the percentage of uncomplexed CD can increase to almost 93%. DPPC vesicles supply a more hydrophobic environment than micelles due to the much lower critical vesicle concentration, as compared to the cmc. Then, we would be able to obtain a vesicle−CD mixed system in which all cyclodextrin would be free and available to bind the organic substrate, as we propose in Scheme 3. It is well-known that the addition of CDs to micellar systems produces changes in its physicochemical properties, due to the formation of inclusion complexes CD−surfactant monomer, and if the [CD] is high enough, this complexation process can lead to destroy the micellar aggregates. To take this interaction into account, the concentration of free or uncomplexed CD (CD not associated to surfactant molecules) was calculated by using the information obtained from the experiments in systems where the benzoyl chlorides are in presence of a single association entity, CD or vesicles.

As pointed out before for the substituted benzoyl chlorides with dissociative mechanism, 4-MeO and 4-Cl, the reactivity of the substrate complexed with the cyclodextrin is negligible, and then in these cases, eq 3 can be rewritten as

$$k_{obs} = \frac{k_w + k_{ves}K_V[DPPC]}{1 + K_V[DPPC] + K_{CD}[CD]_f} \quad (4)$$

For any vesicle concentration it is possible to obtain the concentration of free cyclodextrin ($[CD]_f$), from the following equation derived from eq 4.

$$[CD]_f = \frac{k_w - k_{obs} + K_V(k_{ves} - k_{obs})[DPPC]}{k_{obs}K_{CD}} \quad (5)$$

The combination of the curves for the systems with only CD or DPPC (Figure 2B and Figure 3, parts A and B) can be used as calibration curves and let us obtain the concentration of uncomplexed cyclodextrin in the mixed system formed by CD and a vesicular surfactant. This requires that we assume that the kinetics and equilibrium constants in the individual system are not modified in the mixed system. For each constant concentration of CD of Figure 3, parts A and B, we can obtain the value $[CD]_f$ from the k_{obs} value of each DPPC concentration. In Table 1 are shown the obtained mean values of $[CD]_f$. The percentages of free cyclodextrin are compatible with 100% and are independent of the cyclodextrin concentration of the medium. This result is in accordance with previous results obtained in our research group[45,46] that showed that the percentage of uncomplexed CD increases with the hydrophobic character of the micellar surfactant. DPPC provides a more hydrophobic environment than micelles, or at least its critical vesicle concentration is lower than the cmc of micelles.

On the other hand for the 4-CF_3-substituted benzoyl chloride (with and associative mechanism and therefore a reactive CD−substrate inclusion complex) it is possible to calculate the uncomplexed or free cyclodextrin concentration from eq 6, which is obtained from eq 3.

$$[CD]_f = \frac{k_w - k_{obs} + K_V(k_{ves} - k_{obs})[DPPC]}{k_{obs}K_{CD} - k_{CD}K_{CD}} \quad (6)$$

The Figure 2A values (in absence of DPPC) and Figure 3C (in absence of CD) were used as calibration curves and allow us to obtain the concentration of uncomplexed cyclodextrin. As before, the percentage of free cyclodextrin in the mixed system is compatible with 100% (see Table 1).

The experimental data together with the percentages of free cyclodextrin obtained from eqs 5 and 6 allow us to propose the lack of interaction between cyclodextrin and the vesicular surfactant. Therefore, the concentration of DPPC in eqs 3 and 4 corresponds to the total concentration of vesicular surfactant (we are taking into account the very low critical aggregation concentration of vesicles),[47] and $[CD]_f$ is the total cyclodextrin concentration which is available to react with benzoyl chlorides.

TABLE 2: Results of Fitting Eq 3 or 4 to the Experimental Data for the Solvolysis of Benzoyl Chlorides in CD−Vesicle Mixed Systems Keeping Constant the DPPC Concentration[a]

substrate	[DPPC]/M	k_{CD}/s^{-1}	K_{CD}/M^{-1}
4-MeO	0		391 ± 15
4-MeO	5×10^{-4}		411 ± 7
4-Cl	0		225 ± 2
4-Cl	5×10^{-4}		212 ± 11
4-CF_3	0	0.092 ± 0.003	200 ± 23
4-CF_3	5×10^{-4}	0.085 ± 0.006	268 ± 85

[a] $k_w = 57\ s^{-1}$ (4-MeO), $k_w = 0.195\ s^{-1}$ (4-Cl), $k_w = 0.037\ s^{-1}$ (4-CF_3), $k_{ves} = 0.064\ s^{-1}$ (4-Cl), $k_{ves} = 0.16\ s^{-1}$ (4-CF_3), $K_v = 335\ M^{-1}$ (4-MeO), $K_v = 518\ M^{-1}$ (4-Cl), $K_{CD} = 107\ M^{-1}$ (4-CF_3).

Taking into account the above considerations (that is, the DPPC and CD_f concentrations correspond to the total concentrations) eqs 4 and 3 can be fitted to the experimental data for the 4-MeO/4-Cl and 4-CF_3, respectively. The curves traced in Figure 3, parts A and B, correspond with the fit of eq 4 to the experimental values of k_{obs}. In the case of Figure 3C we fit eq 3 to the observed rate constant. To simplify the fitting procedure, we have only optimized the parameters corresponding with the reaction in the vesicular pseudophase, k_{ves} and K_V (using the values for k_w, k_{CD}, and K_{CD} obtained in the absence of vesicles). The value of k_{ves} for the solvolysis of 4-MeO is much smaller than that in water and compatible with zero. For this reason and to make easier the fitting procedure we have considered these values negligible in the CD−vesicle mixed system. In Table 1 are shown the obtained fitting parameters, and essentially these parameters are independent of [CD] and agree satisfactorily with the values obtained in the absence of CD. These results indicate the validity of the model being applied. Besides, the constancy in the K_V values indicates to us that the properties of DPPC vesicles themselves are not affected by the presence of CD in the medium.

In order to check the validity of the proposed model, we study the effect CD concentration on the solvolysis of substituted benzoyl chloride in the presence of a constant concentration of vesicles, $[DPPC] = 5 \times 10^{-4}$ M. Figure 2A shows the results for 4-CF_3 benzoyl chloride. The observed rate constant increased with increasing CD concentration, as happened in the absence of vesicles, due to the formation of a reactive complex between benzoyl chloride with electron-withdrawing groups and CD. The increase in k_{obs} values in the presence of DPPC vesicles is due the fact of 4-CF_3 benzoyl chloride reacts through an associative channel, which is favored by the vesicles,[39] as we mentioned in the Results section. Figure 2B shows the effect of increasing [CD] on k_{obs} for the solvolysis of 4-MeO in the presence of vesicles. The reaction rate shows inhibition behavior with increasing [CD], due the formation of an unreactive inclusion complex between benzoyl chloride with electron-donating groups and CD. The reaction rate decreases in the presence of DPPC vesicles, which provide a more apolar medium, leading to an inhibition of reaction that goes through the dissociative mechanism, as was commented in the Results section. Similar results were found to 4-Cl benzoyl chloride (see the Supporting Information). The solid lines in Figure 2, parts A and B, represent the best fit of eqs 3 and 4, respectively, to the experimental data. In this case, we optimized the parameters corresponding with the reaction in the presence of cyclodextrins, k_{CD} and K_{CD} (and using the values for k_w, k_{ves}, and K_V obtained in the absence of cyclodextrin). In Table 2 are shown the optimized values for these parameters in cyclodextrin and mixed CD−vesicle systems. As we can observe there is a good agreement between the values of k_{CD} and K_{CD} obtained in the absence and presence of DPPC vesicles. These results support the proposed model.

The results obtained and shown in Tables 1 and 2 let us conclude that (a) the properties of DPPC vesicles themselves are not affected by the presence of CD in the medium and (b) there is no type of interaction between the CD and the vesicular surfactant monomers, and then all cyclodextrin is present in the mixed system as uncomplexed cyclodextrin.

In our model we considered that properties of DPPC vesicles themselves were not affected by the presence of CD in the medium. The characterization of the mixed system and the kinetic result obtained supports this assumption. Previous kinetic studies have shown that there is no interaction between CD and sodium dodecyl sulfate (SDS) or tetradecyltrimethylammonium bromide (TTABr) micelles.[48,49] In the other hand, the results obtained from the study of the enthalpy of transfer of cyclodextrin from water to the aqueous surfactant solutions suggest the existence of interactions between micelles and cyclodextrins by using fluorinated alkanoates.[50] However, the existence of these interactions has been questioned recently,[51] using the self-diffusion NMR technique to study the host−guest interactions between CD and micelles of cationic, anionic, and nonionic surfactants.

As we mentioned, previous studies carried out in our group and by others[52] showed that, rather than being two competitive processes, the association to the cyclodextrin and the autoassociation of surfactant are simultaneous processes, and from the competition between them derives the existence of free cyclodextrin. Our results show that an increase in the hydrophobic-character of the surfactant favors more the autoassociation[45,46] rather the association to the cyclodextrin. With the use of a provesicular surfactant, it is possible to obtain a surfactant−CD mixed system in which all cyclodextrin is free and available to react with the organic substrate. Another explanation for the absence of interactions between DPPC vesicles and cyclodextrins is the high robustness of the vesicles comparing to micelles. Although there is a surfactant concentration threshold below which vesicles do not form,[47] this threshold is not the result of a dynamic equilibrium between free and vesicular surfactant, like the cmc of micellar media. Once formed, vesicles are not destroyed by dilution. The stability of the vesicles is, in this sense, higher than the micelles.

As a last confirmation of our results, we have studied the influence of DPPC on solvolysis of 4-MeO in mixed systems of CD−vesicle, but in this case the vesicular surfactant was cosonicated in presence of $[CD] = 3 \times 10^{-3}$ M. The hydrodynamic diameter of the vesicles prepared in this way was $D_h = 70 \pm 2$ nm (similar to that in the absence of CD). The values of k_{obs} obtained increasing the vesicles concentration were similar (see the Supporting Information) to that obtained, at the same starting reactant concentrations, in the mixed system in which the cyclodextrin is added to the previous formed vesicles, leading to a similar values of K_V (see Table 1). These results reassert our assumption of there is no type of interaction between the CD and the vesicular surfactant monomers. Opposite to what happens with micellar surfactant, the additions of cyclodextrin to the vesicular systems neither destroy nor alter the properties of the vesicles. The results summarized in this work highlight the complexity of CD−vesicle systems, and we consider them of great importance since cyclodextrins and DPPC (or others surfactants) aggregates are potential drug complexing agents. Therefore, formulations that contain both species should take

into deep consideration the possible interaction between both species and the consequences on this interaction on its function.

Conclusions

A study has been carried out on the solvolysis of substituted benzoyl chlorides in cyclodextrins−DPPC vesicle mixed systems. The reaction takes place simultaneously through dissociative and associative mechanisms. A quantitative interpretation of the experimental behavior observed can be carried out by means of the formalism of the pseudophase model, which allowed us to obtain the thermodynamic and kinetic coefficients characteristic of the reaction.

The kinetic proposed model lets us determine the percentages of uncomplexed cyclodextrin in equilibrium with the vesicular system which are compatible with 100% and are independent of the cyclodextrin concentration in the medium. Transmission electron microscopy and DLS measurements showed that the size and shape of the vesicles are not modified by the presence of cyclodextrin. The results obtained let us conclude that the properties of DPPC vesicles themselves are not affected by the presence of CD in the medium, and there is no type of interaction between the CD and the vesicular surfactant monomers, and then all cyclodextrin is present in the mixed system as uncomplexed cyclodextrin. Opposite to what happens with micellar surfactant, the addition of cyclodextrin to the vesicular system neither destroys nor alters the properties of the vesicles.

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Supporting Information Available: Graphs for the solvolysis of 4-Cl in the presence of CD and solvolysis of 4-MeO in the cosonicated CD−vesicles system. This material is available free of charge via the Internet at http://pubs.acs.org.

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