Effect of α-Cyclodextrin on Drug Distribution Studied by Electrochemistry at Interfaces between Immiscible Electrolyte Solutions

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The description and understanding of noncovalent interactions and distribution of potential new drug compounds in an organism is of paramount importance for the successful development of new drugs. In this work, a new procedure based on electrochemistry at the interface between two immiscible electrolyte solutions (ITIES) for addressing and discriminating between drug compound/ligand interactions in aqueous solution and nonspecific ligand effects on oil−water distribution behavior has been developed. The procedure is demonstrated using five drug compounds with different physical chemical parameters and α-cyclodextrin as the aqueous phase ligand. α-Cyclodextrin was chosen as an aqueous phase ligand, as it is frequently used in drug formulations to enhance solubility and bioavailability of drug compounds. Supplementary capillary electrophoresis experiments provided more detailed information on α-cyclodextrin drug complexation and, in combination with the electrochemical studies, provided information on solvation effects affecting the oil−water distribution of the drug compounds. The use of ligand shift ion partition diagrams for data presentation is a convenient format for the visualization of ligand effects on distribution behavior of related drug compounds.

Introduction

Drug action is determined by biological effects, by physicochemical properties of the active drug compound (such as ionization constants, partition coefficients, and solubility), and by the specific pharmaceutical formulation (suspensions, emulsions, and tablets).[1−3] The efficacy of a given drug compound is determined by a complex interplay between these factors. One approach for optimizing drug efficacy is through the use of pharmaceutical excipients such as cyclodextrins.[4,5] The most common cyclodextrins contain six, seven, or eight dextrose molecules (corresponding to α-, β-, and γ-cyclodextrin, respectively) bound in a 1,4-configuration to form ring structures.[5,6] The cyclodextrins have a hydrophilic exterior and a lipophilic core in which appropriately sized organic molecules can form noncovalent inclusion complexes. Complexation of drugs with cyclodextrins has frequently been used to enhance aqueous solubility and chemical stability of drug compounds,[5,6] but the effect of cyclodextrins as solvation modifiers has to our knowledge not previously been addressed. Among the most common unsubstituted cyclodextrins, α-cyclodextrin (α-CD) appears to be ideal to address solvation effects, as it is highly soluble in water. Furthermore, α-CD generally forms relatively weak complexes with drug compounds,[7] which makes it easier to quantify solvation effects.

Electrochemistry at the interface between two immiscible electrolyte solutions (ITIES) is a well-established technique for studying the partitioning of ions and ionizable compounds at water−oil interfaces.[8−13] For example, cyclic voltammetry at ITIES performed at different pH levels of the water phase can provide two-phase partition diagrams.[14−20] These diagrams allow an accurate determination of transfer potentials corresponding to drug compound transfer from one liquid phase to another and provide the standard partition coefficient of the ionic species.

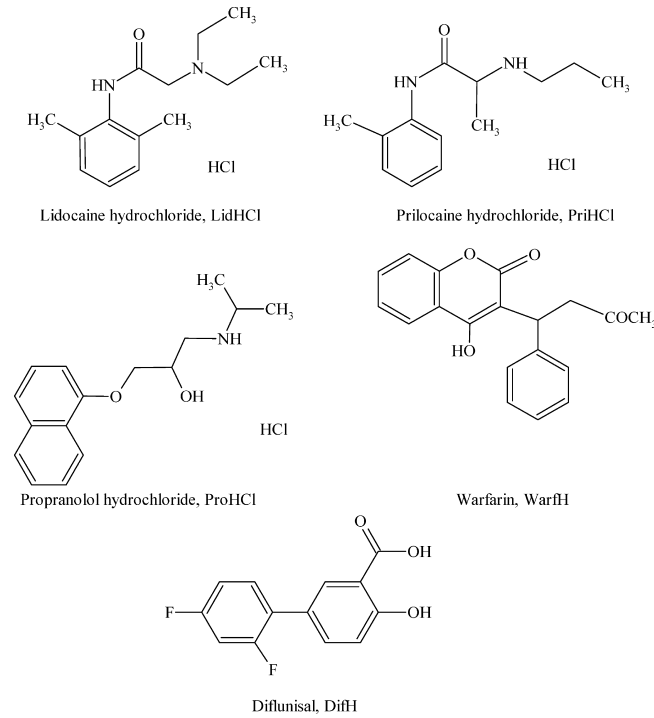

Figure 1. Chemical structures of the studied compounds.

In this work, electrochemistry at ITIES and capillary electrophoresis (CE) were used to study specific drug−ligand interactions as well as ligand-induced nonspecific media effects on physical chemical parameters such as ionization and distribution constants of drug compounds. The present study focuses on the interactions of five drug compounds (Figure 1) with α-CD

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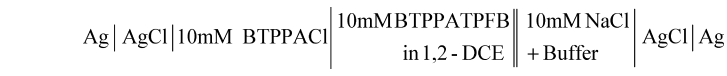

Figure 2. Electrochemical cell diagram.

in water by using a special type of ion partition diagram termed the ligand-shift partition diagram.[20] From the observed electrochemical behavior of the studied drug compounds, it was possible to discriminate between drug/α-CD complexation and solvation effects due to the presence of α-CD in the aqueous phase.

Experimental Methods

Chemicals. All aqueous solutions were prepared using deionized water from a Milli-Q system (Millipore, Bedford, MA), whereas HPLC grade 1,2-dichlorethane (1,2-DCE) (Aldrich, Germany) was used as the organic phase and was handled with all necessary precautions. The organic phase supporting electrolyte salt bis(triphenylphosphoranylidene) ammonium tetrakis(pentafluorophenyl) borate (BTPPATPFB) was prepared by metathesis of equimolar quantities of the corresponding salts, lithium tetrakis(4-chlorophenyl) borate (Boulder Scientific Company, U.S.A.) and bis(triphenylphosphoranylidene) ammonium chloride (BTPPACl) (Fluka, Switzerland) in a minimum amount of a 2:1 methanol/water mixture. The resulting BTPPATPFB precipitate was filtrated and recrystallized from acetone before use. Lidocaine hydrochloride (LidHCl), prilocaine hydrochloride (PriHCl), diflunisal (DifH), warfarin (WarfH), propranolol hydrochloride (ProHCl), and α-CD (all purchased from Sigma, Germany) were of analytical grade. Methyl viologen dichloride ($MVCl_2$) and tetrapropylammonium chloride (TPACl) were supplied by Fluka. All inorganic salts were provided by Merck, Germany.

Electrochemical Instrumentation and Procedures. Cyclic voltammetry experiments were carried out using a four-electrode potentiostat AUTOLAB PGSTAT 30 (Eco-Chemie, The Netherlands) equipped with IR drop compensation connected to a computer. The cell diagram is shown in Figure 2.

A portion of 10 mM NaCl was used as the aqueous electrolyte in combination with 2 mM acetate, phosphate, or borate buffers to fix the pH at the desired level. The volume of each phase was 2 mL, the interface area was about 1.1 cm^2, and the experiments were carried out at room temperature (20 ± 2 °C). The analytes were added to the aqueous phase from concentrated stock solutions; the final concentrations of the studied drug compounds were 0.1 mM. For the drug−ligand interaction studies, α-CD was dissolved in the water phase in various concentrations. All of the ion transfer half-wave potentials were referred to the half-wave potential of the MV^{2+} or TPA^+ ions obtained by addition of $MVCl_2$ and TPACl from concentrated stock solutions (10 mM) to the aqueous phase at the end of each experiment. The measured potentials were converted to Galvani potential differences using a literature value of the standard transfer potential for TPA^+ of −93 mV,[15] and the standard transfer potential for MV^{2+} was referred to the TPA^+ ion and found to be 216 mV.

CE Procedures. CE experiments were performed on a Hewlett-Packard HP 3D CE system equipped with a UV detector. Fused silica capillaries (Polymicro Technologies, Phoenix, AZ) of a 50 μm i.d. and an effective length of 40 cm (total length, 48 cm) were used for the experiments. Conditioning of new capillaries was conducted by flushing with 1 M NaOH for 60 min followed by 0.1 M NaOH and buffer for 30 min each. The capillary cassette temperature was set to 25 °C.

Determination of Drug−α-CD Association Constants Employing a Mobility Shift Assay. Acetate buffer solutions (10 mM, pH 4.0) were used for LidHCl, PriHCl, and ProHCl, whereas phosphate buffer solutions (10 mM, pH 7.4) were employed for DifH and WarfH. To determine binding constants, α-CD at different concentrations (up to 50 mM) was added to the buffer solution. The samples were injected into the capillary for 5 s using a pressure of 50 mbar. The applied voltage was 15 kV. Between the injections the capillary was flushed with 0.1 M NaOH followed by buffer for 2 min each. The UV detector was set to 200 nm, and the samples were run in triplicate.

The presence of additives, such as α-CD, in the electrophoresis buffer changes the viscosity of the solution, which will affect the electrophoretic mobility. This fact is taken into consideration when calculating the effective electrophoretic mobilities, μ', as[21]

$$\mu' = \frac{\eta}{\eta_0}\mu = \frac{\eta}{\eta_0}\frac{L_d L_t}{V}\left(\frac{1}{t_{analyte}} - \frac{1}{t_{EOF}}\right) \quad (1)$$

where L_d is the length of the capillary from the inlet to the detector, L_t is the total capillary length, V is the applied voltage, $t_{analyte}$ and t_{EOF} are the measured migration times of the analyte and the electroosmotic flow marker, respectively, and η and η_0 are the solution viscosities in the presence and absence of α-CD, respectively. The viscosities of the α-CD solutions were obtained from the literature.[22] The obtained effective electrophoretic mobilites were fitted to a 1:1 binding isotherm according to literature procedures:[21,23,24]

$$\mu' = \frac{\mu_D + \mu_{D-\alpha\text{-CD}} K_{1:1}[\alpha\text{-CD}]}{1 + K_{1:1}[\alpha\text{-CD}]} \quad (2)$$

where μ_D is the electrophoretic mobility of the drug compound in the absence of α-CD, $\mu_{D-\alpha\text{-CD}}$ is the electrophoretic mobility of the complex, $K_{1:1}$ is the 1:1 complexation constant, and [α-CD] is the concentration of α-CD, respectively. The percentage of complexed drug can be calculated as[25]

$$\%\ \text{bound} = \frac{K_{1:1}[\alpha\text{-CD}]}{1 + K_{1:1}[\alpha\text{-CD}]} \times 100\% \quad (3)$$

When α-CD is present in a large excess compared to the drug compound, [α-CD] can be approximated by the total α-CD concentration. Procedures for estimating pK_a values and the effect of α-CD on pK_a values using CE and UV spectroscopy are described in the Supporting Information.

Results and Discussion

Five drug compounds were chosen in the present work to include a group of bases (lidocaine, prilocaine, and propranolol) as well as a group of acids (DifH and WarfH). The transfer potential of the drug compounds was measured as a function of pH of the water phase to obtain ionic partition diagrams as has previously been described in the literature.[14−18,20,26,27] The ionic partition diagrams give information on the physical and chemical properties of the drug compounds such as the log P and pK_a values and provide an easy interpretable visual representation of the distribution characteristics of the drug. The theory of ionic partition diagrams has already been described in the literature,[14,18,20] but here we shall briefly review it for the acid DifH. When two immiscible phases such as oil and water are brought into contact, a Galvani potential difference is established across the interface. The Nernst equation describes the relation between the Galvani potential difference $\Delta_o^w\phi$

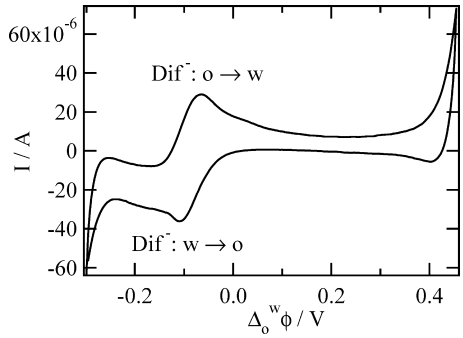

Figure 3. Cyclic voltammogram of the ion transfer of Dif^- between water (2 mM phosphate buffer, pH 7.0) and 1,2-DCE. The concentration of DifH was 100 μM.

between the two liquids and the concentration of an ion, i, in oil, c_i^o, and water, c_i^w, respectively:

$$\Delta_o^w\phi = \phi^w - \phi^o = \Delta_o^w\phi_i^{o'} + \frac{RT}{z_iF}\ln\frac{c_i^o}{c_i^w} \quad (4)$$

where $\Delta_o^w\phi_i^{o'}$ is the formal transfer potential of the ion i. $\Delta_o^w\phi_i^{o'}$ is related to the standard transfer potential of the ion:

$$\Delta_o^w\phi_i^{o'} = \Delta_o^w\phi_i^o + \frac{RT}{z_iF}\ln\frac{\gamma_i^o}{\gamma_i^w} \quad (5)$$

where γ_i^o and γ_i^w are the ionic activity coefficients of i in oil and water, respectively.

The measurable potential is the half-wave potential which is closely related to the formal transfer potential:

$$\Delta_o^w\phi_{1/2} = \Delta_o^w\phi^{o'} - \frac{RT}{z_iF}\ln\sqrt{\frac{D_i^o}{D_i^w}} \quad (6)$$

where D_i^o and D_i^w are the organic and aqueous phase diffusion coefficients of i, respectively. The formal partition coefficient $P_i^{o'}$ of i can be defined as

$$\log P_i^{o'} = -\frac{z_iF}{RT\ln 10}\Delta_o^w\phi_i^{o'} \quad (7)$$

DifH is an acid with a pK_a value of 3.0.[28] At a low pH the compound is thus neutral in the form of DifH, and at a high pH it is deprotonated as Dif^-. The ion transfer potential can be measured using cyclic voltammetry. A cyclic voltammogram corresponding to the transfer of Dif^- is shown in Figure 3. The potential window is limited by the transfer of the aqueous electrolyte anion and cation from water to oil, at low and high potentials, respectively. The two peaks in the cyclic voltammogram correspond to the transfer of the ion from water to oil (forward peak at −65 mV) and back from oil to water (reverse peak at −120 mV). The amphiphilic nature of DifH and Dif^- may lead to adsorption at the aqueous/1,2-DCE interface as suggested by the small post peak observed in Figure 3. However, the measured transfer potential is not likely to be substantially affected by an eventual adsorption, as the cyclic voltammogram has features corresponding to a diffusion controlled transfer of Dif^- between the bulk phases of water and oil.

The partition diagram of DifH is presented in Figure 4. At a high pH (the horizontal boundary line 1) the Nernst equation describes the partitioning of Dif^-. Under these conditions and assuming the diffusion coefficients in water and oil are identical, the measured half-wave potential (obtained as the average of the two peak potentials) corresponds to the formal transfer potential of Dif^-, $\Delta_o^w\phi_{Dif^-}^{0'}$:

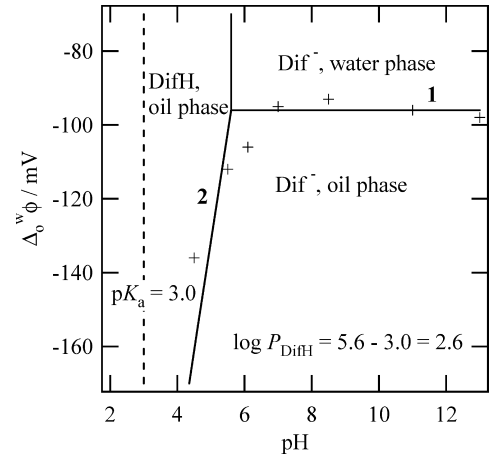

Figure 4. Two-phase partition diagram of DifH obtained at the aqueous buffer/1,2-DCE interface.

$$\Delta_o^w\phi_{1/2} = \Delta_o^w\phi_{Dif^-}^{0'} \quad (8)$$

At this boundary line, the transfer potential is independent of the pH of the water phase. At a low pH (boundary line 2), the transfer potential becomes dependent on pH as

$$\Delta_o^w\phi_{1/2} = \Delta_o^w\phi_{Dif^-}^{o'} + \frac{RT\ln 10}{z_iF}(\mathrm{pH} - \log P_{DifH} - \mathrm{p}K_a) \quad (9)$$

where $\log P_{DifH}$ is the partition coefficient of the neutral form of DifH. The intercept between these two boundary lines can be determined by using eqs 8 and 9:

$$\mathrm{pH} = \mathrm{p}K_a + \log P_{DifH} \quad (10)$$

From a mechanistic point of view, it may be noted that the overall interfacial ion transfer process at a low pH corresponds to an interfacial deprotonation of the lipophilic acid.

Partition diagrams may also be used for drug−ligand interaction studies as has been illustrated for systems with a ligand present in the oil phase.[20] In the present work, α-CD was chosen as an example of a water-phase ligand.

α-CD−Drug Interactions Characterized by Electrochemistry at ITIES. An initial series of experiments revealed that α-CD had an effect on the measured half-wave potentials of the drug compounds under study (see Supporting Information). On the basis of these experiments, it was decided to focus on an α-CD concentration of 50 mM where the most pronounced effect of α-CD was observed. In the following section we shall present the partition data in the form of ligand shift diagrams for bases (Figure 5) and acids (Figure 6) to fully elucidate the impact of α-CD on the partition behavior of the studied drug compounds. All the results have been summarized in Table 1. It should be noted that solution viscosity changes due to the presence of α-CD will only affect the half-wave potential by 1−2 mV (according to eq 6, Walden's rule, and literature values of viscosities[22]), which is close to the experimental uncertainty of the method. We shall thus neglect this effect in the following discussion of the results. Some cyclodextrins have been found to be surface active.[29,30] However, we did not make any experimental observations which indicate strong adsorption of α-CD to the water/1,2-DCE interface under the current conditions.

Basic Drug Compounds. In Figure 5a,b are shown the partition diagrams for the two basic compounds lidocaine and prilocaine. The signal, $\Delta_o^w\phi_{1/2}$, is shifted for both compounds in

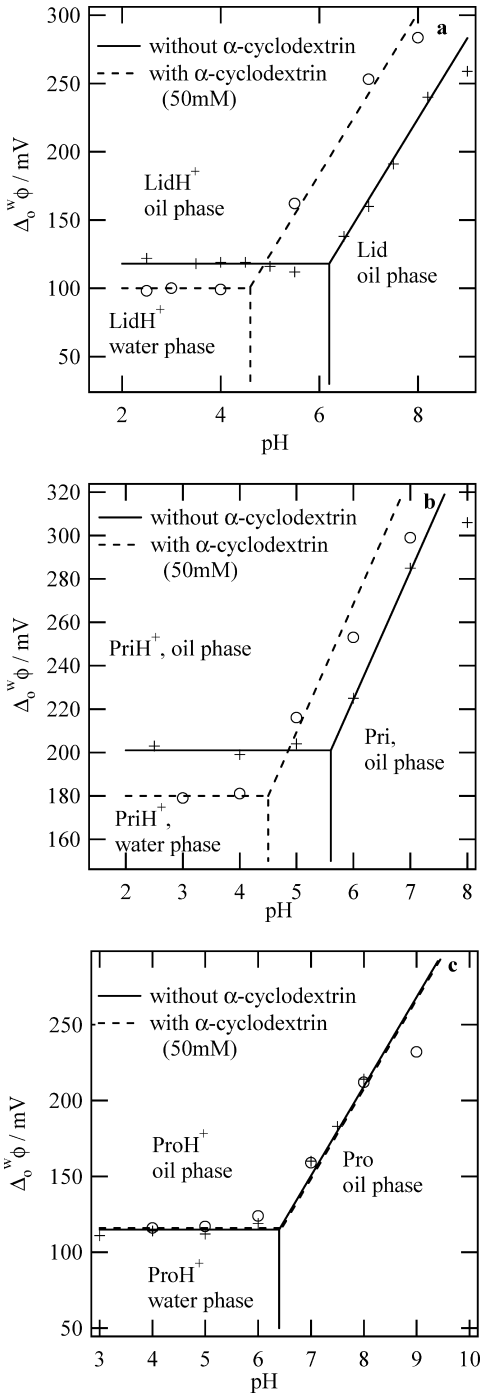

Figure 5. Ligand shift ion partition diagrams for lidocaine (a), prilocaine (b), and propranolol (c) in the presence and absence of 50 mM α-CD in the water phase obtained at an aqueous buffer/1,2-DCE interface.

the presence of α-CD. Moreover, the intercept between the two lines is also changed. For basic drug compounds, the intercept is given by[20]

$$\mathrm{pH} = \mathrm{p}K_a - \log P_N \qquad (11)$$

where P_N is the partition coefficient of the neutral form of the substance.

From a mechanistic point of view, it may be noted that the overall interfacial process at a high pH corresponds to a proton transfer assisted by the lipophilic base. At a low pH the electrochemical signal corresponds to a simple cation transfer of the protonated base.

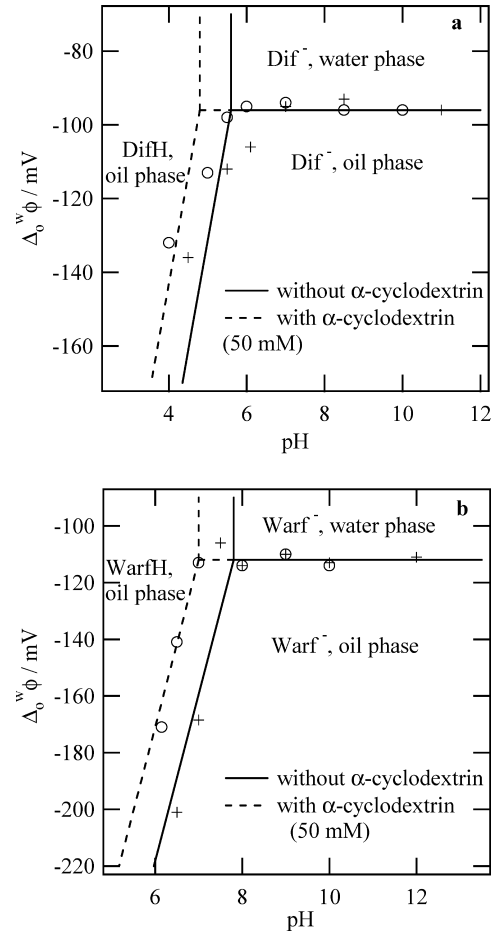

Figure 6. Ligand shift ion partition diagrams for DifH/α-CD (a) and WarfH/α-CD (b) systems obtained at an aqueous buffer/1,2-DCE interface.

TABLE 1: Experimental Results for the Investigated Compounds Obtained by Employing Electrochemistry at ITIES and Mobility Shift Affinity CE

compound	$\Delta_o^w\phi^{o'}$,[a] V	$K_{1:1}$,[b] mol^{-1} L	pK_a[c]	log $P_i^{o'}$[d]	Δ log $P_i^{o'}$[e]	log P_N[f]	Δ log P_N[g]
lidocaine	0.120	<5	7.3	−2.0	0.35	1.1	1.6
prilocaine	0.201	<5	7.5	−3.4	0.35	1.9	1.1
propranolol	0.115	<5	9.2	−1.9	0.0	2.8	0.0
DifH	−0.096	7	3.0[h]	−1.6	0.0	2.6	−0.8
WarfH	−0.112	10	5.1[h]	−1.9	0.0	2.7	−0.8

[a] Formal transfer potential obtained at the aqueous buffer/1,2-DCE interface. [b] 1:1 association constant in aqueous buffer at pH 4.0 ($LidH^+$, $PriH^+$, and $ProH^+$) or pH 7.4 (Dif^- and $Warf^-$) between the α-CD and the charged drug compound obtained from eq 2 by a nonlinear regression analysis. [c] Apparent pK_a values obtained as described in the Supporting Information unless otherwise noted. [d] 1,2-DCE water partition coefficients of the ionized form of the drug compound obtained from the formal transfer potentials using eq 7. [e] Effect of 50 mM α-CD on the 1,2-DCE water partition coefficients of the ionized form of the drug compound (difference between log $P_i^{o'}$ in the presence and absence of α-CD). [f] 1,2-DCE water partition coefficients of the neutral form of the drug compound obtained form the partition diagram and eq 10 (acids) and eq 11 (bases). [g] Effect of 50 mM α-CD on the 1,2-DCE water partition coefficients of the neutral form of the drug compound (difference between log P_N in the presence and absence of α-CD). [h] Literature values.[28]

According to eq 11, the change in intercept suggests that α-CD also affects the distribution of the neutral forms of the compounds and not only the partition coefficient of the ionized

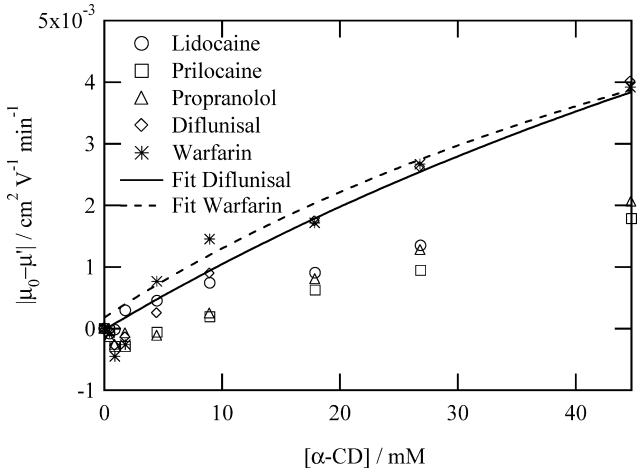

Figure 7. Studies on binding between α-CD and drug compounds using CE. Experiments for acids (◇, *) performed at pH 7.4 (10 mM phosphate buffer) and for bases (○, □, △) at pH 4.0 (10 mM acetate buffer). The corrected mobilities of DifH (full line) and WarfH (broken line) were fitted to eq 2. The data (and fit) have been referred to the mobility in the absence of α-CD and reported as absolute values.

species as defined by eq 7. In the presence of α-CD, both $LidH^+$ and $PriH^+$ are more prone to transfer from water to oil (i.e., lower transfer potentials are measured in the presence of α-CD). This result may seem surprising as a stabilization caused by binding of $LidH^+$ and $PriH^+$ to α-CD would be expected to have the opposite effect. The effect on the intercept (eq 11) may be rationalized both in terms of an effect on the pK_a value or on log P_N. To clarify this point, the pK_a values of the two compounds were measured using CE in the presence and absence of α-CD, but they were found to be similar within the experimental uncertainty (see Supporting Information). The change in intercept (eq 11) thus has to be explained by an effect on log P_N, suggesting that Lid and Pri have a higher log P_N in the presence of α-CD. Finally, we performed binding studies using mobility shift affinity CE at pH 4.0 (Figure 7). The migration data was corrected for changes in the solution viscosity due to the presence of α-CD. However, the data indicated only very weak binding of $LidH^+$ and $PriH^+$ to α-CD (i.e., $K_{1:1}$ is estimated to be less than 5 mol^{-1} L by fitting the data to eq 2). It is only possible to give a rough estimate of the upper limit on $K_{1:1}$ because of the relatively limited α-CD concentration range investigated and the media effects caused by high α-CD concentrations affecting the mobility corrections.[31] Literature data indicate that a weak interaction exists between α-CD and $PriH^+$,[32] but an affinity constant has not been reported.

The effect of complexation of the electroactive compound with a ligand in large excess has previously been described theoretically.[33] For a complexation in water and assuming that α-CD is not distributed to 1,2-DCE, the measured half-wave potential can be expressed as

$$\Delta_o^w\phi_{1/2} = \Delta_o^w\phi_i^{o'} + \frac{RT}{z_iF}\ln(1 + K_{1:1}c_L) \qquad (12)$$

where $K_{1:1}$ is the complexation constant for the formation of a 1:1 complex with the charged drug molecule and c_L is the α-CD concentration in the water phase. It is assumed that the α-CD concentration is much greater than that of the drug compound. Using a $K_{1:1}$ value of 5 mol^{-1} L, it thus follows from eq 12 that $\Delta_o^w\phi_{1/2}$ is affected less than 6 mV. Moreover, the effect on $\Delta_o^w\phi_{1/2}$caused by a complexation is of opposite sign compared to the observed shift in $\Delta_o^w\phi_{1/2}$. We therefore conclude that the results can be interpreted as being mainly due to a medium or solvent effect on the partition coefficient of the neutral and charged forms of the drug compounds. In other words, the introduction of α-CD renders the water phase more nonpolar, thus reducing the energy required to transfer the drug compound from water to 1,2-DCE. The large concentration of α-CD has an impact on water structure and thereby also on the solvation of the analyte ions.[34−36] It has, for example, been shown that approximately 35 water molecules are closely associated to α-CDs in solution[35] which inevitably will affect the solvation of ions. In fact, the presence of structure-creating solutes (also called kosmotropes) based on carbohydrates has previously been found to affect the permeation of analytes through lipid membranes.[34,36] Although it may be accepted that substrates affecting the structure of the solvent could have an impact on the drug permeation through lipid membranes, it has been difficult in most investigations to study this phenomenon in detail as other effects (such as complexations or alterations to the biomembrane) often dominate. The results presented here are interesting as there is growing evidence that cyclodextrins may act as biomembrane penetration enhancers through different mechanisms than has previously been thought, but this, however, remains a subject of debate.[37,38] On the basis of the present study, it may be envisioned that the effect of cyclodextrins as solvation modifiers, at least in some cases, may play a role.

The present set of data does not allow us to quantify on a molecular scale the changes in solvation of lidocaine and prilocaine in the presence of α-CD in a high concentration. We therefore limit ourselves to this qualitative discussion and report the effect in terms of the experimentally observed changes in partition coefficients ($\Delta \log P_i^{o'}$ and $\Delta \log P_N$) as given in Table 1. It thus appears that for both the neutral and the charged species the solvation effect dominates the impact of α-CD on the distribution behavior of lidocaine and prilocaine as positive $\Delta \log P_i^{o'}$ and $\Delta \log P_N$ are measured.

The ligand shift partition diagram for propranolol is presented in Figure 5c. As shown in Figure 7, the mobility shift assay on $ProH^+$ indicated only very weak binding between $ProH^+$ and α-CD ($K_{1:1}$ < 5 mol^{-1} L). The current set of data only allowed giving an upper limit of $K_{1:1}$. It has been reported that $ProH^+$ forms a stronger complex with α-CD,[39] but that study was based on migration data which was not corrected for viscosity changes. The fact that there is no observed effect of α-CD on the partition behavior of this compound can be explained by intramolecular hydrogen bonds of the β-hydroxy group in propranolol which will tend to shield and delocalize the charge and electron pair on the amine group. Therefore, this compound is expected to be much less sensitive to changes in the solvating environment as has also previously been observed.[20]

Acidic Drug Compounds. The partition diagrams in the presence and absence of 50 mM α-CD for the two acids DifH and WarfH are presented in Figure 6. The observed changes in $\Delta_o^w\phi_{1/2}$ cannot be explained by altered pK_a values, as they were found by UV spectroscopy to be similar in the presence and absence of 50 mM α-CD (see Supporting Information). The possible binding of Dif^- and $Warf^-$ with α-CD was addressed using CE at pH 7.4. As shown in Figure 7, a more pronounced effect of α-CD on the viscosity-corrected mobilities of Dif^- and $Warf^-$ as compared to $LidH^+$, $PriH^+$, and $ProH^+$ is observed. It thus appears that whereas $LidH^+$, $PriH^+$, and $ProH^+$ only complex weakly (or not at all) with α-CD, Dif^- and $Warf^-$ seem to form stronger complexes with α-CD. The data corresponding to Dif^- and $Warf^-$ were fitted to a 1:1 binding isotherm (eq 2)[21] as shown in Figure 7. In this way binding

constants of 7 mol^{-1} L and 10 mol^{-1} L were determined for Dif^- and $Warf^-$, respectively. It should be stressed that these value are estimates due to uncertainties in the mobility corrections. The fact that only up to approximately 30% (calculated using eq 3)[25] of the drug compound is complexed with α-CD introduces additional uncertainty in the estimate. The determined values are in good accordance with literature values of 17 mol^{-1} L for Dif^-[40,41] and 10 mol^{-1} L for $Warf^-$,[42] respectively, determined under similar conditions using other techniques.

Even though the CE experiments indicate binding between $Warf^-$ or Dif^- and α-CD, we could not observe an effect in the cyclic voltammograms as shown in Figure 6 (high pH values). By using $K_{1:1} = 7$ or 10 mol^{-1} L (corresponding to the complexes involving Dif^- and $Warf^-$, respectively) and $c_L = 0.050$ M, we can calculate using eq 12 that the complexation will affect the half-wave potential in the order of 7 and 9 mV, respectively. The fact that no significant effect on the half-wave potential of Dif^- and $Warf^-$ is observed at high pH can thus be explained by the low complexation constant and possibly a small solvation effect which would tend to reduce the effect on the half-wave potential caused by a complexation. Apparently, there is not a relatively strong medium effect as observed for prilocaine and lidocaine. This finding is likely to be due to charge delocalization in $Warf^-$ and the presence of a hydroxyl group in the ortho position to the carboxylic acid group in Dif^- which can form an intramolecular hydrogen bond to the carboxylate group. This type of intramolecular interaction has been observed before[43,44] and will tend to reduce the influence of the medium on solvation. On the other hand, both WarfH and DifH seem to form a stronger complex with α-CD as the half-wave potentials at a low pH are affected to a larger extent. Thus, for WarfH and DifH, complexation with α-CD affects the distribution to a larger extent than the solvation effects. From the observed shifts in intercepts due to the presence of α-CD and eq 10, the changes in apparent log P_N values (Δ log P_N) can be calculated to be −0.8 for both WarfH and DifH (Table 1). In theory it might be possible to calculate a complexation constant on the basis of the observed shifts in log P_N values, but as solvation effects may also contribute, we shall not make such an attempt.

Conclusions

Electrochemistry at ITIES has been used in the present investigation to assess drug compound/ligand interactions in an aqueous solution. Furthermore, using complementary techniques it has been shown that α-CD present in a high concentration in the water phase affects the solvation of the two compounds $LidH^+$ and $PriH^+$. Using mobility shift affinity CE, Dif^- and $Warf^-$ were found to form weak complexes with α-CD, but the complexation was not strong enough to be quantified using the present electrochemical technique. On the other hand, DifH and WarfH form stronger complexes with α-CD which affect their apparent log P_N value.

It can be concluded that the charge (de)localization in the analyte compound determines if it is sensitive to changes in the solvating media caused by α-CD. When the charge was delocalized as in $Warf^-$, Dif^-, and $ProH^+$, no (or very limited) solvation effects were observed on the distribution behavior. On the other hand, the partitioning of the compound having a localized charge ($PriH^+$ and $LidH^+$) into the organic phase is significantly affected by α-CD. On the basis of the present set of data, we may thus suggest that α-CD may act as a penetration enhancer for drug transport across biomembranes if the drug compound has a localized charge.

For the bases as well as the acids, the ligand shift partition diagrams provide an overview of partition behavior over a range of pH values. This type of diagram can thus be used in itself as a means to characterize effects of ligands (such as pharmaceutical excipients) on partition behavior. In relation to studying the effect and mechanism of action of pharmaceutical excipients, the present procedure should thus be widely applicable.

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Supporting Information Available: Determination of pK_a values in the presence and absence of 50 mM α-CD, spectrophotometric investigation of the effect of α-CD on pK_a values, and initial studies of the effect of α-CD on the measured half-wave potentials. This material is available free of charge via the Internet at http://pubs.acs.org.

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