Characterization of inclusion complexes of organic ions with hydrophilic hosts by ion transfer voltammetry with solvent polymeric membranes

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ABSTRACT

The quantitative characterization of inclusion complexes formed in aqueous phase between organic ions and hydrophilic hosts by ion-transfer voltammetry with solvent polymeric membrane ion sensors is studied, both in a theoretical and experimental way. Simple analytical solutions are presented for the determination of the binding constant of the complex from the variation with the host concentration of the electrochemical signal. These solutions are valid for any voltammetric technique and for solvent polymeric membrane ion sensors comprising one polarisable interface (1PI) and also, for the first time, two polarisable interfaces (2PIs). Suitable experimental conditions and data analysis procedures are discussed and applied to the study of the interactions of a common ionic liquid cation (1-octyl-3-methyl-imidazolium) and an ionisable drug (clomipramine) with two hydrophilic cyclodextrins: α-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin. The experimental study is performed via square wave voltammetry with 2PIs and 1PI solvent polymeric membranes and in both cases the electrochemical experiments enable the detection of inclusion complexes and the determination of the corresponding binding constant.

1. Introduction

Association or complexation of ions with hydrophilic large hosts such as cyclodextrins (CDs) [1], cucurbit[n]urils [2], dendrimers [3], DNA [4], etc. are frequently found in nature as well as employed to control (even selectively [5]) the activity and availability of the guest species in solution. Thus, for example, cyclodextrins are used in a tremendous number of applications and fields [6]: enhancement of the aqueous solubility of drugs, biomedicine, bioconversion and fermentation, environmental sciences and technologies (removal of organic pollutants and heavy metals from soil, water and atmosphere), catalysis, analytical separation, cosmetics, food and textile industry, etc. Besides natural cyclodextrins (α-, β- and γ-cyclodextrin), there are thousands of derivatives of variable ring size and random or site-specific chemical functionalization for tweaking their solubility and molecular interactions, which broaden even more the fields of applications [6,7].

The characterization of the affinity between the host and guest molecules is essential to understand, predict and optimize the behaviour of the above systems in real media. This is usually carried out by spectroscopic techniques including UV–vis, fluorescence, circular dichroism and nuclear magnetic resonance [2,3,8,9]. Dynamic electrochemical methods have also been employed in the study of inclusion complexes as a fast, simple and economic alternative when the guest species can be electrolysed [8,10–13]. The development of the electrochemistry at liquid|liquid interfaces [14] enables us to extend the use of electrochemical techniques to non-redox ionic molecules, in some cases enhancing the selectivity [15] and facilitating the interpretation of results (usually, ion transfer processes follow simpler mechanisms than redox electrode reactions). Moreover, additional information is obtained about the lipophilicity of the transferable species with important implications in the design of pharmaceutical compounds. Accordingly, the interactions of ionisable drugs and dopamine with DNA [16], dendrimers [17] and proteins [18] have been examined at externally polarized water|oil interfaces.

Considering all the above, in this work the use of solvent polymeric membranes [19] with two (2PIs) or one (1PI) polarisable interfaces for the study of inclusion complexes is developed with a joint theoretical and experimental approach. With respect to ‘conventional’ liquid|liquid systems [14] employed in previous studies, these membranes provide more robust, easy-to-handle electrochemical devices [19] with durable interfaces, high mechanical stability and negligible vapour
pressure. As illustrated in Scheme 1, the W/O interface is polarisable in both 2PIs- and 1PI-systems, the differences between them lying in the polarizability of the interface O/W′ as a result of the nature of the electrolyte employed in the water solution W′. Thus, in 1PI-systems the organic and W′ phases contain one common ionic species such that the corresponding equilibrium interfacial potential difference is determined by the distribution of such ion. Regarding 2PIs-systems, in phase W′ there is not any substantial amount of a common ion with the organic phase such that the interface O/W′ behaves as polarisable. Although this makes the theoretical modelling of the response more complicated, 2PIs-systems are of practical interest with its polarization window being twice the size of that of an equivalent 1PI-system[20,21] and with more lipophilic salts (RY in Scheme 1) being employed as supporting electrolyte in O since no permanent reversible partition equilibrium is to be established. As a result, very large potential windows are available for electrochemical studies with 2PIs-systems[19].

First, a new analytical theory for the voltammetric response at 2PIs systems associated with the transfer of ionic species undergoing labile complexation in solution is derived. No restrictions are made to the values of the diffusion coefficients such that the analytical equations obtained are applicable to the case of very large ligands (including macromolecules) where the complexes show notably lower diffusivity than the free species. The response at 2PIs-systems in cyclic voltammetry (CV) and square wave voltammetry (SWV) affected by the homogeneous association process is studied and critically compared with that at 1PI-systems, establishing optimum conditions and procedures for the determination of the binding constant.

Next, the theoretical results are applied to the experimental voltammetric study of the association between 1-octyl-3-methyl-imidazolium and protonated clomipramine with two different cyclodextrins: α-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin, respectively (see Scheme 2). These hydrophilic cyclodextrins are considered nontoxic and employed as drug solubilizers [7] as well as in environmental applications (e.g., fertilizers [22] and remediation of soil and water [23,24]). The analysis of the variation of the SWV signal position with the concentration of cyclodextrin enables us to confirm the formation of inclusion complexes as well as to determine the corresponding binding constant.

2. Experimental

2.1. Ion sensor

The design of the voltammetric ion sensor has been described elsewhere [19]. Briefly, a Pt-wire counter electrode was accommodated inside the inner solution compartment of a Fluka ion-selective electrode (ISE) body. A glass ring of 28 mm inner diameter and 30 mm height and a glass plate were purchased from Fluka for the construction of the membranes.

2.2. Reagents and solutions

Poly (vinyl chloride) high molecular weight, 2-nitrophenyl octyl ether (NPOE) and tetrahydrofuran (THF) were Selectophore products from Fluka. Sodium tetraphenylborate (NaTPB), sodium picrate (NaPic), tetrabutylammonium tetrakis-(4-chloro-phenyl) borate (TDDA-TCPB), 3-chloro-10,11-dihydro-N,N-dimethyl-5H dibenz[b,f]azepine,5-propanamine hydrochloride (clomipramine, Cmp. HCl), 1-octyl-3-methyl-imidazolium chloride ([C8C1Im]Cl) and α-cyclodextrin were purchased from Sigma. Crystal violet was purchased from Riedel-de Haën in the form of chloride (CVCl) and (2-hydroxypropyl)-β-
cyclodextrin (\( \langle M_\text{w} \rangle \approx 1380 \text{ g mol}^{-1} \)) from Aldrich. All the other reagents used were of analytical reagent grade. Nanopure water (18 M\( \Omega \)) from a Milli-Q (Millipore) system was used throughout.

Crystal violet tetraphenylborate (CV-TPB) was obtained as a precipitate by mixing an aqueous solution of crystal violet chloride with an aqueous solution of sodium tetraphenylborate in equimolar amounts.

2.3. Membrane preparations

Two membranes were prepared by dissolving 200 mg NPOE, 100 mg PVC and 10.4 mg CV-TPB (for the system of a single polarized interface) or 17.2 mg TDDA-TClPB (for that of two polarized interfaces) in 3 ml of tetrahydrofuran. These solutions were poured into a glass ring resting on a glass plate and were left overnight to allow the solvent THF to evaporate slowly. A 6 mm diameter piece of each membrane was cut out with a punch and incorporated into the modified ISE body as described above.

2.4. Electrochemical measurements

The electrochemical cell used for the system with two polarisable interfaces can be expressed as:

\[
\text{Ag|AgCl|3 M KCl} \parallel 5 \times 10^{-3} \text{ M LiCl}, 10^{-4} \text{ M I}^+\text{Cl}^- (\text{w}) | 5 \times 10^{-2} \text{ M TDDA TCIPB} (\text{o}) | 5 \times 10^{-2} \text{ M LiCl} (\text{w'}) | \text{AgCl|Ag (Cell I)}
\]

and for that of a single polarisable interface as:

\[
\text{Ag|AgCl|3 M KCl} \parallel 5 \times 10^{-2} \text{ M LiCl}, 10^{-4} \text{ M I}^+\text{Cl}^- (\text{w}) | 5 \times 10^{-2} \text{ M CV TPB} (\text{o}) | 5 \times 10^{-2} \text{ M CVC} (\text{w'}) | \text{AgCl|Ag (Cell II)}
\]

with I' = \( \text{C}_6\text{C}_1\text{Im}^+ \) and Cmp'.

The applied potential, \( E \), is maintained at the pre-set value by a four-electrode set-up and an in-house custom-built potentiostat that applies the necessary potential between the counter electrodes and it allows for automatic compensation of the ohmic potential drop. A positive current corresponds to the transfer of positive charge from the aqueous phase which contains the target ion \( \text{C}_6\text{C}_1\text{Im}^+ \) or Cmp' to the organic phase. All computer programs were written in our laboratory.

3. Theory

As illustrated in Schemes 3 and 4, we will consider that the complexing agent (L) is of hydrophylic nature so that neither the transfer of the ligand nor that of the complexes (IL\( \text{n} \)) to the organic phase are expected. This is the case of hydrophilic cyclodextrins [25], dendrimers [17,26] and cucurbit[n]urils [27]. Note that the response of the mirror image situation (i.e., I\( ^+ \) species in an organic medium associating with a lipophilic ligand such as crown ethers or rotaxanes) is equivalent to that predicted by the theory below.

3.1. Two polarized interfaces systems (2PIs)

In the case of 2PIs-systems, the transfer of the target ion I\( ^+ \) at the interface W|O is coupled to a concomitant ion transfer at the interface O|W' in order to keep electroneutrality in the organic phase [19], which hereafter will be assumed to involve the ion of the membrane electrolyte with the same charge as I\( ^+ \) that is transferred.

Let us consider the application of a constant potential difference \( E \) between both aqueous phases (W and W') so that the transfer of I\( ^+ \) takes place. It is well-reported that the kinetics of inclusion/release...
with cyclodextrins [28–32] (and also with other large hosts such as dendrimers [33], cucurbit[n]urils [2] and sequences of DNA [4] depending on the nature of the substrate) are fast since no covalent bonds are usually involved. Therefore, it can be assumed that chemical equilibrium conditions hold (close-to-equilibrium conditions [29]) in any point of phase W and time of the experiment. Thus, under an excess of species L in the aqueous phase such that $c_1^L (x, t) \approx c_0^L \forall t, x \leq 0$ [34], the concentrations of the different complexes can be related to that of the free ion through the corresponding overall complexation constant as follows [35]:

$$\frac{c_{m}^L (x, t)}{c_{n}^L (x, t)} = \beta_m = (c_1^L)_{m} \prod_{n=1}^{m} K_n \quad \text{for } m \geq 1 \quad (1)$$

where $K_n = \frac{c_{m}^L}{c_{n}^L}$ Taking into account the relationships given by Eq. (1), a new variable can be defined as the sum of the concentrations of the free species and all the possible complexes

$$c_{r}^L (x, t) = c_1^L (x, t) + \sum_{n=1}^{m} c_{n}^L (x, t) \quad (2)$$

This new variable $c_{r}^L$ can be viewed as a pseudo-species of bulk concentration $c_1^L = c_0^L + \sum_{n=1}^{m} c_{n}^L$ with an effective diffusion coefficient $D_{eff}^L$ (Eq. (10)) and an apparent formal potential $\Delta \phi^L_{app}$ (Eq. (11)). Thus, the variation of the species concentrations as a result of diffusion, homogenous chemical reactions and interfacial charge transfer can be expressed by the following boundary value problem:

$$\frac{\partial c_{r}^L (x,t)}{\partial t} = D_{eff}^L \frac{\partial^2 c_{r}^L (x,t)}{\partial x^2} \quad (3)$$

$$x \leq 0, t = 0 \quad \frac{c_{r}^L (x,0)}{c_{r}^L (x,0)} = c_1^L (x,0) \approx c_0^L \quad (4)$$

$$0 \leq x \leq d, t = 0 \quad \frac{c_{r}^L (x,0)}{c_{r}^L (x,0)} = 0 \quad (5)$$

$$x \geq d, t = 0 \quad \frac{c_{r}^L (x,0)}{c_{r}^L (x,0)} = 0 \quad (6)$$

$$x = 0, t = 0 \quad D_{eff}^L \frac{\partial c_{r}^L (x,0)}{\partial x} = D_{eff}^L \frac{\partial c_{r}^L (x,0)}{\partial x} \quad (7)$$

$$c_{r}^L (0) = c_1^L (0)e^{\eta_{W/O}} \quad (8)$$

$$x = d, t = 0 \quad c_{r}^L (d,0) = c_1^L (d,0)e^{\eta_{W/O}} \quad (9)$$

$$D_{eff}^L = \frac{D_{eff}^L + \sum_{n=1}^{m} \beta_n}{1 + \sum_{n=1}^{m} \beta_n} \quad (10)$$

$$\Delta \phi_{app}^L = \Delta \phi_{app}^L + \frac{RT}{zF} \ln \left(1 + \sum_{n=1}^{m} \beta_n\right) \quad (11)$$

$$\eta_{W/O} = \frac{zF}{RT}(E_{W/O} - \Delta \phi_{app}^L) \quad (12)$$

$$E = E_{W/O} + \Delta \phi_{app}^L \quad (13)$$

Also note that the concentration of $R^+$ is assumed to be in excess such that it does not change significantly in the organic phase $(o)$ as a result of the current flow: $c_{R^+}^L(x, t) \approx c_0^L \forall t, 0 < x < d$ [19].

Attending to that the above mathematical problem is formally identical to that of a simple ion transfer, the current response of the system in Scheme 3 when applying a potential pulse $E_i$ of duration $\tau$ ($0 \leq t \leq \tau$) can be calculated from [19]:

$$I_{2^{nd}} = \zeta FA D_{eff}^L \left(\frac{\partial c_{r}^L (x,0)}{\partial x}\right)_{x=0} = \zeta FAc_1^L \frac{D_{eff}^L}{zF} \frac{\sqrt{(e^n)^2 + 8e^h - e^n}}{4} \quad (14)$$

where

$$\eta = \frac{zF}{RT}(E_i - E_{1/2,app}^{2nd}) \quad (15)$$

with the apparent half-wave potential, $E_{1/2,app}^{2nd}$ being:

$$E_{1/2,app}^{2nd} = E_i + \frac{RT}{zF} \ln \left(1 + \sum_{n=1}^{m} \beta_n\right) - \frac{RT}{zF} \ln \left(\frac{2[D_{eff}^L D_{eff}^L c_0^L]}{D_{eff}^L c_1^L}\right) \quad (16)$$

and

$$E_i^D = \Delta \phi_{app}^L - \Delta \phi_{app}^L \quad (17)$$

Attending to the linearity of the operators in (3) and to the fact that the interfacial concentrations of the (pseudo)species $c_{r}^L$ are only dependent on the applied potential and independent of the history of the experiment [19], the superposition principle applies [36] and the following expression is obtained for the current response when applying an arbitrary sequence of potential pulses $E_1, E_2, ..., E_p$ of duration $\tau$:
where the potential-dependent functions are given by:

\begin{equation}
 g^{2PI}(\eta_i) = 0 \quad \text{for } \eta_i \leq 1

g^{2PI}(\eta_i) = \frac{\sqrt{\eta_i^2 + 4 \eta_i}}{4} \quad \text{for } \eta_i > 1
\end{equation}

with \( \eta_i = \frac{\psi_i}{RT} \).

### 3.2. One polarisable interface systems (1PI)

In the case of 1PI-systems, the analytical solution of the problem depicted in Scheme 4 when a single potential pulse \( E_1 \) is applied for \( 0 < t \leq \tau \) under chemical equilibrium conditions was obtained in a previous work and it is given by [35]

\begin{equation}
 I_{1PI}^p = z FA \frac{D_{eff}}{RT} \int_{-\tau}^{\tau} \frac{g^{1PI}(\eta_i)}{1 + c_i^0} \, dx
\end{equation}

where:

\begin{equation}
 \eta_i^p = \frac{\psi_i}{RT} (E_1 - E_{1PI}^\text{app})
\end{equation}

with the apparent or conditional half-wave potential at 1PI-systems being:

\begin{equation}
 E_{1/2,app}^{1PI} = \Delta \phi_{app}^{1PI} + RT \ln \left( 1 + \sum_{i=1}^{n} \beta_i \right) - RT \ln \left( \frac{D_{eff}^1}{D_{eff}^0} \right)
\end{equation}

As in the case of two polarisable interfaces, under chemical equilibrium conditions the superposition principle holds and the following analytical solution is deduced for the current response when applying an arbitrary sequence of potential pulses \( E_1, E_2, \ldots, E_p \) of length \( \tau \)

\begin{equation}
 I_{p}^{1PI} = z FA \frac{D_{eff}}{RT} \sum_{i=1}^{p} \frac{g^{1PI}(\eta_i)}{1 + c_i^0}
\end{equation}

where the potential-dependent functions are:

\begin{equation}
 g^{1PI}(\eta_i^p) = 1 \quad \text{for } \eta_i \leq 1
\end{equation}

with \( \eta_i^p = \frac{\psi_i}{RT} (E_1 - E_{1PI}^\text{app}) \).

## 4. Results and discussion

### 4.1. Theoretical results

Analytical solutions (18) and (23) enable us to calculate the response in any voltammetric technique whatever the values of the diffusion coefficients of the species in each phase. The latter is particularly important given that the diffusivity of the complex is likely to be notably slower than that of the free ion in the case of large ligands.
are established for 2PIs-systems: increases (for example, $K_p$ and $K_z$). Therefore, $I_p$ depends on $c_L$ and $K_z$, to assure a sensitivity higher than 10% with respect to $E_z$ and the use of the potential duration of the potential pulses in SWV (the frequency is defined as $f = 1/2r$). Therefore, $I_p$ decreases when the proportion of the species that diffuses more slowly (IL) increases leading to the diminution of $D^w_{IL}$, according to Eq. (10).

Regarding the peak potential ($E_p$), this takes more positive values as $c_L^*$ and $K$ increase due to the high hydrophilic nature of the complex IL. This shift of the SWV and CV peak position as a result of the complexation process is described by the following relationships in 2PIs-systems:

$$I_{p,SWV}^{2PI} = 0.383 \, zFAc_L^* \frac{D^w_{IL}}{\tau^{1/2}} \left(\frac{FE_m}{RT}\right)^{1/2} = 0.973$$ (25)

$$I_{p,SWV}^{1PI} = 0.382 \, zFAc_L^* \frac{D^w_{IL}}{RT} \left(\frac{FE_m}{RT}\right)^{1/2} = 0.973$$ (26)

$$I_{p,CV}^{1PI} = 0.538 \, zFAc_L^* \frac{D^w_{IL}}{RT} \left(\frac{FE_m}{RT}\right)^{1/2} = 0.973$$ (27)

$$I_{p,CV}^{2PI} = 0.446 \, zFAc_L^* \frac{D^w_{IL}}{RT} \left(\frac{FE_m}{RT}\right)^{1/2} = 0.973$$ (28)

with $v$ being the scan rate in CV and $r$ the duration of the potential pulses in SWV (the frequency is defined as $f = 1/2r$). Therefore, $I_p$ decreases when the proportion of the species that diffuses more slowly (IL) increases leading to the diminution of $D^w_{IL}$, according to Eq. (10).

The effects of the complexation of $I^+$ on the CV and SWV signals for 2PIs- and 1PI-systems are shown in Fig. 1 for a binding constant $K = 10^5$ M$^{-1}$ and a ratio between the diffusion coefficient of the free and bound species $D^w_{IL}/D^w_{I^+} = 5$. Both the position and the magnitude of the signals are affected, the effect being more apparent as the concentration of species L ($c_L^*$) and the binding constant ($K$, not shown) are larger. Thus, the peak current ($I_p$) under linear diffusion depends on the square root of the effective diffusion coefficient. From the analysis of the theoretical peak currents predicted by Eq. (18), the following relationships for $I_p$ are established for 2PIs-systems:

$$E_{p,SWV}^{2PI} = E_{1/2,app}^{2PI} \pm 0.467 \frac{RT}{2F} \left(\frac{FE_m}{RT}\right)^{1/2} = 0.973$$ (29)

$$E_{p,CV}^{2PI} = E_{1/2,app}^{2PI} \pm 1.868 \frac{RT}{2F} \left(\frac{FE_m}{RT}\right)^{1/2} = 0.973$$ (30)

and in 1PI-systems [41]:

$$E_{p,SWV}^{1PI} = E_{1/2,app}^{1PI}$$ (31)

$$E_{p,CV}^{1PI} = E_{1/2,app}^{1PI} \pm 1.109 \frac{RT}{2F}$$ (32)

where the positive sign applies to cations and the negative sign to anions and $E_{1/2,app}$ depends on $c_L^*$ and $K$ according to Eqs. (16) and (22). It is worth noting that the SWV peak potential in 2PIs-systems differs from the apparent (half-wave) potential, this discrepancy decreasing as the square wave amplitude $E_m$ increases (for example, $E_{p,SWV}^{2PI} = E_{p,SWV}^{2PI} \pm 0.311 \frac{RT}{2F}$ for $E_m = 1.946$).

According to the above, the characterization of the complexation equilibrium in solution is possible from the analysis of the variation of the peak position and current with $c_L^*$ [34], as shown in Fig. 2. Thus, the difference between the values of the peak potential and peak current in the presence and absence of species L depend on the value of the binding constant and also on the ratio $D^w_{IL}/D^w_{I^+}$. Then, the fitting of experimental $I_p$ and $E_p$ data obtained at different concentrations of species L with K as fitting parameter (and also $D^w_{IL}/D^w_{I^+}$ in case this is unknown) enables us to characterize the association process. For this, the optimum conditions in the $I_p$-based approach are $c_L^* = 0.6/k$ to 6.5/$K$, to assure a sensitivity higher than 10% with respect to the peak current in absence of ligand as well as with respect to the plateau reached for very large $c_L^*$. In regard to the $E_p$-based strategy, it is found that suitable conditions correspond to $c_L^* > 0.1/K$ and the use of differential techniques (such as SWV) is recommended given that they provide peak-shaped signals with reduced distortions due to background currents. This makes the determination of the signal position and height easier and more accurate.

Obviously the $I_p$-based approach is only adequate when the diffusion coefficients of the free ($I^+$) and bound (IL) species are different enough (see Fig. 2a) so that it can be suitable for the study of interactions between charged molecules and high weight macromolecules such as DNA [37]. When the diffusivity of species $I^+$ and IL is not so different and the $E_p$-based strategy is employed, 2PIs-systems have a
bit lower sensitivity to $K$ than 1PI-systems (compare Fig. 2b and c) but they offer wider electrochemical windows, which can be a key advantage (see Section 4.3). Attending to all the above, the $I_p$- and $E_p$- strategies can be viewed as complementary depending on the characteristics of the system (mainly on $D_{D / I}^{2}$) and on which experimental magnitude can be determined more accurately.

### 4.2. Experimental study of the complex C8C1Im+–αCD

The theoretical results are applied to the study of the interaction between the cation 1-octyl-3-methyl-imidazolium (C8C1Im+) and α-cyclodextrin in aqueous solution (50 mM in LiCl) with both 2PIs- (Cell I) and 1PI- (Cell II) membranes:

$$C_{8}\text{C}_{1}\text{Im}^+(w) + \alpha\text{CD}(w) \rightleftharpoons C_{8}\text{C}_{1}\text{Im}^− + \alpha\text{CD}(w)$$

(33)

such that in this case $F^+ \equiv C_{8}\text{C}_{1}\text{Im}^+$ and $L \equiv \alpha\text{CD}$ in Schemes 3 and 4. The diffusion coefficient of C8C1Im+ was determined by chronoamperometry in the absence of αCD taking the transfer of the anion picrate ($D = 8.2 \times 10^{-6}$ cm$^2$/s [42]) as internal reference to correct for any uncertainty in the value of the area of the membrane. The value obtained of $1.5 \times 10^{-5}$ cm$^2$/s coincides well with those reported in the literature for similar compounds [43]. With respect to the diffusion coefficient of the complex C8C1Im+–αCD, it was assumed to be equal to that of αCD (i.e., $D_{\text{C}_{8}\text{C}_{1}\text{Im}^-}$ [38]) due to its large size in comparison with the guest molecule.

Fig. 3a and b show the experimental baseline-corrected SWV voltammograms corresponding to the transfer of C8C1Im+ in two and one polarisable interfaces, respectively, under different excess concentrations of α-cyclodextrin ($c_{\text{CD}}^*$) and on which experimental magnitude can be determined more accurately.

![Experimental baseline-corrected SWV voltammograms](image)

**Fig. 3.** (a and b) Experimental baseline-corrected SWV voltammograms corresponding to the transfer of C8C1Im+ at membranes with two (Cell I) and one (Cell II) polarisable interfaces under different concentrations of α-cyclodextrin in the aqueous phase (0, 1, 3 and 25 mM). (c and d) Experimental values of $E_{p,2PI}$ and $E_{p,1PI}$ (circle points) at different concentrations of α-cyclodextrin and best-fit theoretical curve (solid lines, Eqs. (29) and (31)) corresponding to two different sets of experiments. $c_{\text{CD}}^*$ = $c_{\text{CD}}^{+} + c_{\text{CD}}^{-} = 100$ μM, $E_{0} = E_{\text{sw}} = 10$ mV, $f = 0.5$ Hz. $E_{\text{index}} = (E_1 + E_2)/2$, with $E_1$ and $E_2$ corresponding to the pulse with odd index and to the consecutive pulse with even index that comprises each cycle in SWV, respectively [41].

The quantitative analysis of the experimental data for the determination of the binding constant is shown in Fig. 3c and d. This is based on the fitting of the variation with $c_{\text{CD}}^*$ of the SWV peak potential, which could be measured more accurately than the peak current and showed better reproducibility. The fitting was performed by minimizing the value of the sum of the squared differences between the values of the experimental and theoretical values of the peak potential ($\chi^2$) with the binding constant $K$ as the only fitting parameter:

$$\chi^2 = \sum_{i=1}^{N}(E_{p,\text{exp}} - E_{p,\text{theor}})^2$$

(34)

with $N$ being the number of different concentrations of αCD assayed in the experiments and the theoretical values $E_{p,\text{theor}}$ being calculated from Eqs. (29) and (31). As can be seen in Fig. 3c and d, the theoretical curves match very satisfactorily the experimental data in both types of membranes ($R^2 > 0.99$) and good agreement exists between the values for the binding constant of C8C1Im+–αCD obtained with both membranes: $K = (6.44 \pm 0.09) \times 10^3$ M$^{-1}$ with the 2PIs-membrane (Cell I) and $K = (5.8 \pm 0.1) \times 10^3$ M$^{-1}$ with the 1PI-membrane (Cell II), the error bars corresponding to the standard deviation obtained from two different sets of experiments. The above two values show reasonably satisfactory agreement with that obtained by isothermal titration.
3.5 \times 10^{-3} \text{M}^{-1} \equiv H \text{P} \text{C} \text{D} \quad (35)

which is consistent with the data reported 0.5 Hz. E_{\text{index}} = (E_1 + E_2) / 2, with E_1 and E_2 corresponding to the pulse with odd index and to the consecutive pulse with even index that comprises each cycle in SWV, respectively [41].

\[ R^2 = 0.9922 \]
\[ R^2 = 0.9997 \]
\[ R^2 = 0.9948 \]

Fig. 4. (a) Experimental baseline-corrected SWV voltammograms corresponding to the ion transfer of Cmp+ at a membrane with two (Cell I) polarisable interfaces under different concentrations of HP-β-cyclodextrin in the aqueous phase. (b) Experimental values of \( K_{\text{HPCD}} \) (circle points) at different concentrations of HP-β-cyclodextrin and best-fit theoretical curve (solid line, Eq. (29)) corresponding to three different sets of experiments. \( c_{\text{w}} = c_{\text{p}} + c_{\beta} = 100 \, \mu \text{M}, E_0 = E_{\text{rev}} = 10 \, \text{mV}, f = 0.5 \, \text{Hz, } E_{\text{index}} = (E_1 + E_2) / 2, \) with \( E_1 \) and \( E_2 \) corresponding to the pulse with odd index and to the consecutive pulse with even index that comprises each cycle in SWV, respectively [41].

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