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Electrochemical sensing of ammonium ion at the water/1,6-dichlorohexane interface

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ABSTRACT

In this work, ion transfer and facilitated ion transfer of ammonium ion by a lipophilic cyclodextrin is investigated at the water/1,6-dichlorohexane micro-interface, using electrochemical approaches (cyclic voltammetry, differential pulse voltammetry and square wave voltammetry). The association constant has been obtained for the complex between ammonium ion and the cyclodextrin. Experimental conditions for the analytical determination of ammonium ion were established and a detection limit of 0.12 μM was obtained. The amperometric sensor gave a current response proportional to the ammonium ion concentration in the range from 4.2 to 66 μM.

1. Introduction

Determination of ammonia or ammonium ion is important in clinical [1–3], environmental [4–6] and industrial [7] processes. Furthermore, measurement of ammonium ions has found an increasing application in the development of biosensors because ammonium ions are a metabolic product in many enzymatic reactions (for example, urea can be detected by sensing its metabolic product, i.e., ammonium ions [8–12]).

Accordingly, the detection of ammonium ions has been widely reported in the literature where spectrophotometric [13–16], fluorimetric [17], chemiluminescence [18], conductimetric [19–21], potentiometric [8,22–27] and amperometric [9–11,28–33] methods can be found and their performance can be compared.

As an example of potentiometric determination of ammonia, Meyerhoff et al. [34,35] reported the use of an ammonium-ion selective poly(vinylchloride) (PVC) membrane, both containing the antibiotic nonactin and the ammonia-gas sensing electrode. Ammonium sensors are also used frequently as potentiometric biosensors where enzymes or microbial cell are immobilized on the surface of the sensor [36,37].

Contrasting with the potentiometric sensors, amperometric sensors generally present a higher sensitivity and a lower detection limit. Various types of amperometric procedures were applied to the detection of ammonium ions in aqueous solutions, including: enzymatic sensing materials [9,28], Nafion® film cation selective membrane [30] and conducting polymers sensing materials [10,11,29,31]. Most of the NH_4^+ sensors were designed to be detectors for FIA systems [6,10,11,30–32].

There are also reports in the literature demonstrating the possibility of detecting ammonium ion by voltammetry at the polarized Interface between Two Immiscible Electrolyte Solutions (ITIES). The first example of facilitated ammonium ion transfer across aqueous/organic solution interfaces was reported in 1987 by Yoshida and Kihara [38]. The transfer of Li^+, Na^+, K^+, NH_4^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, from aqueous solution to 1,2-dichloroethane (DCE) or nitrobenzene (NB), facilitated by polyoxyethylene ethers, Triton X, was studied by current-scan polarography using the aqueous electrolyte dropping electrode. Kihara's group published another work [39] based on ion transfer reactions across the interface water/DCE in the presence of a phospholipid layer adsorbed on the interface. The transfer of hydrophilic cations, such as ammonium, from water to DCE was found to be facilitated by the formation of a complex between the cation and the phospholipid layer, followed by desorption of the complex from the interface. In 1987, an amperometric ammonia sensor was constructed by Osakai et al. [40] based on the ammonium-ion selective PVC–nitrobenzene gel electrode containing dibenzo-18-crown-6 (DB18C6). The electrode was covered with by a gas-permeable membrane to give the electrode high selectivity for ammonium ion. The detection scheme for the assay of ammonium ion used in their work could be applied to the ultimate detection of urea and creatinine. Subsequent

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publications by Senda and Yamamoto [41,42] detail further investigation and development of the proposed detection scheme. In addition, facilitated ion transfer reactions at liquid–liquid micro-interface of the ammonium ion, produced in the urea–urease enzymatic reaction, was investigated by Senda et al. [43] for amperometric urea sensor fabrication. In 1992, Thomas et al. [44] studied the facilitated transfer of alkali metal ions from water to NB phase containing various crown ethers, using cyclic voltammetry (CV). The transfer of NH_4^+ facilitated by 4′-picrylamino-5′-nitrobenzo-18-crown-6 was also included in the study. Girault et al. studied the transfer reactions of sodium, ammonium and potassium ions, either direct or facilitated by the various ionophores (such dibenzo-18-crown-6 and valinomycin) at the water/DCE [45,46] interface and at water/2-nitrophenyloctylether (NPOE) [47]–PVC gel micro-interface, using CV. The same authors have also developed enzyme sensors, using liquid–liquid micro-interface arrays, for the assay of urea [12] and creatinine [48], where the transducer relies on the amperometric detection of ammonium ion at the water/DCE interface. More recently, in 2002, Shao et al. [49] used a functionalized fullerene derivative containing a monoaza-18-crown-6 moiety for the investigation of facilitated ion transfer, including the ammonium ion, across the water/NB micro-interface supported at the tip of a micropipette. The interaction between the cation and the ionophore used was evaluated by CV and Osteryoung square wave voltammetry (OSWV).

Various examples of lipophilic ionophores for the facilitated ammonium ion transfer across the water/oil interface are shown in the literature. In this work we explore the possibility of the use of a cyclodextrin (CD) as ionophore, present in the organic stationary phase.

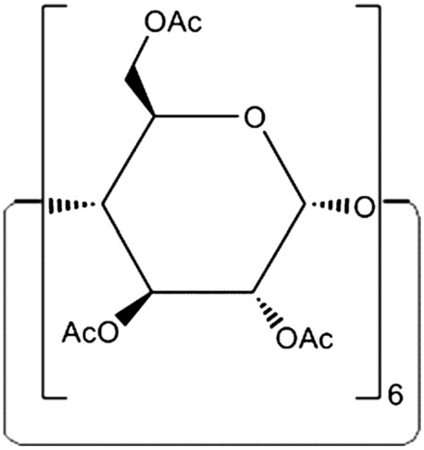
Cyclodextrins have been intriguing due to their unique physicochemical characteristics, including the ability to form host-guest complexes with a wide variety of organic compounds, controlling the solubility and presenting extremely low toxicity against living systems [50,51]. They can interact with almost every class of compound including hydrocarbons, aliphatic alcohols, diols, amines and acids, oligopeptides, sugars, phenols, aromatic amines, azo compounds, naphthalene derivatives and various drugs [50]. As a result, complexation reactions involving CDs molecules are very important in various fields of application and have been widely used in the pharmaceutical, food, cosmetic, and agricultural industries, in analytical chemistry and catalysis [52,53]. Many methods for CD immobilization have been used including physical adsorption of CD [54,55], polymeric films [56,57], immobilization in composites materials [58], Langmuir–Blodgett films [59], use of self-assembled monolayers (SAMs) [60–64] and lipophilic membranes [65,66] of CDs. In liquid–liquid interface processes, Heptakis(2,3,6-tri-O-acetyl)-α-cyclodextrin was used as an alternative method of chiral detection and separation at the water/DCE interface [67].

In the present work, we demonstrate the ammonium ion facilitated transfer across the water/1,6-dichlorohexane (DCH) interface which can be achieved by the use of Hexakis(2,3,6-tri-O-acetyl)-α-cyclodextrin (HexαCD) as an ionophore. The association constant between the ionophore and the transferring ion was calculated as well as the analytical performances of the electrochemical amperometric sensor for ammonium ion detection.

2. Experimental

2.1. Reagents and preparation of solutions

Ammonium chloride (normapur) was purchased from VWR - Prolabo.



Scheme 1. Molecular structure of Hexakis(2,3,6-tri-O-acetyl)-α-cyclodextrin (HexαCD).

The organic phase supporting electrolyte salt used in this study was the bis(triphenylphosphoranylidene) ammonium tetrakis(4-chlorophenyl)borate (BTPPATPBCl) which was prepared by the metathesis of BTPPACl (Aldrich) and the KTPBCl (Fluka). Hexakis(2,3,6-tri-O-acetyl)-α-cyclodextrin (HexαCD), represented in Scheme 1, was purchased from Cyclolab LTD (Hungary). NaCl, KCl, $MgCl_2$ and $CaCl_2$ were obtained from Merck (p.a.) and HCl (suprapur) from Sigma–Aldrich. Aqueous solutions were prepared with water purified with a Milli-RO3 Plus and Milli-Q systems to a specific resistance greater than 18 MΩ cm. 1,6-Dichlorohexane (DCH) was obtained from Aldrich and was purified according to a procedure described elsewhere [68]. The organic solvent used in this work was handled with all necessary precautions.

2.2. Apparatus

Electrochemical data were obtained using a computer controlled potentiostat Autolab PGSTAT302N (Eco Chemie B.V., Utrecht, Netherlands).

2.3. Procedure

The experimental electrochemical cell for the investigations with arrays of micro liquid–liquid interfaces (μITIES) was a four-electrode cell (see ref. [69]); the two reference electrodes were Ag/AgCl (prepared by electrochemical oxidation of an Ag wire in NaCl 1 M solution) and the two counter electrodes were Pt, one in each phase.

In this system, a 12 μm thick PET membrane with 66 holes, 10 μm diameter, 100 μm separation between pore centres was used. The micro-hole arrays were kindly supplied by Prof. Hubert Girault, EPFL, Switzerland. The electrochemical cell used had a geometrical water/organic solvent interface of 5.2×10^{-5} cm^2. The microporous membrane was sealed with a fluorosilicone sealant (Dow Corning 730) onto a glass cylinder which was filled with a small volume (4.0 mL) of the aqueous phase, where the aliquots from concentrated ammonium ion solution were added in order to change it concentration in the aqueous phase. The membrane was then immersed into the organic phase contained in the cell.

The reference solution for the organic phase (2 mM BTPPACl + 2 mM NaCl) was mechanically stabilized by a gel as described elsewhere [70]. The cell used in the electrochemical studies of ammonium ion is schematically represented in Scheme 2.

Ag	AgCl	NaCl 2 mM BTPPACl 2 mM (Water)	*x* mM HexαCD BTPPATPBCl 1 mM (1,6-DCH)	*y* mM NH_4^+ HCl 1 mM (Water)	AgCl	Ag'

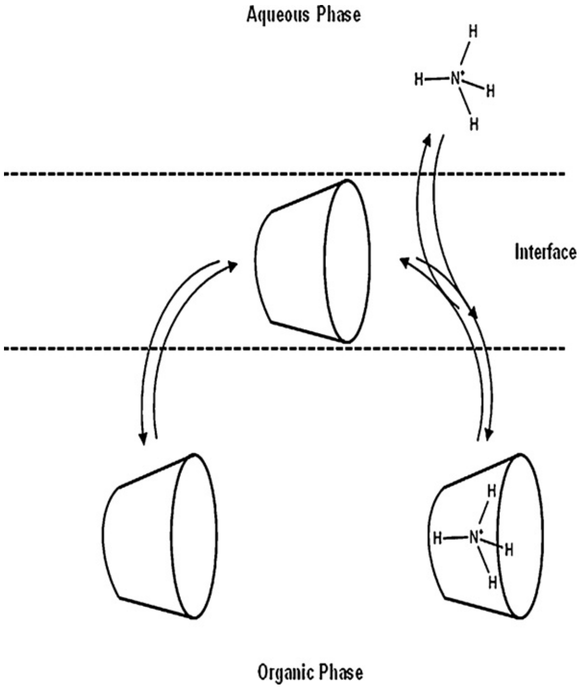
Scheme 2. Schematic representation of the electrochemical cell used in this work.

CV was performed at a scan rate of 50 mV s^{-1}. The operating conditions for square wave voltammetry (SWV) were: step potential of 2 mV, pulse amplitude of 50 mV and a frequency of 25 Hz. The experimental conditions for differential pulse voltammetry (DPV) were: step potential of 2 mV, pulse amplitude of 50 mV and scan rate of 5 mV s^{-1}. All the electrochemical data were then monitored by the General Purpose Electrochemical System (GPES) version 4.9, software package. All electrochemical experiments were performed at room temperature. The electrochemical cell was placed in a Faraday cage in order to minimize the contribution of background noise to the analytical signal.

3. Results and discussion

Ammonium ion facilitated transfer was attributed to the formation of the hydrophobic complex of ammonium ion with the synthetic cyclodextrin adsorbed at the aqueous/organic solution interface, followed by the transfer of the complex from the interface into the organic solution, as represented in Scheme 3. This ligand is capable to form a complex with the ammonium ion by sequestering the ion within the structure.

Scheme 3 illustrates the concept of ammonium ion transfer by the formation of the hydrophobic complex of ammonium ion and the HexαCD adsorbed at the aqueous/organic solution interface. The ammonium ion is sequestered within the structure of the ligand which improves it solvation in the organic phase and then transported to the organic phase.



Scheme 3. Concept of ammonium ion transfer reaction facilitated by HexαCD at the water/DCH micro-interface.

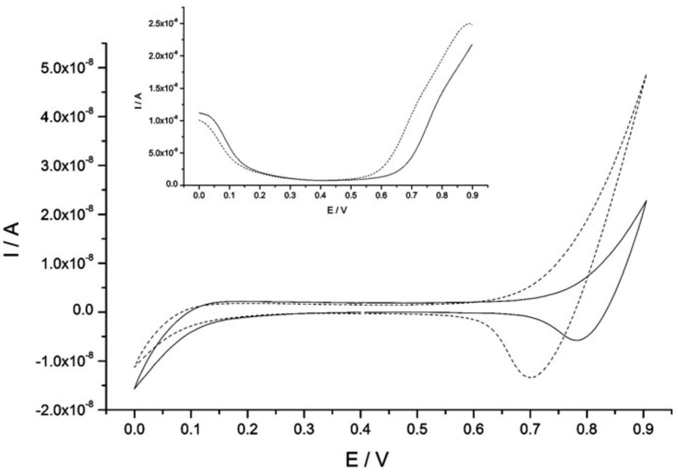


Fig. 1. CVs obtained at the water/DCH micro-interface in the absence (–) and presence (—) of ammonium ion 0.64 mM. HexαCD 10 mM. Inset: DPVs obtained for the assisted transfer of ammonium ion in the same conditions.

3.1. *Characterization of the NH_4^+:HexαCD complex at the water/DCH micro-interface*

In order to test the ability of the ionophore HexαCD to assist the electrochemical transfer of ammonium ion at the water/DCH polarized interface, preliminary studies were carried out by CV.

Fig. 1 compares the cyclic voltammograms (CVs) recorded in absence and presence of ammonium ion in the aqueous solution. The organic phase contains a concentration of 10 mM in HexαCD. The increase in current at the positive potentials limit observed when ammonium ion is present in aqueous phase may be considered as corresponding to the assisted transfer of ammonium ion from the water to DCH. Differential pulse voltammograms (DPVs) were also collected in the same conditions, after the CVs measurements, and are represented in the inset of the Fig. 1.

At the less positive end of the potential window the presence of ammonium ion in the aqueous phase seems to decrease the current of the transfer of the supporting electrolyte ions.

Changing the ammonium ion concentration, when the $C_{\mathrm{ION}} \gg C_{\mathrm{LIGAND}}$, results in the displacement of the peak potential while the limiting current is independent of ion concentration. As a result, the half-wave potential of the transfer, $\Delta_o^w\phi_{1/2}$, shifts away from the transfer potential of the species studied in the absence of the ionophore.

For each experiment, a predetermined amount of tetrabutylammonium (TBA^+) was added to the aqueous phase providing a reference for all half-wave potential measurements. The selection of the tetraalkylcation was made accordingly to the transfer potential of the ammonium ion complex in order to avoid superposition of both voltammetric waves. The values measured were transposed to the Galvani potential scale taking into account the following relationship [71]:

$$E_{1/2} - \Delta_o^w\phi^0 = E_{1/2}(\mathrm{TBA}^+) - \Delta_o^w\phi^0(\mathrm{TBA}^+) \qquad (1)$$

where $\Delta_o^w\phi^0(\mathrm{TBA}^+)$ represents the value of the transfer potential of the TBA^+ ion. $E_{1/2}$ and $E_{1/2}(\mathrm{TBA}^+)$ are the experimental half-wave potentials of the charge transfer reactions and TBA^+ transfer, respectively.

As reference for our measurements the value of $\Delta_{\mathrm{DCH}}^w\phi^0(\mathrm{TBA}^+) = -0.193$ V [72] was used.

Fig. 2 shows the DPVs for the assisted transfer of ammonium ion when using a concentration of HexαCD ligand of 2.0 mM and varying the ammonium ion concentration from 2 to 25 mM, by consecutive addictions of ion to the electrochemical cell. The DPVs

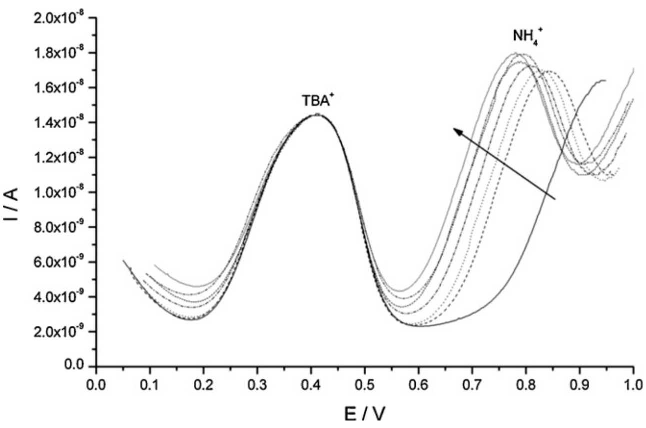


Fig. 2. DPVs obtained for ammonium ion transfer assisted by HexαCD (C = 2.0 mM) for different ion concentration and in the presence of 0.25 mM of TBA$^+$. NH$_4^+$ concentrations: 0, 2.0, 4.0, 8.0, 12, 17 and 25 mM. The arrow indicates the increase in ammonium ion concentration.

also shows a peak for the transfer of TBA$^+$ ion from the aqueous to the organic phase that is used to establish the reference to convert from the experimental potential scale to the thermodynamic potential scale. The simple ammonium ion transfer was also studied in this work. However, since the ammonium ion transfer occurs very close to the end of the potential window it is of reduced utility for analytical purposes. Recently, Katano et al. [68] presented a study where they have determined the half-wave potential of the species limiting the potential window of ion-transfer at the water/DCH, water/1,4-dichlorobutane (DCB) and at the water/DCE interface. According to these authors, the midpoint potentials of the hydrophilic ammonium cation are: $\Delta_{\mathrm{DCH}}^{w}\phi_m' = 0.559\,\mathrm{V}$, $\Delta_{\mathrm{DCB}}^{w}\phi_m' = 0.515\,\mathrm{V}$ and $\Delta_{\mathrm{DCE}}^{w}\phi_m' = 0.488\,\mathrm{V}$.

According to Reymond et al. [73], the slope of the following plot provides information of the complexation stoichiometry: $-(zF/2.303RT)(\Delta_o^w\phi_{1/2,[\mathrm{NH_4L}]^+} - \Delta_o^w\phi_{1/2,\mathrm{NH_4^+}})$ *vs* of log $(C_{\mathrm{NH_4^+}}/M)$ (where z is the ion charge, F is the Faraday constant, R is the gas constant and T is the temperature). As shown in Fig. 3 a slope of 1.0 was obtained for the studies involving the complex between NH$_4^+$ and HexαCD indicating the formation of a 1:1 complex at the interface.

Fig. 4 shows the change in the half-wave potential ($\Delta_{\mathrm{DCH}}^{w}\phi_{1/2}$) when the concentration of excess ammonium ion is varied. Using the methodology proposed by Reymond et al. [73], further analysis of the voltammetric data represented in Fig. 4 allows the evaluation of the complexation equilibrium constant (β) of NH$_4^+$:HexαCD complex in the organic phase. In the case of ammonium ion excess, β is the association constant of the 1:1 complex and can be evaluated solving the following equation:

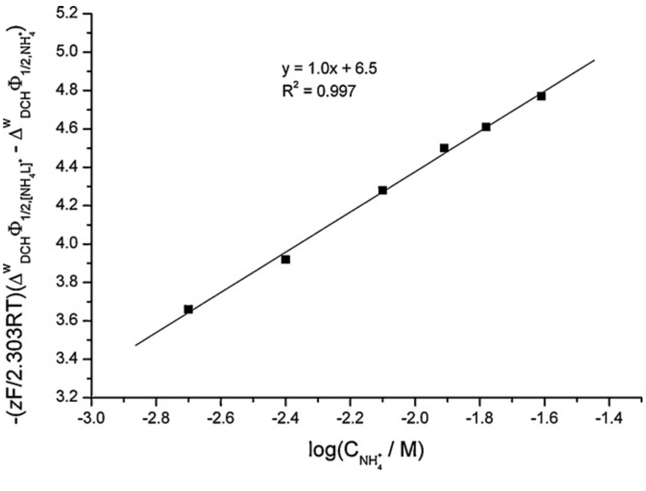


Fig. 3. Plot of $-(zF/2.303RT)(\Delta_{\mathrm{DCH}}^{w}\phi_{1/2,[\mathrm{NH_4L}]^+} - \Delta_{\mathrm{DCH}}^{w}\phi_{1/2,\mathrm{NH_4^+}})$ *vs* log $(C_{\mathrm{NH_4^+}}/M)$ for the assisted ammonium ion transfer.

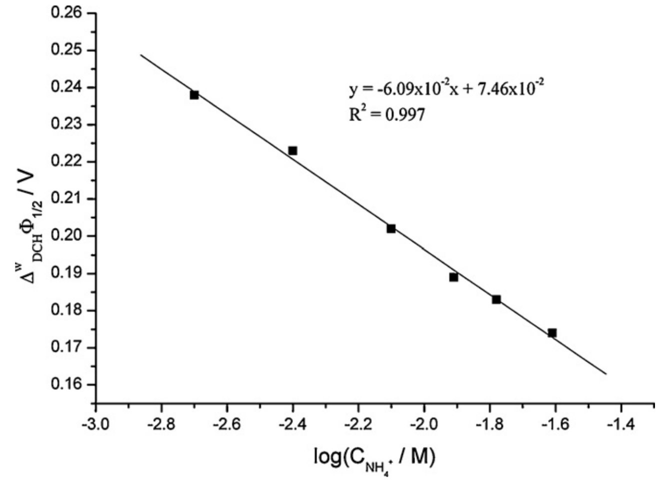


Fig. 4. Dependence of half-wave potential on the log $(C_{\mathrm{NH_4^+}}/M)$ when ammonium ion is in excess for a ligand concentration of 2.0 mM in the organic phase.

$$\Delta_o^w\phi_{1/2,[\mathrm{NH_4L}]^+} = \Delta_o^w\phi_{1/2,\mathrm{NH_4^+}} - \frac{2.303RT}{zF}\log(\beta C_{\mathrm{NH_4^+}}) \qquad (2)$$

where $\Delta_o^w\phi_{1/2,[\mathrm{NH_4L}]^+}$ represents the half-wave Galvani potential for the transfer of the complex [NH$_4$L]$^+$, $\Delta_o^w\phi_{1/2,\mathrm{NH_4^+}}$ the half-wave Galvani potential for the ammonium ion and $C_{\mathrm{NH_4^+}}$ the concentration of the ammonium ion.

From the intercepts of the plots in Fig. 4, considering the value of $\Delta_o^w\phi_{1/2,\mathrm{NH_4^+}} = 0.559\,\mathrm{V}$ (as proposed by Katano et al. [68]) and using Eq. (2), a value of log β = 7.9 was obtained for the NH$_4^+$:HexαCD complex at the water/DCH interface.

As this system has not been studied at other liquid–liquid interfaces, the experimental data obtained was compared with data found in literature for ammonium ion facilitated transfer with other ionophores, in various types of water/organic solution interfaces. The calculated association constant is consistent with values reported by Thomas et al. [44] and Girault et al. [45,47] who reported values for the ammonium ion of 6.34 (ligand: 4′-picrylamino-5′-nitrobenzo-18-crown-6; interface: water/NB), 8.6 (dibenzo-18-crown-6; water/DCE), 13.1 (valinomycin; water/DCE), 14.1 (nonactin; water/DCE), 9.4 (dibenzo-18-crown-6; water/NPOE) and 14.6 (valinomycin; water/NPOE).

3.2. Determination of ammonium ion at water/DCH micro-interface

The micro-ITIES setup was used for sensing ammonium ions by using the assisted transfer of ammonium ion by HexαCD at the water/DCH interface. As the concentration of the ionophore in the organic phase is in excess, any steady-state current measured is proportional to the concentration of species in the aqueous phase.

The analytical properties of the assisted ammonium ion transfer at the micro-ITIES were investigated by SWV, as this electrochemical technique allows the detection of lower concentrations of target analyte when compared to CV and DPV. In order to further increase the sensitivity of the SWV technique, the background subtraction was employed, i.e., the blank experiment (in the absence of ammonium ion) was subtracted from each of the recorded SWV curve. Fig. 5 shows the voltammograms (Fig. 5A) and the background-subtracted voltammograms (Fig. 5B) recorded, for the ammonium ion concentrations ranging from 4.2 to 327 μM. The peak for the

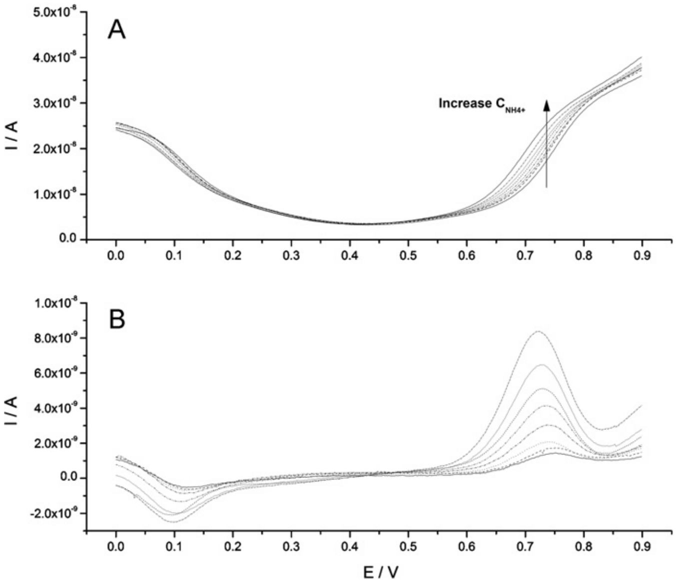


Fig. 5. SWVs obtained for ammonium ion transfer assisted by HexαCD (C = 10 mM) (A). NH_4^+ concentrations in the aqueous phase (from bottom to top): 0, 4.2, 8.4, 17, 33, 66, 106, 183 and 327 μM. Background subtracted SWVs (B). NH_4^+ concentrations (from bottom to top): 4.2, 8.4, 17, 33, 66, 106, 183 and 327 μM.

assisted transfer of ammonium ion was found to be 0.737 ± 0.009 V. The negative current peak observed at approximately 0.1 V in Fig. 5B is due an increase of Cl^- concentration, after each addition of ammonium chloride standard solution to the aqueous phase, which results in a negative current peak after mathematical background subtraction of the SWVs collected.

For the calibration curve development, a mathematical model was used in this work to process the electrochemical signal. The conventional numerical derivation technique is very simple, and has been widely used as a powerful enhancement method in analytical chemistry [74–77] for achieving higher resolution [78,79]. An example of the numerical derivative of SWV (dSWV), after background subtraction method, is presented in Fig. 6 after smoothing by the Savitzky and Golay [80–83] algorithm. The noise is greatly reduced while the peak itself is hardly changed, making it easier to measure the peak position, height, and width. The method for the determination of the numerical derivative of the peak current (dI_{peak}) is also demonstrated in Fig. 6.

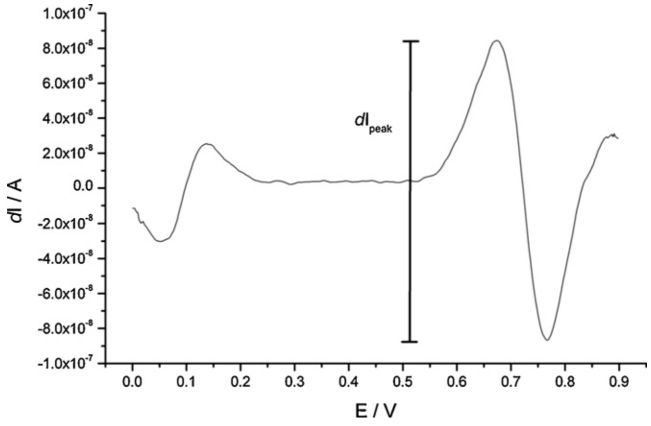


Fig. 6. Example of dSWV, after backgroud subtraction method, for NH_4^+ 327 μM in aqueous solution.

Table 1
Comparison of the two methods used for experimental SWV and dSWV peak current determination.

Ammonium ion conc. (μM)	Peak potential (E_{peak}) (V)	SWV peak current (I_{peak}) (10^{-9} A)	dSWV peak current (dI_{peak}) (10^{-8} A)
4.2	0.752	1.45	2.46
8.4	0.752	1.73	3.03
16.7	0.742	2.06	3.85
33.3	0.738	3.04	6.10
66.0	0.730	4.13	8.68
105.9	0.730	5.11	11.1
183.0	0.726	6.48	13.6
326.6	0.728	8.29	17.2

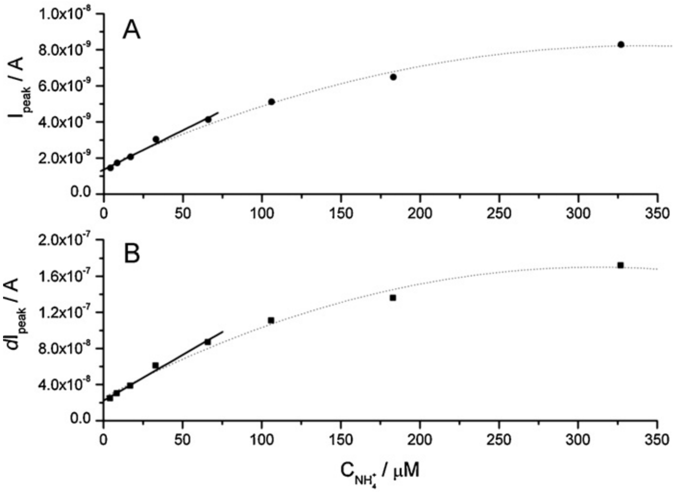


Fig. 7. Calibration curve for ammonium ion transfer at the water/DCH micro-interface, after background subtraction, using SWV (A) and dSWV (B) peak current.

The methods for the experimental peak current determination were compared. The values of the SWV peak current (I_{peak}) obtained, after background subtraction, and the correspondent dSWV peak current (dI_{peak}) value are presented in Table 1, for the different ammonium ion concentrations. The values of the dI_{peak} obtained are about one order of magnitude higher than the correspondent common I_{peak}, and were used for the analytical determination of ammonium ion.

Fig. 7 shows the SWV peak current (Fig. 7A) and the dSWV peak current (Fig. 7B) as a function of the ammonium ion concentration in the aqueous solution. Good linearity was achieved over the same concentration range 4.2–66 μM for both the calibration curves. A non-linear increase of the I_{peak} and dI_{peak} with the concentration of ammonium ion was observed for concentrations values higher than 66 μM. A similar behaviour was also observed by Girault's group in their studies for liquid–liquid detection of urea [12] and creatinine [48].

A summary of analytical performances obtained when using SWV and the dSWV, is presented in Table 2. The comparison of the analytical parameters including the slope, the intercept of the linear fitting, the linear range and limits of detection (LOD) is shown. The LOD was calculated according to Analytical Methods Committee recommendations [84,85].

The limit of detection (LOD) calculated from the regression line was found to be 0.12 μM, using SWV technique and the dSWV

Table 2
Analytical results for the detection of ammonium ion, in the linear range of concentrations used, using the SWV and dSWV peak current.

	Linear range (μM)	Slope (A μM^{-1})	Intercept (A)	R^2	LOD (μM)
SWV	4.2–66 (N = 5)	4.35×10^{-11}	1.36×10^{-9}	0.985	2.9
dSWV	4.2–66 (N = 5)	1.01×10^{-9}	2.22×10^{-8}	0.987	0.12

Table 3
Linear fitting parameters from the $I_t = f(C_{NH_4^+})$ plot and amperometric selectivity coefficient calculated from Eq. (5).

Ion	Slope (A μM^{-1})	Intercept (A)	k_{ij}^{amp}
Mg^{2+}	7.0×10^{-10}	-8.0×10^{-10}	1.1×10^{-4}
Ca^{2+}	7.2×10^{-10}	6.0×10^{-9}	8.3×10^{-4}
Na^{+}	3.4×10^{-11}	1.2×10^{-9}	1.8×10^{-2}
K^{+}	3.8×10^{-10}	1.1×10^{-9}	2.9×10^{-4}

peak current (dI_{peak}) as electrochemical signal. Since the values of the *d*SWV peak current (dI_{peak}) are about one order of magnitude higher than the correspondent SWV peak current (I_{peak}), the LOD obtained using the processed signal was improved from 2.9 to 0.12 μM. The LOD obtained for ammonium ion is of the same order of magnitude of those found by other investigators using potentiometric [5,8,22] and amperometric [10,29,31,32] devices. No references to LODs were found in literature for the electrochemical determination of ammonium ion by means of facilitated ion transfer at liquid–liquid interfaces. However, Osborne et al. [45] have found a linear relationship between the steady-state plateau current and the ammonium ion concentration over the range from 1 μM to 500 μM of NH_4^+ ions, at a single microhole water/DCE interface. Osakai et al. [40] obtained a current response proportional to the ammonia concentration in the range from 2 to 200 μM at water/PVC–NB gel interface.

3.3. Interference investigation

The evaluation of the analytical performance of a sensor requires the assessment of the selectivity towards interfering ions that can potentially be present in the sample to be measured. In this work the evaluation will be made using the methodology proposed by Wang [86]. Wang defined the selectivity coefficient for amperometric sensors (k_{ij}^{amp}). For any amperometric device, the total current response in the presence of interfering species (*j*) can be described by the following equation:

$$I_t = K(C_i + \sum k_{ij}^{amp} C_j) \qquad (3)$$

where *i* is the target analyte, I_t corresponds to the analyte peak current in the presence of the interferents, *K* is the slope of the calibration curve and C_i and C_j are respectively the concentrations of the analyte and interfering species.

Magnesium, calcium, sodium and potassium were tested as interferents and their effect on the ammonium ion determination was evaluated. Other amines, namely dopamine noradrenaline and adrenaline were tested however no effect could be observed.

The procedure used in this work consisted in making constant the concentration of the interfering ion (a value of 10 mM was used in order to test the system using a large excess of interfering species). Under the experimental conditions used, Eq. (3) can be expressed in the following way:

$$I_t = KC_i + Kk_{ij}^{amp} C_j \qquad (4)$$

From the plot of I_t as a function of C_i, it is possible to extract the value of k_{ij}^{amp} using the following equation:

$$k_{ij}^{amp} = \frac{\text{Intercept}}{\text{Slope} \cdot C_j} \qquad (5)$$

Fig. 8 displays the plot of I_t as a function of $C_{NH_4^+}$, in the presence of the interferents under test. Table 3 summarizes the parameters evaluated from the linear fitting of I_t as a function of ammonium ion concentration, as well as the value of the amperometric selectivity coefficient calculated from Eq. (5).

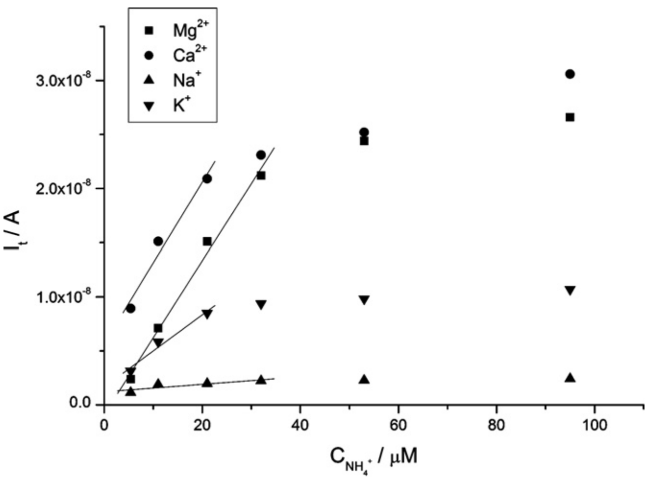


Fig. 8. Calibration curve for ammonium ion transfer at the water/DCH micro-interface in the presence of 10 mM Mg^{2+}, Ca^{2+}, K^+ and Na^+.

Although the presence of the interfering species can difficult the direct determination of ammonium in aqueous samples, the values of the selectivity constant are reasonably low and therefore we can consider the proposed electrochemical sensor as a valid option for the amperometric detection of ammonium ion in aqueous solutions. Furthermore, the interference can be minimized if the proposed sensor is used as an amperometric detector in ion chromatography as demonstrated by Lee and Girault for a similar system [87].

4. Conclusions

Voltammetric behaviour of ammonium ion transfer facilitated by complexation with a hydrophobic cyclodextrin was studied at polarized water/DCH micro-interface. This ionophore proved to form stable complexes with ammonium ion, with an association constant (log β) of 7.9. Comparing to simple ion transfer, this ionophore produces well-defined facilitated ammonium ion transfer waves over a large range of concentrations, which occur near the centre of the potential window.

Taking advantage of SWV, ammonium ion was determined with a pulsed technique with well-known capability for achieving lower detection limits. The limit of detection (LOD) of 0.12 μM was achieved and a linear response from 4.2 to 66 μM of added ammonium ion was obtained. The LOD obtained in this study at the water/DCH is comparable to that obtained with other amperometric and potentiometric biosensors.

The results point to the development of a useful sensor based on ITIES that can be used for detection of ammonium ion in environmental systems or for determination of ammonium ion as a metabolite in clinical systems in particular if the sensor is combined with a separation technique such as ion chromatography.

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References

[1] J.M.C.S. Magalhães, A.A.S.C. Machado, Analyst 127 (2002) 1069–1075.
[2] G. Palleschi, M. Mascini, E. Martinez-Fabregas, S. Alegret, Anal. Lett. 21 (1988) 1115–1129.

[3] J.F.C.C. Lima, C. Delerue-Matos, C.V.M. Cristina, Anal. Chim. Acta 385 (1999) 437–441.
[4] P.W. Alexander, T. Dikitrakopoloulos, D.B. Hibbert, Electroanalysis 9 (1997) 1331–1336.
[5] N. Jaffrezic-Renault, A. Senillou, C. Martelet, K. Wan, J.M. Chovelon, Sens. Actuators B 59 (1999) 154–164.
[6] H.S. Twu, T.R. Ling, T.C. Chou, M.C. Yang, Ultrason. Sonochem. 8 (2001) 41–47.
[7] T. Suzuki, T. Yasuda, T. Yamane, S. Shimizu, J. Ferment. Technol. 64 (1986) 63–70.
[8] A. Senillou, N. Jaffrezic-Renault, C. Martelet, S. Cosnier, Talanta 50 (1999) 219–226.
[9] A.K. Abass, J.P. Hart, D.C. Cowell, A. Chappell, Anal. Chim. Acta 373 (1998) 1–8.
[10] B. Strehlitz, B. Grundig, H. Kopinke, Anal. Chim. Acta 403 (2000) 11–23.
[11] W.-J. Cho, H.-J. Huang, Anal. Chem. 70 (1998) 3946–3951.
[12] M.D. Osborne, H.H. Girault, Electroanalysis 7 (1995) 714–721.
[13] N. Stromberg, S. Hulth, Sens. Actuators B 90 (2003) 308–318.
[14] N. Stromberg, S. Hulth, Anal. Chim. Acta 443 (2001) 215–225.
[15] E. Wang, L. Zhu, L. Ma, H. Patel, Anal. Chim. Acta 357 (1997) 85–90.
[16] J. Reichert, W. Sellien, H.J. Ache, Sens. Actuators A 26 (1991) 481–482.
[17] Z. Genfa, P.K. Dasgupta, Anal. Chem. 61 (1989) 408–412.
[18] P.R. Krous, S.R. Crouch, Anal. Lett. 20 (1987) 183–200.
[19] L. Cardoso de Faria, C. Pasquini, Anal. Chim. Acta 245 (1991) 183–190.
[20] J.J.R. Rohwedder, C. Pasquini, Analyst 116 (1991) 841–845.
[21] C. Pasquini, L. Cardoso de Faria, Anal. Chim. Acta 193 (1987) 19–27.
[22] L.Y. Heng, S. Alva, M. Ahmad, Sens. Actuators B 98 (2004) 160–165.
[23] J.H. Shin, S.Y. Yoon, I.J. Yoon, S.H. Choi, S.D. Lee, H. Nam, G.S. Cha, Sens. Actuators B 50 (1998) 19–26.
[24] P.C. Pandey, G. Singh, Talanta 55 (2001) 773–782.
[25] J. Saurina, S. Hernández-Cassou, S. Alegret, E. Fàbregas, Biosens. Bioelectron. 14 (1999) 67–75.
[26] M.L. Hamlaoui, K. Reybier, M. Marrakchi, N. Jaffrezic-Renault, C. Martelet, R. Kherrat, A. Walcarius, Anal. Chim. Acta 466 (2002) 39–45.
[27] M.L. Hamlaoui, R. Kherrat, M. Marrakchi, N. Jaffrezic-Renault, A. Walcarius, Mater. Sci. Eng. C 21 (2002) 25–28.
[28] P. Bertocchi, D. Compagnone, G. Palleschi, Biosens. Bioelectron. 11 (1996) 1–10.
[29] Y.-C. Luo, J.-S. Do, Biosens. Bioelectron. 20 (2004) 15–23.
[30] R. Liu, B. Sun, D. Liu, A. Sun, Talanta 43 (1996) 1049–1054.
[31] Y.T. Shih, H.J. Huang, Anal. Chim. Acta 392 (1999) 143–150.
[32] A. Walcarius, V. Vromman, J. Bessiere, Sens. Actuators B 56 (1999) 136–143.
[33] Y.-C. Luo, J.-S. Do, Sens. Actuators B 115 (2006) 102–108.
[34] M.E. Meyerhoff, Anal. Chem. 52 (1980) 1534–1535.
[35] M.E. Meyerhoff, R.H. Robins, Anal. Chem. 52 (1980) 2383–2387.
[36] M. Ikeda, H. Hichaya, S. Ito, Y. Asanao, T. Imato, Biosens. Bioelectron. 13 (1998) 531–537.
[37] T. Okada, I. Karube, S. Suzuki, Anal. Chim. Acta 135 (1982) 159–163.
[38] Z. Yoshida, S. Kihara, J. Electroanal. Chem. 227 (1987) 171–181.
[39] Y. Yoshida, H. Yoshinaga, N. Ichieda, A. Uehara, M. Kasuno, K. Banu, K. Maeda, S. Kihara, Anal. Sci. 17 (2001) 1037–1039.
[40] T. Osakai, T. Kakutani, M. Senda, Anal. Sci. 3 (1987) 521–526.
[41] Y. Yamamoto, M. Senda, Sens. Actuators B 13 (1993) 57–60.
[42] Y. Yamamoto, M. Senda, Electroanalysis 5 (1993) 775–779.
[43] T. Osakai, T. Kakutani, M. Senda, Anal. Sci. 4 (1988) 529–530.
[44] N.K. Harris, S. Jin, G.J. Moody, J.D.R. Thomas, Anal. Sci. 8 (1992) 545–551.
[45] M.D. Osborne, Y. Shao, C.M. Pereira, H.H. Girault, J. Electroanal. Chem. 364 (1994) 155–161.
[46] M.D. Osborne, H.H. Girault, Electroanalysis 7 (1995) 425–434.
[47] H.J. Lee, C. Beriet, H.H. Girault, J. Electroanal. Chem. 453 (1998) 211–219.
[48] M.D. Osborne, H.H. Girault, Mikrochim. Acta 117 (1995) 175–185.
[49] B. Su, S. Zhang, Y. Yuan, J. Guo, L. Gan, Y. Shao, Anal. Chem. 74 (2002) 373–378.
[50] K.A. Connors, Chem. Rev. 97 (1997) 1325–1357.
[51] K. Uekama, F. Hirayama, T. Irie, Chem. Rev. 98 (1998) 2045–2076.
[52] A.R. Heges, Chem. Rev. 98 (1998) 2035–2044.
[53] J. Szejtli, Chem. Rev. 98 (1998) 1743–1753.
[54] L. Pospisil, M. Svestka, J. Electroanal. Chem. 366 (1994) 295–302.
[55] L. Pospisil, M. Svestka, J. Electroanal. Chem. 426 (1997) 47–53.
[56] W. Kutner, H. Wu, K.M. Kadish, Electroanalysis 6 (1994) 934–944.
[57] Q. Chen, P.V.A. Pamidi, J. Wang, W. Kutner, Anal. Chim. Acta 306 (1995) 201–208.
[58] A. Ferancová, E. Korgová, R. Mikó, J. Labuda, J. Electroanal. Chem. 492 (2000) 74–77.
[59] K. Odashima, M. Kotato, M. Sugawara, Y. Umezawa, Anal. Chem. 65 (1993) 927–936.
[60] O. Crepo-Biel, B. Dordi, P. Maury, M. Péter, D.N. Reinhoudt, J. Huskens, Chem. Mater. 18 (2006) 2545–2551.
[61] O. Crepo-Biel, C.W. Lim, B.J. Ravoo, D.N. Reinhoudt, J. Huskens, J. Am. Chem. Soc. 128 (2006) 17024–17032.
[62] C.A. Nijhuis, F. Yu, W. Knoll, J. Huskens, D.N. Reinhoudt, Langmuir 21 (2005) 7866–7876.
[63] F.S. Damos, R.C.S. Luz, L.T. Kubota, Electrochim. Acta 53 (2007) 1945–1953.
[64] T. Fukuda, Y. Maeda, H. Kitano, Langmuir 15 (1999) 1887–1890.
[65] R. Kataky, S. Palmer, D. Parker, D. Spurling, Electroanalysis 9 (1997) 1267–1272.
[66] D. Parker, R. Kataky, P.M. Kelly, S. Palmer, Pure Appl. Chem. 68 (1996) 1219–1223.
[67] R. Kataky, P. Lopes, Chem. Commun. (2009) 1490–1492.
[68] H. Katano, H. Tatsumi, M. Senda, Talanta 63 (2004) 185–193.
[69] J.A. Ribeiro, I.M. Miranda, F. Silva, C.M. Pereira, Phys. Chem. Chem. Phys. 12 (2010) 15190–15194.
[70] O. Dvořak, V. Mareček, Z. Samec, J. Electroanal. Chem. 284 (1990) 205–215.
[71] E.S. Ferreira, A. Garau, V. Lippolis, C.M. Pereira, F. Silva, J. Electroanal. Chem. 587 (2006) 155–160.
[72] H. Katano, M. Senda, Anal. Sci. 17 (2001) 1027–1029.
[73] F. Reymond, P.A. Carrupt, H.H. Girault, J. Electroanal. Chem. 449 (1998) 49–65.
[74] T.C. O'Haver, T. Begley, Anal. Chem. 53 (1981) 1876–1878.
[75] J.K. Kauppinen, D.J. Moffatt, H.H. Mantsch, D.G. Camerlon, Anal. Chem. 53 (1981) 1454–1457.
[76] J.A. Howell, L.G. Hargis, Anal. Chem. 66 (1994) 445R–461R.
[77] L.G. Hargis, J.A. Howell, R.E. Sutton, Anal. Chem. 68 (1996) 169R–183R.
[78] X. Zhang, J. Jin, Electroanalysis 16 (2004) 1514–1520.
[79] A.K. Leung, F. Chau, J. Gao, Anal. Chem. 70 (1998) 5222–5229.
[80] A. Savitzky, M.J.E. Golay, Anal. Chem. 36 (1964) 1627–1639.
[81] J. Steiner, Y. Termonia, J. Delfour, Anal. Chem. 44 (1972) 1906–1909.
[82] H.H. Madden, Anal. Chem. 50 (1978) 1383–1386.
[83] P.A. Gorry, Anal. Chem. 62 (1990) 570–573.
[84] Analytical Method Committee, Analyst 112 (1987) 199–204.
[85] J. Mocak, A.M. Bond, S. Mithell, G. Scollary, Pure Appl. Chem. 69 (1997) 297–328.
[86] J. Wang, Talanta 41 (1994) 857–863.
[87] H.J. Lee, H.H. Girault, Anal. Chem. 70 (1998) 4280–4285.