Effect of charge of quaternary ammonium cations on lipophilicity and electroanalytical parameters: Task for ion transfer voltammetry

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A B S T R A C T

The electrochemical behavior of three differently charged drug molecules (zwitter-ionic acetylcarnitine, bi-cationic succinylcholine and tri-cationic gallamine) was studied at the interface between two immiscible electrolyte solutions. Tetramethylammonium was used as a model mono cationic molecule and internal reference. The charge and molecular structure were found to play an important role in the drug lipophilicity. The studied drugs gave a linear correlation between the water – octanol \((\log P_{\text{octanol}})\) partition coefficients and the electrochemically measured water – 1,2-dichloroethane \((\log P_{\text{DCE}})\) partition coefficients. Comparison with tetraalkylammonium cations indicating that the correlation between \(\log P_{\text{octanol}}\) and \(\log P_{\text{DCE}}\) is molecular structure dependent. The highest measured sensitivity and lowest limit of detection were found to be 0.543 mA dm\(^{-3}\) mol\(^{-1}\) and 6.25 \(\mu\)M, respectively, for the tri-cationic gallamine. The sensitivity for all studied ions was found to be a linear function of molecular charge. The dissociation constant of the carboxylic group of zwitter-ionic acetylcarnitine was calculated based on voltammetric parameters and was found to be 4.3. This study demonstrates that electrochemistry at the liquid – liquid interface is a powerful technique when it comes to electroanalytical or pharmacokinetic drug assessment.

1. Introduction

Electrochemistry at the liquid – liquid interface or the interface between two immiscible electrolyte solutions (ITIES) is considered as a biomimetic approach [1,2]. This is due to discontinued properties of the liquid – liquid interface that find analogy to lipid membranes in contact with aqueous solutions. Another similarity lies in the ionic transport. ITIES allows studying interfacial ion transfer reactions, as this gives rise to an electric current that can be quantified via a number of electrochemical techniques, including ion transfer voltammetry. Detection – that is not restricted to oxidation and reduction reactions – avoids straightforward and direct molecular sensing, which is not always feasible with conventional, solid-state electrodes. Examples include a broad class of molecules containing a quaternary ammonium group (e.g., nicotinic drugs [3]) or illicit amphetamine drugs, which are inactive at a glassy carbon electrode [4], but do give a signal at the ITIES [5,6]. Furthermore, a list of analytes detectable at electrified liquid – liquid interfaces span a different class of molecular species. For example, macromolecules, such as dendrimers [7,8], polyelectrolytes [9,10,11] and proteins [12,13] were found to give a characteristic voltammetric behavior indicating interfacial adsorption processes [7,14,15]. Direct electrochemical detection of alkali and alkaline earth metals from aqueous solutions is also possible [16]. A commonly used methodology involves hydrophobic ionophores dissolved in the organic phase [17,18,19]. The presence of ionophores lowers the standard Gibbs energy of ion transfer from one phase to the other, which is of ultimate interest since alkali and alkaline earth cation transfers are usually hindered by a potential window limiting current or is beyond the available potential window. Ionophores were also used for the detection of inorganic anions, as in the work by Kivlehan et al. where a urea-calix[4]arene was used for phosphate sensing [20]. A lot of attention was given to the interfacial behavior of bio- and bio-relevant molecules as described in a comprehensive review by Arrigan et al. [21].

Besides sensing, electrochemistry at the ITIES plays an increasingly prominent role in pharmacokinetics, especially when it comes to the evaluation of partitioning coefficients \(P\), expressed as \(\log P\). Such an approach was for the first time proposed by Kontturi and Murtomäki [22] and further developed by others [23,24,25]. As octanol, typically used as a hydrophobic phase, is inappropriate for electrochemical measurements, other solvents like 1,2-dichloroethane (DCE), ortho-nitrophenyl n-octyl ether (onPOE) and nitrobenzene (NB) were used [26]. Besides being fast, accurate and relatively easy, current state-of-
the-art highlights the advantages of the ITIES over other conventionally used methods for logP determination (e.g., shake flask or HPLC measurements): (i) Pharmacological activity of drugs is directly related to its lipophilicity as is the half-wave potential of the ion transfer reaction [24], (ii) Partition of ionic drugs (containing ionizable functional group) as function of pH can be readily established in a form of so-called Pourbaix-like pH – Galvani potential difference diagrams [27,28], (iii) According to the Nernst-like equation for ion transfer reaction [29], ion (e.g., a charged drug) distribution across two immiscible phases (or lipid bilayer contacted with aqueous fluids by analogy) is Galvani-potential dependent and easily measurable with electrochemistry.

The logP_{DCE} available from electrochemical experiments can be correlated with the logP_{octanol} [22]. Interestingly, two good linear correlations were found: (1) for drugs being able to form hydrogen bonds and (2) for drugs without hydrogen-bond formation ability. In recent work, Nakamura and Osakai studied the interfacial behavior of nine drugs containing amine groups [30]. logP_{octanol} and logP_{PAMPA} (parallel artificial membrane permeation test) were correlated with the standard Galvani potential of ion transfer (\(\Delta_{\text{org}} P_{\text{fl}} \phi_i P_{\text{aq}} \phi_i \alpha \log P_{\text{DCE}}\)) as well as the distribution constant (\(K_i\)) of its neutral equivalent. Once again, some deviations from logP_{octanol} vs logP_{PAMPA} were attributed to the presence of hydroxyl groups being able to form hydrogen bonds. On the other hand, good correlation between \(\Delta_{\text{org}} P_{\text{fl}} \phi_i\) and logP_{PAMPA} suggested that DCE gives better representation of the nonpolar part of lipid bilayers as compared with the octanol.

Drugs and other molecules containing a quaternary ammonium functionality play an important role in neuromuscular blocking and transmission [31]. Some of these drugs interact with acetylcholine receptors, which results in a deficiency of their functioning – the action of utmost interest for anesthesia. Potential candidates for neuromuscular blocking need to be characterized in terms of (i) duration of action; (ii) time of onset; (iii) built-in, self-destruction mechanism, and (iv) possible side effects. In other words, the link between physiology and pharmacology has to be addressed and the electrochemistry at the liquid – liquid interface is a potential method in this regard. In this study, we have investigated the electrochemical behavior of quaternary ammonium drugs (zwitterionic acetylcholine, bi-cationic succinylcholine and tri-cationic gallamine) at the water – 1,2-dichloroethane (DCE) interface. In Section 3.1 we present and discuss logP_{DCE} values calculated based on the standard ion transfer potential. Next, these logP_{DCE} values were correlated with calculated logP_{octanol} data. In Section 3.2 the effect of molecular charge on the electroanalytical properties (sensitivity and limit of detection (LOD)) is evaluated and discussed. In Section 3.3 we report on the calculation of the acid dissociation constant for carbonyl group of acetylcholine based on diffusion coefficient values measured at pH below and above its pK_a.

2. Experimental section

2.1. Chemicals

Potassium tetrakis(4-chlorophenyl)borate (KTPBCl, Sigma-Aldrich, 98%) and bis(triphenylphosphoranyliden)e)ammonium chloride (BT-PACl, Sigma-Aldrich, 97%) were used to prepare the organic phase electrolyte bis(triphenylphosphoranyliden)e)ammonium tetrakis(4-chlorophenyl)borate (BTTPATPBCl) via a simple metathesis reaction according to the protocol published elsewhere [27]. The aqueous phase electrolyte was sodium chloride (NaCl, Sigma Aldrich, ≥ 99%). Studied ions: tetramethylammonium chloride (TMACl, purity, 97%), O-Acetyl-1-carnitine hydrochloride (ACHCl, ≥ 99%), succinylcholine chloride dihydrate (SCCG2, 98–102%) and gallamine triethiodide (GalT, ≥ 99%) were all purchased from Sigma Aldrich. The pH of the aqueous phase was adjusted with 1 M HCl. Ag/AgCl reference electrodes were made via potentiostatic silver oxidation in a 1 M HCl solution.

2.2. Electrochemistry at ITIES

All measurements were performed in a four-electrode glass cell with an interface having a surface area equal to 1.13 cm\(^2\). The cell configuration was as follows:

| Ag/AgCl | x mM Drug, |
|---------|------------|
| Reference electrode (aq) | 10 mM NaCl or 10 mM HCl | Aqueous phase |
| 10 mM BTPPA TPBCl in 1, 2-dichloroethane | 10 mM BTPPA/ACl\(^-\), 10 mM NaCl | Organic phase | Aqueous supporting phase |

| Ag/AgCl | Cell I |
|---------|-------|
| Reference electrode (org) | |

The reference electrodes were Ag/AgCl immersed into the Luggin capillaries of each phase. The organic phase reference electrode was immersed into the aqueous supporting electrolyte containing NaCl and BTPPA\(^+\) (Cl\(^-\) was the counter-ion) as the common ion with the organic phase, which resulted in the formation of the unpolariised interface [29]. Counter electrodes were coiled Pt wires (\(e = 2 \text{ mm, } \approx 6 \text{ cm in length}\)). The organic phase counter electrode was additionally embedded in the glass capillary in order to avoid the short circuit with the aqueous phase. The potential difference across the water||1,2-dichloroethane interface was controlled with an Autolab potentiostat PGSTA-T302N. Voltammograms used to measure the half-wave potential of ion transfer (\(E^{1/2}\)) were calibrated according to:

\[
\Delta E = \Delta_{\text{org}} P_{\text{fl}} \phi_i P_{\text{aq}} \phi_i \alpha P_{\text{DCE}} + \Delta_{\text{org}} P_{\text{fl}} \phi_i P_{\text{aq}} \phi_i \alpha P_{\text{DCE}}^0
\]  

(2.1)

The \(\Delta E\) is the potential difference measured between the reference electrodes in the aqueous and the organic phase. The \(\Delta_{\text{org}} P_{\text{fl}} \phi_i\), is the Galvani potential difference across water/DCE interface. The \(\Delta_{\text{org}} P_{\text{fl}} \phi_i P_{\text{aq}} \phi_i \alpha P_{\text{DCE}}^0\) is the reference potential equal to the standard potential of the ion transfer for TMA\(^+\) (\(\Delta_{\text{org}} P_{\text{fl}} \phi_i P_{\text{aq}} \phi_i \Delta E = 0\) = 160 mV) [2,32]. Fig. 1 shows a blank voltammogram calibrated according to Eq. (2.1). Three characteristic regions can be distinguished here: (b) potential window that is determined by (a and c) potential window limiting currents.

Fig. 1. Blank cyclic voltammogram recorded at the ITIES composed from a 10 mM NaCl (aq, blue, top) and a 10 mM organic electrolyte (org, orange, bottom). The forward scan was from a less positive to a more positive potential. The scan rate was 10 mV/s. See Cell I (X = 0 mM) for detail on the configuration of the cell. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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By convention, the sign of the current is directly related to the direction of ion transfer: positive current is when cations transfer from the aqueous to the organic phase or anions transfers from the organic to the aqueous phase. In contrary, a negative sign of the current is attributed to reversed direction, transfer of cation from the organic to the aqueous phase or anions transfers from the organic to the aqueous phase. Accordingly, currents that are limiting the potential window on the more negative side of voltammogram are due to the Cl⁻ transfer (positive current = Cl⁻ → org; negative current = Cl⁻ → aq) whereas the Na⁺ transfer takes place at the more positive site of the potential window (positive current = Na⁺ → aq; negative current = Na⁺ → org) [34].

Fig. 2. Cyclic voltammograms recorded for all studied quaternary ammonium drugs. The potential scale was calibrated to Δφa/φorg = 160 mV. Forward scan is from less positive to more positive potential. Scan rate was 10 mV/s.

2.3. Theory

Electrochemistry at the ITIES gives an easy approach to measure the lipophilicity of charged or ionizable molecules. It is especially useful for drug molecules of which affinity towards the desired environment (apolar interior of the lipid bilayer or their external hydrophilic space) dictates their pharmacological activity. The parameters directly available from voltammetric measurements can be related to the partition of charged species between water and its immiscible counter phase. At a polarized interface, the ion transfer reaction is governed via the following Nernst-like equation for the ion transfer reaction [2]:

\[ \Delta \phi_{\text{aq/org}} = \Delta \phi_{\text{aq}} - \Delta \phi_{\text{org}} + \frac{2.303RT}{z_iF} \log \frac{a_{i\text{org}}}{a_{i\text{aq}}} \]  

(2.2)

where \( \Delta \phi_{\text{aq/org}} \) is the Galvani potential difference between the aqueous and the organic phase, \( \Delta \phi_{\text{aq}} \) is the standard Galvani potential of ion (i) transfer, \( R \) is the gas constant, \( T \) is the absolute temperature, \( z_i \) is the ion charge, and \( F \) is the Faraday constant. The last term, \( a_{i\text{org}}/a_{i\text{aq}} \), gives the ratio of activities of ion (i) in the organic and the aqueous phase that, interestingly, can be directly related to the partition coefficient:

\[ \log P_{\text{aq/org}} = \log \frac{a_{i\text{org}}}{a_{i\text{aq}}} \]  

(2.3)

For charged molecules substitution of Eq. (2.3) in Eq. (2.2) results in a potential-dependened parameter:

\[ \log P_{\text{aq/org}} = \frac{\Delta \phi_{\text{aq}} F}{2.303RT} + \frac{\Delta \phi_{\text{org}} F}{2.303RT} - \frac{(\Delta \phi_{\text{aq}} - \Delta \phi_{\text{org}}) z_i F}{2.303RT} \]  

(2.4)

Based on Eq. (2.4), one can also define the standard partition coefficient:

\[ \log P_{\text{iq/ij}}^{0\text{/ij}} = -\frac{\Delta \phi_{\text{iq}} F}{2.303RT} \]  

(2.5)

To measure \( \log P_{\text{aq/org}}^{0\text{/ij}} \) one needs to know \( \Delta \phi_{\text{aq}}^{0\text{/ij}} \), which can easily be measured by ion transfer voltammetry since [2]:

\[ \Delta \phi_{\text{aq}}^{0\text{/ij}} = \Delta \phi_{\text{aq}}^{0\text{/ij}} - \frac{2.303RT}{z_iF} \log \frac{I_{\text{aq}}}{I_{\text{org}}} - \frac{2.303RT}{z_iF} \log \frac{D_{\text{aq}}^{0\text{/ij}}}{D_{\text{org}}^{0\text{/ij}}} \]  

(2.6)

where \( \phi_{1/2}^{0\text{/ij}} \) is the half-wave potential of the reversible ion-transfer reaction directly accessible from the voltammogram. \( D_{\text{aq}}^{0\text{/ij}} \) and \( D_{\text{org}}^{0\text{/ij}} \) are diffusion coefficients of ion (i) in the aqueous and the organic phase, respectively. Activity coefficients \( \gamma_{\text{aq}}^{0\text{/ij}} \) and \( \gamma_{\text{org}}^{0\text{/ij}} \) can be calculated from the Debye–Hückel equation:

\[ \ln \frac{\gamma_{\text{aq/ij}}^{\text{aq/ij}}}{\gamma_{\text{org/ij}}^{\text{org/ij}}} = -25.83 \frac{z_i^2}{\epsilon_{\text{aq/ij}}} \left( \frac{1}{\epsilon_{\text{aq/ij}}} - \frac{1}{\epsilon_{\text{org/ij}}} \right) \]  

(2.7)

The constant \(-25.83\) was calculated for a temperature of 25 °C, \( I \) is the ionic strength in the corresponding phase and \( \epsilon_i \) is the relative permittivity of the solvent. For \( \gamma_{\text{aq}}^{0\text{/ij}} \) we assumed that ion pair formation in the organic phase can be neglected. Diffusion coefficients of ion (i) in the aqueous or the organic phase can be measured via voltammetry from the dependency of the peak current \( I_p \) to square root from the scan rate \( (\sqrt{v}) \) governed by the Randles-Sevcik equation:

\[ I_p = 268600A \sqrt{D_{\text{aq}}^{0\text{/ij}}} C_i \sqrt{v} \]  

(2.8)

where \( A \) is the interfacial surface area and \( C_i \) is the bulk concentration. If \( D_i \) in one of the phases is known, then the Waldner rule [29], stating that the diffusion coefficients of a solute for two different solvents is inversely proportional to viscosities of these solvents, can be applied:

\[ \frac{D_{\text{aq}}^{0\text{/ij}}}{D_{\text{org}}^{0\text{/ij}}} = \frac{\eta_{\text{aq}}^{0\text{/ij}}}{\eta_{\text{org}}^{0\text{/ij}}} \]  

(2.9)
3. Results and discussion

3.1. Partitioning coefficients

As shown in Fig. 2, all ions studied in this work gave a characteristic pair of symmetric peaks within the available potential window. The potential axis was calibrated to the standard ion transfer potential of TMA⁺ ($\Delta_{\text{org}}^{\text{aq}}\phi_{\text{TMA}^+} = 160 \text{ mV}$) [32]. All studied ions were initially dissolved in the aqueous phase and hence the direction of polarization is in agreement with the positive charge of the studied drugs, in all cases from less positive to more positive potential. A positive current is, by convention, attributed to cation transfer from the aqueous to the organic phase. Leaving out the structural dissimilarities, the main difference between all investigated drugs is the number of quaternary ammonium groups ($z_{\text{TMA}^+} = 1$, $z_{\text{AC}^+} = 1$, $z_{\text{SC}^2+} = 2$, $z_{\text{GA}^+} = 3$) and the presence of negative charge located on carboxylic group of zwitterion AC⁺⁻.

In general the determination of partition coefficient is straightforward for the neutral form of drugs. However, this is less straightforward for molecules containing a permanently charged functional group(s) or molecules that behave as weak acids or bases. In such scenarios, the equilibrium distribution is affected by the arising potential gradient across the boundary between the two phases and/or by changes in pH of the adjacent aqueous phase. For such a situation, electrochemistry at the ITIES gives a straightforward way for the determination of the acting partition coefficients. The half-wave potentials for each drug read out from Fig. 2 were used to calculate $\Delta_{\text{org}}^{\text{aq}}\phi_i$ according to Eq. (2.6), which — in turn — allowed the calculation of the standard partition coefficient — ($\log P_{\text{octanol}}$) from Eq. (2.5). The thus-obtained values for both, $\Delta_{\text{org}}^{\text{aq}}\phi_i$ and $\log P_{\text{octanol}}$, are presented in Table 1, which summarises all experimentally extracted electrochemical, analytical and thermodynamic properties of studied ions. Direct estimation of lipophilicity can be made based on the location of the ion transfer within the potential window. A tentative rule for like-charged drugs is in agreement with the positive charge of the studied drugs, in all cases from less positive to more positive potential. A positive current is, by convention, attributed to cation transfer from the aqueous to the organic phase. Leaving out the structural dissimilarities, the main difference between all investigated drugs is the number of quaternary ammonium groups ($z_{\text{TMA}^+} = 1$, $z_{\text{AC}^+} = 1$, $z_{\text{SC}^2+} = 2$, $z_{\text{GA}^+} = 3$) and the presence of negative charge located on carboxylic group of zwitterion AC⁺⁻.

Fig. 3. Correlation between $\log P_{\text{octanol}}$ and $\log P_{\text{DCE}}$ for studied drugs and family of quaternary ammonium cations. Drugs studied in this work: (1) SC²⁺, (2) AC⁺, (3) GA⁺⁻; and values for quaternary ammonium cations taken from literature: (4) tetraethylammonium (TMA⁺), (5) tetraethylenammonium (TEA⁺), (6) tetrapropylammonium (TPA⁺), (7) tributylammonium (TBA⁺) and (8) tetratetradecylammonium (TPD⁺)

Table 1

| Molecule | $z_i$ | $\Delta_{\text{org}}^{\text{aq}}\phi_i / \Delta_{\text{aq}}^{\text{org}}\phi_i$ (mV) | $\Delta_{\text{org}}^{\text{aq}}\phi_i$ (mV) | $\Delta_{\text{aq}}^{\text{org}}\phi_i$ (kJ/Mol) | $\log P_{\text{DCE}}$ | Sensitivity (nA/M) | LOD (µM) | Lowest measured (µM) | pH | Diffusion coefficients (cm²/s⁻¹) |
|----------|------|-------------------------------|----------------|----------------|----------------|-----------------|----------|-----------------|-----|------------------|
| TMA⁺ | 1 | 158 | 161 | 15.5 | −2.76 | 0.15 (± 6%) | 14.16 | 27.23 | 6 | 10.27 (± 3%) |
| AC⁺ | 1 | 230 | 234 | 22.6 | −4.03 | 0.14 (± 4%) | 21.94 | 49.20 | 2 | 12.06 (± 3%) |
| AC⁻⁻ | −1 | 254 | − | − | − | 0.13 (± 6%) | 41.18 | 81.37 | 6 | 3.7 (± 2%) |
| SC²⁺ | 2 | 137 | 142 | 27.4 | −4.89 | 0.36 (± 5%) | 19.56 | 59.55 | 6 | 1.48 (± 6%) |
| GA⁺⁻ | 3 | 50 | 56 | 16.2 | −2.89 | 0.54 (± 2%) | 5.94 | 6.25 | 6 | 0.20 (± 1%) |

* Calculated using Eq. (2.6).
* Calculated according to $\Delta_{\text{org}}^{\text{aq}}G_i = z_iF \Delta_{\text{org}}^{\text{aq}}\phi_i$.
* Calculated using Eq. (2.5).
* Calculated using Eq. (2.11).
* Errors correspond to the standard error of the linear fit.
* Calculated for AC total concentration.
different correlation than drugs which are deprived from this ability [22]. Similar reasoning was also used to explain deviations for similar correlations of nine amine drugs studied by Nakamura and Osaki [30].

Also for the present study, the difference of slopes between two class of species (1.084 for the quaternary ammonium drugs from this work and 0.899 for the TAA+ family), can be attributed to the hydrogen bond formation ability. All three drugs employed in this work possess group being able to form hydrogen bonding, i.e., the ether group for GA3+, ester group for SC2+ and both ester and hydroxyl group for AC+. The logPoctanol/logP DCE correlation from work of Kontturi and Murтомäki gave a slope of linear fitting equal to 1.89 for drugs containing a hydroxyl group and 1.09 for drugs without this group [22]. This comparison leads to a conclusion that correlation output is molecular class dependent. Molecules that are able to form hydrogen bond with the hydroxyl groups of octanol have a higher affinity to this solvent, resulting in a higher slope of the correlation study. The smaller difference in a slope between two correlations obtained in this work can be related to hydrogen-bonding ability constants (I ′ H) introduced by Seiler [39]. According to the given hydrogen bond descriptor, hydroxyl substituents (I ′ H = 1.82) form stronger hydrogen bonds than molecules containing for instance an ether bond (I ′ H = 0.11) [40]. This clearly indicates the relevance of hydrogen bond formation ability on drug partitioning. Moreover, the relatively hydrophilic nature of the studied drugs, as indicated by the partition coefficient values, may suggest their permeability and adsorption properties in the context of cell membranes. Since all measured logP DCE values were in the range from −5 to −2 one can expect poor transcellular transport properties and higher affinity of the drugs towards polar environments of the lipid membrane [41]. As it will be shown in following section, additionally to the logP DCE determination, electrochemistry at the liquid–liquid interface can be used to describe interfacial behavior of drug species.

3.2. Electroanalytical parameters

In addition to pharmacological insights that clearly relate the logP DCE with logP octanol for different molecular classes, ion transfer voltammetry can be used to study the electroanalytical behavior of quaternary ammonium ions, including sensitivities, limits of detection and diffusion coefficients. Leaving out the polymeric species and proteins being able to adsorb at ITIES [9], voltammetric studies revealed that the potential-dependent ion transfer reaction of small quaternary ammonium cations exhibits reversible characteristics: (i) ratio of the forward and reversed peak current are close to one, (ii) peak-to-peak separation approaches the expected 59 mV/z value and (iii) f < 1 (for a diffusion-limited process).

Fig. 4 shows the voltammograms recorded for SC2+ (Fig. 4A) and GA3+ (Fig. 4B) in the concentration range from around 40 to 200 µM. The sensitivity taken as the slope of the calibration curve (see inserts of the graphs in column 1 Fig. 4) for GA3+ was found to be 1.5 times higher compared to SC2+, which is as expected, since GA3+ holds 1.5 times more charge than SC2+. Occasionally at higher concentrations, interfacial transfer of GA3+ gave a broad peak (see the red curve in Fig. 4 – 1B). This may indicate some additional resistance to the ion transfer reaction. If we assume that GA3+ is spherical molecule, then the hydrodynamic radius can be deduced from the Stokes–Einstein relation:

\[ r = \frac{k_B T}{6\pi\eta D} \]  

(2.10)

where \( k_B \) stands for the Boltzmann constant (1.3810e-23 m² kg s⁻¹ K⁻¹), \( T \) is temperature in Kelvin, \( \eta \) is dynamic viscosity of water (8.9010-4 Pa s), and \( D \) is the diffusion coefficient in m² s⁻¹. For relatively low \( D_{GA3+} = 0.2 \times 10^{-2} \) (calculated based on Eq. (2.8) from linear fitting of the insert from Fig. 4 – 2B) the obtained hydrodynamic radius equals 1.23 nm, which is in the same order of magnitude as dendritic molecules that strongly adsorb at the interface [15,42,43]. This results might suggest that at higher concentrations GA3+ is able to weakly adsorb at the liquid – liquid interface.

Next, Fig. 5 shows the voltammograms recorded for an AC molecule at two pH values: \( \text{pH} = 2 \) (Fig. 5 – 1A), which is below the acid dissociation constant of AC and accordingly positively charged (i.e., the carboxylic group is protonated) and \( \text{pH} = 6 \) (Fig. 5 – 1B) at which the zwitterion is expected to be present in the aqueous phase (i.e., the carboxylic group is deprotonated). At \( \text{pH} = 2 \), on the more positive potential side, the potential window was limited by a H⁺ interfacial transfer instead of Na⁺ [44]. Full protonation of AC⁺ at lower pH values caused a slight shift in the transfer potential, 34 mV lower (i.e., a less positive potential value), indicating a higher hydrophobicity. Voltammetric sensitivity of monocharged AC⁺ was 2.6 and 4 times lower as compared with SC2+ and GA3+ respectively. Diffusion coefficient values calculated from scan rate experiments (see insert of Fig. 5 2A and 2B) significantly differ between \( \text{pH} = 2 \) and 6. This phenomenon was attributed to the presence of a weak acid group and is furthermore discussed in Section 3.3. For all studied drugs the limit of detection (LOD) was calculated from the linear fit equation: [42]

\[ \text{LOD} = \frac{3.35D}{S} \]  

(2.11)

where \( S \) is the standard deviation of the intercept and \( S \) is the slope of the linear fit. The calculated LOD values for all drugs studied in this work are in µM and are relatively close to the lowest measured concentration (Table 1). LODs for macroscopic ITIES are suitable to investigate the plasma concentration of muscle relaxants that are required for 50% neuromuscular block [45]. A summary of all electroanalytical parameters calculated for GA3⁺, SC2⁺ and AC⁺ (at \( \text{pH} = 2 \) and 6) including sensitivities, charge, LODs and diffusion coefficients can be found in Table 1.

For all studied molecules, the peak-to-peak separation (ΔEpp), defined as the difference between the forward and reversed peak potentials (\( E_{p, \text{forward}} − E_{p, \text{reversed}} \)), linearly increased with increasing analyte concentration of the aqueous phase. The highest ΔEpp = 114 mV was obtained for [GA3⁺] = 245.5 µM. This result is due to the uncompensated resistance of the organic phase, rather than an indication for irreversibility of the ion transfer reaction [46,47]. In order to find the value for the peak-to-peak separation for each ion, ΔEpp was plotted against each corresponding concentration. From the intercept of linear fitting it was found that \( \Delta E_{\text{p, GA}3+} = 22.8 \text{ mV}, \Delta E_{\text{p, SC}2+} = 28.7 \text{ mV}, \Delta E_{\text{p, AC}+} = 55.4 \text{ mV}, \Delta E_{\text{p, TMA}+} = 56.3 \text{ mV} \), all being very close to expected value of 59 mV/z. Fig. 6 shows the effect of charge and diffusion coefficient on the voltammetric sensitivity for TMA⁺, AC⁺, SC2⁺ and GA3⁺. From Fig. 6A it is obvious that the sensitivity (defined as the slope of the calibration curve) increased linearly with the charge of quaternary ammonium drug. As predicted by the Randles – Sevcik equation (Eq. (2.8)), the sensitivity of diffusion-controlled ion transfer reaction should be proportional to \( z^2D^{1/2} \), which was, e.g., found for macro-molecular dendrimers and proteins [15]. However, given correlation was not observed in the present study as becomes clear from Fig. 6B. If compared to quoted example of macromolecules [15], a great variety of investigated generations (periodically increasing molecular weight and charge) gave sensitivities spanning over 4 orders of magnitude, leaving more space for an error. It was also observed that the correlation between sensitivity and \( z^2D^{1/2} \) is molecular class dependent. It can be concluded that for small ionic drugs holding quaternary ammonium cation the sensitivity is mainly governed by molecular charge, leaving a less significant role for diffusion.

3.3. Dissociation constant of AC⁺

The presence of a carboxylic group within the AC⁺ structure caused the electroanalytical properties to be pH dependent. As already discussed, the protonated form of the drug (at \( \text{pH} = 2 \)) appeared to
be slightly more hydrophobic, as the standard ion transfer potential was shifted to more negative values. Interestingly, also the diffusion coefficients — as obtained from the scan rate experiments — significantly differs and are $12.06 \times 10^{-6}$ cm$^2$/s measured at pH = 2 and $2.37 \times 10^{-6}$ cm$^2$/s measured at pH = 6 for the forward scan. These findings can be related to the presence of a carboxylic group ($-\text{COOH}$) that at pH = 6 is dissociated, lowering the overall concentration of interfacially active AC$^+$ species (assumption can be taken that a deprotonated carboxylic group neutralizes the positive charge of the quaternary ammonium cation group, causing the drug to be electrochemically inactive). These findings allow a very straightforward method for dissociation constant calculations. The concentration of AC$^+$ ions at pH = 6 (which is the same as the concentration of non-dissociated -COOH groups) can be calculated as we know diffusion coefficient value obtained at pH = 2:

$$C_{AC^+} = \frac{S_{iAC^+}}{268600 \sqrt{D_{AC^+} \cdot z_i}}$$  \hspace{1cm} (2.12)

where $S_{iAC^+}$ is the slope of the peak current versus the square root of the scan rate dependency recorded for AC$^+$ ion transfer at pH = 6, $D_{AC^+}$ is the aqueous phase diffusion coefficient obtained at pH = 2, $A$ is the electroactive surface area (1.12 cm$^2$) and $z_i$ is the charge of the transferring molecule. Subtracting the number of AC$^+$ ions calculated from Eq. (2.12) from the theoretical concentration of AC$^+$ + AC$^0$ (zwitterion with zero net charge) gives us the concentration of the AC$^0$ that hold dissociated (R-COO$^-$) group. According to the following reaction:

$$\text{R-COOH} + H^+ \leftrightarrow \text{R-COO}^- + H_2O$$ \hspace{1cm} (2.13)

The dissociation constant can be easily calculated via:

$$K_a = \frac{[\text{R-COO}^-]}{[\text{R-COOH}]}$$ \hspace{1cm} (2.14)

where [R-COO$^-$] and [H$^+$] each is equal to $4.25 \times 10^{-5}$ M, whereas the overall concentration of [R-COOH] holds $7.61 \times 10^{-5}$ M (concentration fixed during scan rate experiment). Accordingly the degree of dissociation $\alpha$ (defined as $[\text{R-COO}^-]/[\text{R-COOH}]$) equals 0.56, resulting in a p$K_a$ of 4.3.

This value is only slightly higher than 3.6 reported in the literature [48] although still lower than 4.8 for butanoic acid [49]. Compared to butanoic acid, an increased acidity of AC$^+$ is governed by the presence of electron-attracting substituents. Deviation of the p$K_a$ value for AC$^+$ in our study can be attributed to local change in the dielectric properties of the interfacial region, which is known to have an effect on the acid dissociation constant value [50]. To further verify this.

Fig. 4. Voltammetric behavior of SC$^{2+}$ (entry A) and GA$^{3+}$ (entry B). Standard addition experiments were performed at 10 mV/s and can be found in column 1. Inserts of graphs from column 1 are the corresponding calibration curves together with linear fit equations. Column 2 gives scan rate experiments recorded at [SC$^{2+}$] = 50.6 μM and [GA$^{3+}$] = 49.6 μM at 5; 10; 15; 20 and 25 mV/s. Inserts of the graphs in column 2 give the current as a function of square root from the scan rate dependency together with linear fit equations. Linear fit equations marked with (+) and (−) are given for the positive and negative current values respectively. For all graphs forward polarization was from a more negative to a more positive potential.
value, we measured the forward peak current of AC⁺ in the pH range from 2 up to 8 (Fig. 7). The pH at which the peak current reached 50% of its maximum value was found to be 4.5 and is very well in agreement with the value calculated using diffusion coefficients measured at two pH values.

4. Conclusions

In this work, we have studied the electrochemical behavior of several drugs involved in the neurotransmission blockage, all being classified as quaternary ammonium cations. We have shown that ion transfer voltammetry is a very useful and straightforward technique for the electroanalytical parameters determination – including sensitivity and LODs – as well as pharmacological properties of these compounds.

Partitioning of ionic species between water – DCE possessing structural fragments allowing them to form hydrogen bonding (ester or ether groups) gave different correlations with the respective water – octanol partition coefficients than molecules without the ability to form hydrogen bonding.
hydrogen bonds (in present study comparison was made with tetraalkylammonium cation family). In pharmacokinetics, the partition coefficient is used to predict the so-called ADME (adsorption, distribution, metabolism and excretion) properties of the drugs. For all studied molecules we found that their partition coefficient values are in the range from −5 to −2 which indicates their hydrophilic nature. This in turn can be translated into poor transcellular transport properties and their increased affinity for the adsorption to the external lipid bilayer surface including transmembrane proteins. It was also found that sensitivity (i.e., the slope of voltammetric calibration curve) increases linearly with the charge of studied ion (from 0.137 mAmD−1mol−1 for mono-charged to 0.573 mAmD−1mol−1 for tri-charged quaternary ammonium cation). The effect of diffusion coefficient, anticipated from the Randles–Sevcik equation was found to have a minor effect. Based on parameters calculated from voltammetric studies at two different pH values (pH = 2 and 6) the dissociation constant of carboxylic group of acetylcarnitine was calculated to be between 4.3 and 4.5. To conclude, this study shows that voltammetry at ITIES can be routinely used as an electroanalytical method for ionized drug screening and characterization.

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