

Facilitated cation transfer at a three-phase junction and its applicability for ionophore evaluation

Marta Podrażka^{1†}, Julia Maciejewska^{1†}, Wojciech Adamiak¹, Emilia Witkowska Nery^{*1},
Martin Jönsson-Niedziółka^{*1}

¹Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224
Warsaw, Poland

*Email: ewitkowskanery@ichf.edu.pl, martinj@ichf.edu.pl, Phone: +48 22 343 33 06

† These authors contributed equally to the work.

Abstract

Although many redox probes were proposed for anion transfer studies in three-phase electrode setup, only few molecules were applied for cation transfer. In this work we show that the fullerene C₆₀ is an efficient redox system for this application. We performed ionophore assisted ion-transfer studies in a droplet system, a disposable setup with a cylindrical electrode, as well as a novel paper-based device. The disposable setup is easy to assemble and thanks to the cylindrical electrode creates a more stable and reproducible phase boundary than the standard droplet setup. With this device we were able to perform studies of unassisted cation-transfer at the three-phase boundary. Additionally, the proposed system was applied to compare performance of three popular potassium ionophores: Valinomycin, dibenzo-18-crown-6 ether (DB18C6) and Potassium Ionophore III (BME-44). Here, ionophores normally applied in ion-selective electrodes (ISE), can be easily evaluated and compared without the influence of other components of the ISE membrane such as plasticizers or lipophilic salts. Three-phase ion-transfer voltammetry is a convenient mean to assess the effect of each ionophore on both the selectivity and Gibbs energy of transfer.

Keywords: Facilitated ion-transfer; Fullerene C₆₀; Three-phase electrode; Interface between Two Immiscible Electrolyte Solutions (ITIES); Pencil graphite electrode

1. Introduction

Facilitated ion-transfer is the transfer of ions between two phases, such as an aqueous and organic phase or an aqueous phase and a polymer membrane, which is promoted with the help of a compound that lowers the transfer energy. These ionophores are lipophilic, usually macrocyclic, compounds, which are able to selectively bind to certain ions. The selectivity derives from the difference in binding strength between the cavities or semi-cavities of the ionophore and different ions [1]. Ionophores, also known as ion-carriers, can be neutral, such as hexyl trifluoroacetyl benzoate used to selectively bind carbonate and Valinomycin used for potassium ions, or charged such as metal porphyrins often applied for nitrate determination. In case of potassium four classes of ionophores were developed: antibiotics (e. g. Valinomycin), mono- (e.g. dibenzo-18-crown-6) and biscrown ethers (e.g. Potassium Ionophore III) and hemispherands [1]. Valinomycin is the most used K⁺ ionophore due to its superior selectivity and is most often applied in clinical analyzers. Sensors with monocrown ethers in many cases show low level of selectivity enhancement but are still widely applied due to their low price. Much better results are obtained with biscrown ethers, which in addition to the interactions with the internal cavity of the crown ether ring are able to form intramolecular sandwich complexes. The most selective of this latter group of compounds is Potassium Ionophore III, studied in this work, which has smaller discrimination capability towards sodium compared to Valinomycin but superior lipophilicity [1].

The most common use of ionophores is to provide the selectivity in ion-selective electrodes (ISEs). Here the ionophore is usually incorporated in a polymer membrane into which the ions should be transported. Although the selectivity is principally attributed to ionophores it is also influenced by all other constituents of the membrane, such as plasticizer

and ionic additives [2–4]. Additionally, the transport process is not only dependent on the type of ion and the ionophore but also on the composition of the organic phase, as the equilibrium constant of the complex formation is solvent dependent [2].

Because of these complications it can be arduous to determine whether a newly synthesized ionophore is more selective or just the membrane composition was more favorable. Traditionally, complexing equilibrium constants were measured calorimetrically, potentiometrically or by means of spectroscopy [5]. In the 1990s Bakker and co-workers proposed a series of methods, both optical and potentiometric which allowed to assess complex formation constants also in the case of highly lipophilic and immobilized ionophores using sandwich membranes [6]. Recently Amemiya and co-workers proposed a different method in which voltammetric characterization is performed using thin polymeric membranes with a cation-to-electron transducer immobilized underneath [7].

Another option is to study ionophores at the interface between two immiscible electrolyte solutions (ITIES) [8,9] as this gives access to the Gibbs energies of ion-transfer. Studies at the liquid-liquid interface were also used to determine ion-to-ligand stoichiometry [10]. In the present work we propose to compare the selectivity of different ionophores using ion-transfer at a three-phase junction. The liquid-liquid interface can be studied using either a four-electrode setup, in which a pair of working and reference electrodes is positioned in each phase or with three electrodes. In the latter case we differentiate between thin film electrodes, in which the working electrode is fully covered by the organic phase [11], and a three-phase electrode setup (TPE), in which both the organic and aqueous phases are in contact with the working electrode [12]. In both systems the reference and counter electrodes are placed in the aqueous phase.

In a typical three-phase electrode setup, the organic phase does not contain any supporting electrolyte, therefore the reaction is only possible at the three-phase junction where a redox

probe present in the organic solution and the electrolyte in the aqueous phase are in the contact with the electrode. When the redox molecule is oxidized or reduced the charge generated in the organic phase has to be compensated by ion transfer from the aqueous phase or expulsion of the charged probe to the aqueous environment. These processes to maintain the electroneutrality of the organic phase are the base of ion transfer studies [13]. Usually the TPE comprises a droplet or an array of droplets not fully covering the working electrode area. Measurements with this kind of system are possible only with a limited number of solvents where a stable droplet can be formed on the electrode. Also, the length of the three-phase junction, which determines the current, is very hard to control. Thus a cylindrical electrode passing through both phases were proposed [14]. In this work, experiments were performed using a standard droplet setup, but also a simple and disposable system, which is a variation of the cylindrical electrode approach. A pencil graphite electrode immobilized in a small glass vial, formed from a Pasteur pipette allowed for more reproducible and sensitive measurements. Pencils are a low-cost, and easily available source of good-quality graphite, resulting in a growing number of applications of pencil graphite electrodes (PGE) [15,16]. Additionally we also performed tests in a paper-based setup, in which both phases are stabilized by enclosure in the polymeric matrix of paper. This recently developed system [17] for anion transfer studies at the three phase junction is also applicable to cation and assisted ion transfer studies.

Although scientific literature is rich in publications concerning anion transfer studies, there are far fewer studies concerning cation transfer. This fact is mainly related to the lack of proper redox probes which are sufficiently insoluble in water in both oxidized and reduced forms. Among the few publications available we find the use of iron(III) tetraphenyl porphyrin chloride [18], lutetium bis(tetratert-butylphthalocyaninato) [19] and 7,7',8,8'-tetracyanoquinodimethane [20]. In case of porphyrin derivatives expulsion of the anion (e.g.

chloride) after the reduction can complicate the analysis of the results, and the 7,7',8,8'-tetracyanoquinodimethane can be transferred to the aqueous phase upon reduction. In this work we used fullerene C₆₀ which, despite its obvious advantages such as ability to undergo multiple reduction steps and extremely low solubility in water of all of the forms, was scarcely studied at the liquid-liquid interface. Until now fullerene C₆₀ redox behavior at the interface was analyzed using scanning electrochemical microscopy [21] and in a thin film setup where it was used for anion-transfer studies [22].

This work is the first time fullerene C₆₀ is successfully applied as a redox probe for cation transfer studies. To the best of our knowledge it is also the first time three-phase junction setup was used for assisted ion transfer studies using ionophores. Ionophores are mainly applied in thin films, which are used for voltammetric ion-transfer studies [2,23] (with liquid or gelled organic phase) or in potentiometric ion-selective electrodes. Here we show that the three-phase electrode allows easy assessment of ionophore selectivity.

2. Experimental Section

2.1. Chemicals and Materials

Fullerene C₆₀ (99,9%, Sigma-Aldrich), 1,2-dichlorobenzene (DCB) (99%, Sigma-Aldrich), inorganic and organic salts of analytical grade: KNO₃ (99%, POCh), KCl (>99.99%, Sigma-Aldrich), NaCl (pure p.a., POCh), NaNO₃ (pure p.a., ChemPur), LiCl (99%, Sigma-Aldrich), NH₄NO₃ (pure p.a., Chempur), MgCl₂ (99%, Sigma-Aldrich), CaCl₂ (99%, POCh), AlCl₃ (≥98%, ROTH), Tetramethylammonium chloride (TMA) (≥99%, Fluka), Tetraethylammonium chloride (TEA) (≥99%, Fluka), Tetrapropylammonium chloride (TPA) (≥98%, Sigma-Aldrich) were used as received. The ionophores dibenzo-18-crown 6 (DB18C6) (≥98%) and Valinomycin were purchased from Sigma-Aldrich, while 2-Dodecyl-2-methyl-1,3-propanediyl bis[N-[5'-nitro(benzo-15-crown-5)-4'-yl]carbamate] (Potassium Ionophore III, BME-44) was obtained from Fluka. Water was filtered and demineralized with

ELIX system (Milipore). HB graphite pencil leads of 0.7 mm diameter and 16 mm length were purchased from Pentel.

2.2. Electrochemical Measurements

Cyclic voltammetry (CV) and square wave voltammetry (SWV) were performed with an Autolab potentiostat (Metrohm Autolab B.V., Netherlands) controlled by the NOVA software (version 2.1.2). Parameters for SWV were as follows: step potential -0.005 V, modulation amplitude 0.025 V, frequency 25 Hz. All measurements were performed in a three-electrode system with a silver-silver chloride reference electrode (Ag|AgCl|saturated KCl) and a platinum wire as a counter electrode.

2.3. Experimental setups

2.3.1 Droplet-modified electrode

A glassy carbon (GC) electrode with a diameter of 1.5 mm was polished with 1, 0.3, and 0.05 μm Al_2O_3 slurry, then rinsed with demineralized water and sonicated in ethanol. Next, a 2 μL droplet of the organic phase was deposited on the electrode surface. The droplet-modified electrode was immersed into the various aqueous electrolyte solutions, in which the reference and counter electrodes were also positioned (Fig. 1A). As the surface of the electrode is not fully covered with the organic solvent there is a region of the electrode which is in contact with both the organic and aqueous phases thus forming the three-phase junction.

2.3.2 Pencil graphite electrode

As the droplet on the electrode surface is sometimes quite unstable and can be used only with a very limited number of organic solvents, we developed a device in which the organic solution is placed in a small glass vial made from a Pasteur pipette with a pencil lead as working electrode. First, the pencil lead was inserted into one end of the glass pipette and then the end of the pipette was melted off using a Bunsen burner to prevent leakage of the organic solution during measurements. Electrical contact was made at the end of the pencil lead using

copper tape. This glass vial was filled with the organic solvent and immersed in a beaker with the aqueous electrolyte to create an interface between the two immiscible liquids (Fig. 1B). In this way it is possible to obtain a stable three-phase junction using a small amount of the organic.

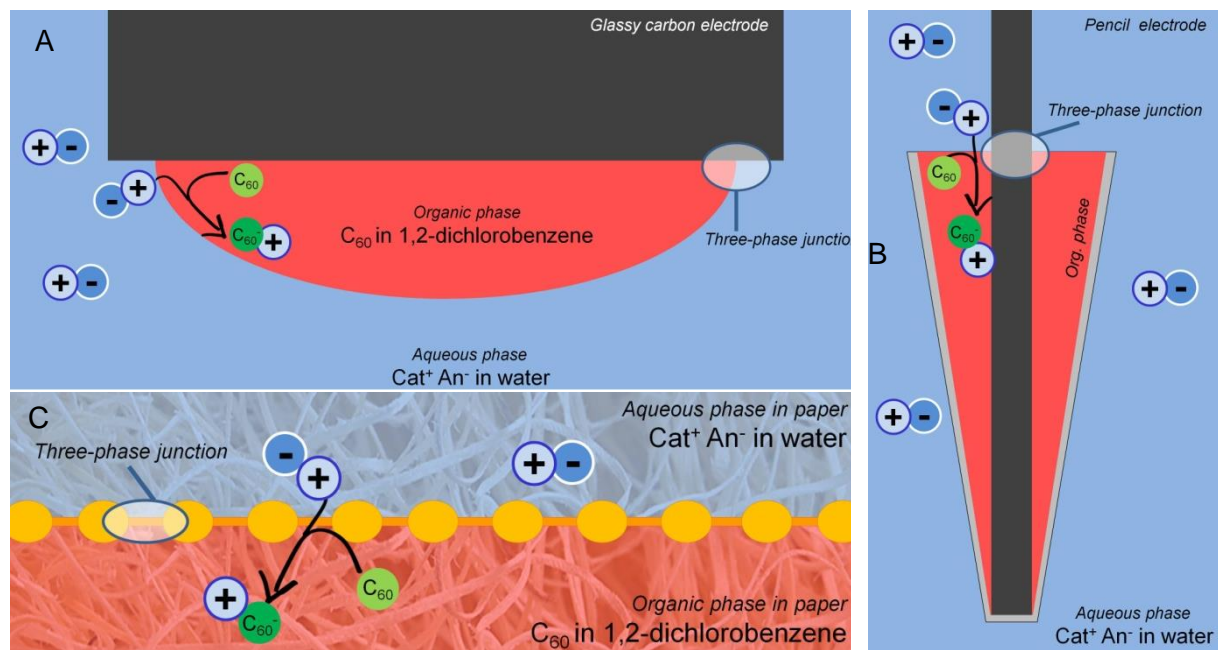


Fig. 1 Schematic representation of the measurement setups used: A. droplet-modified electrode, B. Pencil graphite electrode, C. Paper-based setup; Organic phase marked in red; Aqueous phase in blue.

2.3.3 Paper-based system

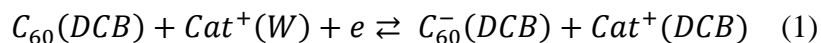
A paper-based three-electrode setup (Fig. 1C) was constructed in the same way as the system previously described for the study of anion transfer [17]. In case of facilitated cation transfer measurements one of the papers was soaked with DCB solution containing 1 mM fullerene C_{60} and 10 mM DB18C6 and the other with the aqueous solution of the studied electrolyte.

3. Results and Discussion

3.1. Direct and assisted (DB18C6) transfer of inorganic cations

Droplet-based three phase electrode is one of the most widely used configuration for investigation of ion transfer processes. For that reason, first we performed experiments in this

well-known system to confirm that fullerene reduction can be accompanied by cation transfer across the water|dichlorobenzene interface, according to the equation:



This equation can be used to describe the transfer process only if the redox probe does not escape to the aqueous phase. No redox reaction could be recorded in the absence of ionophore in the organic phase, using the droplet setup. If the ionophore DB18C6 is added to the organic droplet a reduction reaction can be measured. From the lack of peak shift and the stable current over subsequent cyclic voltammetry scans (SI Fig. S1) we can conclude that this is likely due to the reduction of C_{60} accompanied by a cation transfer into the organic phase. As can be seen in Fig. 2A, when DB18C6 is present in the organic phase, the first reduction potential of the fullerene is dependent on the type of the cation (potassium -0.7 V and sodium -0.8 V). Moreover, comparison of SWV plots for four different sodium and potassium salts (KNO_3 , $NaNO_3$, KCl , $NaCl$) shows that type of anion does not have any influence on the transfer potential (SI Fig. S2), further supporting this interpretation. A second reduction is also visible, however the peaks are less well developed and the potential difference between them is significantly smaller than in case of the first reduction.

Analogous experiments were performed in the glass cell with PGE as working electrode (Fig. 2B). The current related to the transfer processes is one order of magnitude higher than in the droplet-based setup. Moreover, the peak of the K^+ transfer is much sharper and better defined than in the previous case. Additionally, SWV plots without ionophore were registered to check whether it is possible to study non-facilitated cation transfer using this setup. Surprisingly, in the pencil-based system we observed a broad peak around -0.9 V for both Na^+ and K^+ , while in the droplet-based setup there was no sign of the transfer.

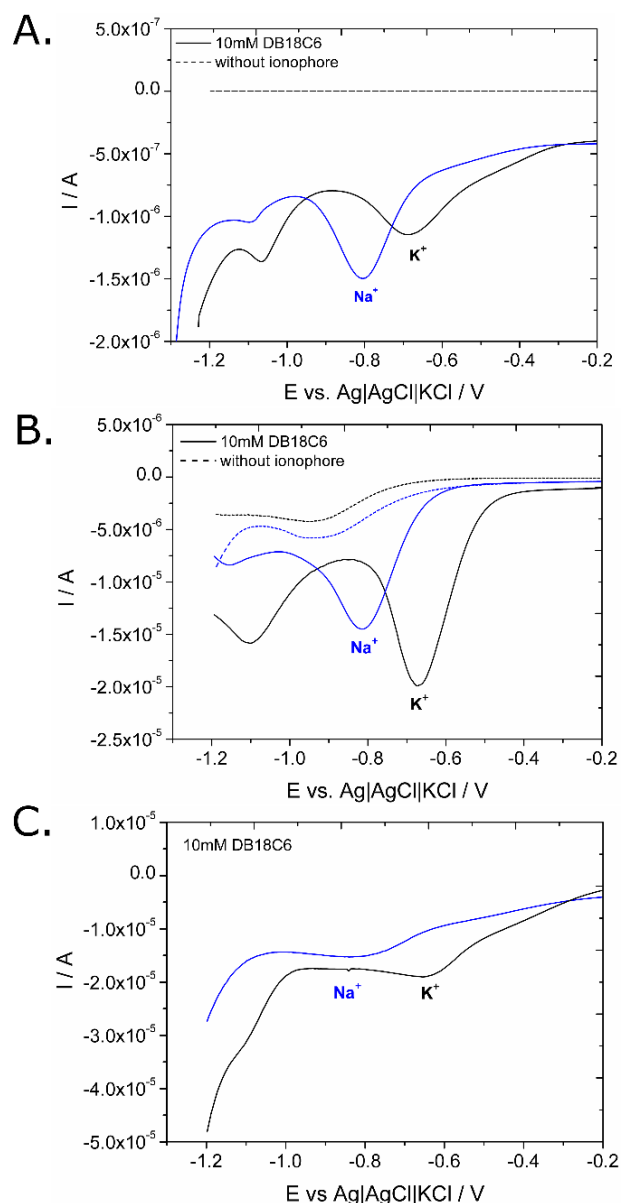


Fig. 2 SWV curves measured for transfer of K^+ and Na^+ in A. droplet-based electrode system, B. glass cell with pencil graphite electrode and C. paper-based system. In all setups aqueous phase contained 0.1M KNO_3 or 0.1M NaNO_3 . The solid lines represent results obtained when organic phase contained 1mM fullerene C_{60} and 10mM DB18C6. The dashed lines are blank experiments for non-facilitated cation transfer without ionophore.

Those differences, as well as higher current values observed for the pencil device can be explained by the increased stability of the interface in the glass cell. Mechanical stability of the ITIES is one of the crucial factors for the ion transfer studies, especially when the three-

electrode configuration is used. The organic phase does not contain any electrolyte so electrochemical reaction can occur only at the three-phase junction and therefore it is very important to create a well-defined boundary between phases. Measurements with droplet-modified electrodes are not trivial and require experience, great care and proper preparation of the electrode surface. When the electrode is immersed into the electrolyte solution there is a high risk of droplet displacement or even detachment, which can hinder the creation of a stable interface and as a consequence severely decrease the reproducibility of such measurements. The disposable glass capillary with the pencil lead introduced in this manuscript helps to overcome those technical problems. The ion-transfer measurements as above were also performed in the paper-based system, confirming its applicability to facilitated cation transfer studies, albeit with lower sensitivity than the other systems.

3.2. Impact of the ionophore concentration

Although it is possible to observe non-facilitated cation transfer, the presence of ionophore is essential for discrimination between different ions. In the absence of ionophore, peaks related to the fullerene reduction are very wide hindering exact determination of the peak potential (Tab. 1).

Novel ionophores are usually synthesized in small amounts and the price of commercially available compounds is often quite high. Thus a tool enabling comparison of selectivity of different ionophores should use as small amount as possible. For that reason we decreased the concentration of the ionophore in the organic phase from 10 mM to 1 mM. The impact of this change is visible on the SWV plots as a negative shift of the peak potential (Fig. 3A). It is known that ion-ionophore complexes can present different stoichiometries depending on the ratio of both molecules in the system, which can affect the transfer potential [24]. The main role of the ionophore is to lower the Gibbs energy of transfer which in voltammetric experiments is observed as a less negative peak potential. When the ratio between the

ionophore and the cation decreases this effect becomes less pronounced and the reduction peak appears at a more negative potential.

Further experiments with 1 mM DB18C6 show that apart from Na^+ and K^+ also NH_4^+ , Li^+ and two divalent cations Ca^{2+} and Mg^{2+} can be distinguished by means of ion transfer measurements in the pencil-based system (Fig. 3B). The order in which peaks appear is governed by hydrophilic properties of the cation and its radius, which is the ability to interact with the cavity of the DB18C6 ionophore and the solvation in the organic phase [24]. In case of DB18C6 assisted transport the least negative transfer potential is observed for potassium ions, which is in the agreement with the literature on binding selectivity of this ionophore [25]. The radii of the other monovalent cations decrease as follows $\text{NH}_4^+ > \text{Na}^+ > \text{Li}^+$ what exactly reflects the order observed on the SWV plot. Sizes of Ca^{2+} and Mg^{2+} are similar to Na^+ , however peak potentials are shifted to the more negative values. This can be explained by other effects than size matching, such as electrostatic interaction between the cation and the cavity, conformation of the ionophore and type of solvation patterns [26]. It should be also remembered that in case of Mg^{2+} and Ca^{2+} doubly charged ions are compensating for a single charged reduction of the redox probe.

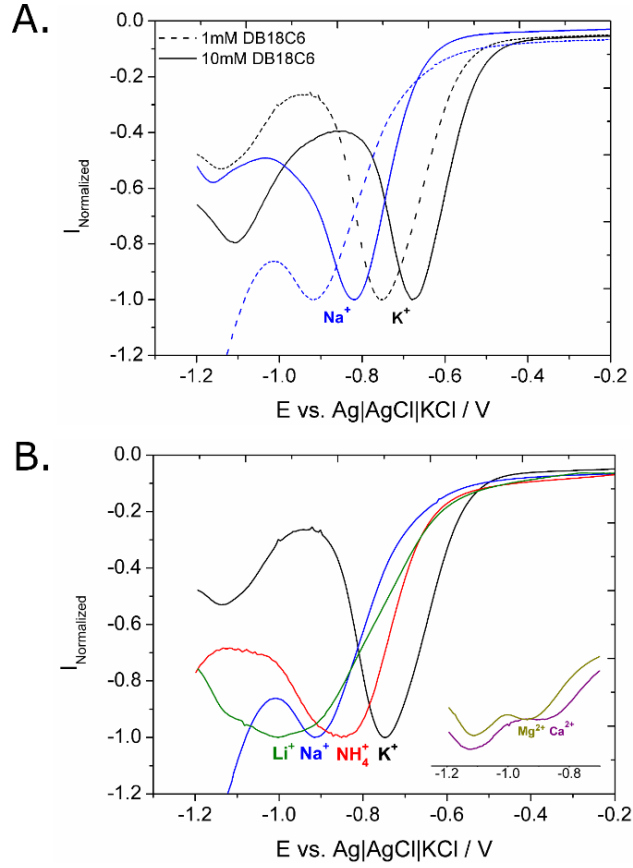


Fig. 3 SWV curves measured for A. transfer of Na⁺ and K⁺ in presence of 1 mM and 10 mM DB18C6 in organic phase; B. transfer of different single charged inorganic cations in presence of 1 mM DB18C6 in organic phase. Inset shows plots for double charged cations. Concentration of all inorganic salts was 0.1 M.

As mentioned before, the hydrophilicity of the cation is another important aspect. In general the more hydrophobic the species, the lower potential needs to be applied to transport it from the aqueous to the organic phase. To quantitatively characterize this effect the standard transfer potential of the cation $\Delta_{\text{aq}}^{\text{org}} \phi_{\text{Cat}^+}^0$ is used. It is one of the components in the Nernst-like equation describing the ion transfer process in the absence of ionophore [27]:

$$E_p = E_{C_{60}/C_{60}^-}^0 + \Delta_{\text{aq}}^{\text{org}} \phi_{\text{Cat}^+}^0 + \frac{RT}{F} \ln c_{\text{Cat}^+}(\text{aq}) + \frac{RT}{F} \ln \frac{2}{c_{C_{60}}} \quad (2)$$

where $E_{C_{60}/C_{60}^-}^0$ is the standard redox potential of C₆₀/C₆₀[−] couple, c_{Cat^+} is the concentration of the cation in the aqueous phase, and $c_{C_{60}}$ is the concentration of C₆₀ in the organic phase.

Since cation transfer is rarely studied, especially in the three-electrode system, there is no available data on $\Delta_{\text{aq}}^{\text{org}} \phi_{\text{Cat}^+}^0$ in dichlorobenzene. Therefore it is hard to determine whether dependence between E_p and $\Delta_{\text{aq}}^{\text{org}} \phi_{\text{Cat}^+}^0$ is linear or not.

3.3. Selectivity of different K^+ ionophores

Facilitated cation transfer in the TPE system can be used as a convenient tool to compare the selectivity of different ionophores. Although comparison of ionophores is a widely discussed topic, vast majority of the studies concerns the use of potentiometric ion-selective electrodes. Rarely voltammetric membrane sensors are applied [29]. All these methods require immobilization of the ionophore in a membrane which composition might affect the ionophores' activity and selectivity.

Ion transfer studies in TPE enable estimation of the selectivity of the ionophore only influenced by the organic solvent, since measurements are done in the liquid phase without other additives. Although the organic phase also contains a redox probe it has been shown that if sufficiently hydrophobic, the rate of the ion transfer is the limiting step of the overall coupled electron-ion transfer reaction [30]. To demonstrate the applicability of the three-phase ion-transfer system in this field we performed experiments in the presence of the most studied potassium ionophores from three different classes: DB18C6 (monocrown ether), Potassium Ionophore III (biscrown ether) and Valinomycin (antibiotic). Their structures are presented in Fig. 4.

Similarly to DB18C6, the other two ionophores are selective for potassium, therefore it is not surprising that peak potentials obtained for monovalent cations follow the same order as described before (SI Fig. S3). Detailed analysis of SWV plots enable qualitative comparison of the ionophores. As can be seen in Tab. 1 the most significant lowering of the transfer potential, as compared with the unassisted cation transfer, was achieved in presence of

Valinomycin. In case of K^+ the potential difference between the direct and facilitated processes was more than 0.5 V.

In 2016 Amemiya [29] proposed a method for calculation of voltammetric selectivity coefficients based on the separate solution method (SSM):

$$\log K_{I,J}^{vol} = \frac{z_I F (\Delta_w^m \phi_J - \Delta_w^m \phi_I)}{2.303 RT} \quad (3)$$

where, $\Delta_w^m \phi_J$ and $\Delta_w^m \phi_I$ are the corresponding phase boundary potentials for the interfering (J) and primary (I) ion respectively, z_I is the charge of the primary ion, F is the Faraday constant, R the gas constant and T the temperature in [K]. All values calculated against the peak potential obtained for potassium.

As can be seen in Tab.1 although the lowest peak potentials are observed for Valinomycin, this ionophore possesses slightly lower selectivity towards sodium and ammonium ions than Ionophore III. Calculated voltammetric selectivity coefficients are compared in the table to traditionally used potentiometric selectivity coefficients $\log K_{K,J}^{pot}$ obtained with PVC membrane ion-selective electrodes, also by means of SSM. High discrepancies between values of $\log K_{K,J}^{pot}$ found in the literature are, as mentioned before, mainly related to the composition of the ion-selective membrane.

Valinomycin is long considered as one of the best neutral-carriers since it exhibits selectivities similar to those observed in biological systems [31]. Although Valinomycin is a very good carrier of monovalent cations it is not so effective in supporting transfer of divalent species. Peaks obtained in presence of Ca^{2+} and Mg^{2+} are very wide and difficult to distinguish from each other (SI Fig. S4). Much better results were achieved for Ionophore III, peaks are sharper and thanks to that both cations can be identified. The use of Ionophore III, is not as common but the few measurements of selectivity found in the literature are in agreement with our findings. Even though higher potential needs to be applied than in case of Valinomycin assisted transfer, the higher lipophilicity of Potassium Ionophore III and its

higher selectivity against sodium and ammonium ions makes it an interesting alternative to the more widely used Valinomycin.

Crown ether DB18C6 is much less selective than Valinomycin [2], but as shown in our study it is still a valid choice for low-cost voltammetric discrimination of cations.

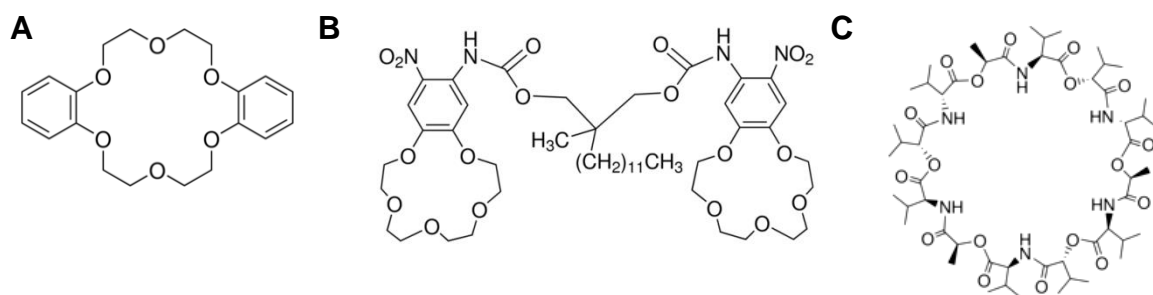


Fig. 4 Structure of studied ionophores: A. dibenzo-18-crown 6 (DB18C6), B. Potassium Ionophore III, C. Valinomycin

Tab. 1 Values of the peak potential and selectivity coefficients for facilitated and non-facilitated transfer of inorganic cations. Peak potential (Ep) vs Ag/AgCl.

J	No ionophore		Valinomycin			Ionophore III			DB18C6		
	Ep	$\log K_{K,J}^{vol}$	Ep	$\log K_{K,J}^{vol}$	$\log K_{K,J}^{pot}$ [32,33]	Ep	$\log K_{K,J}^{vol}$	$\log K_{K,J}^{pot}$ [34,35]	Ep	$\log K_{K,J}^{vol}$	$\log K_{K,J}^{pot}$ [36,37]
K ⁺	-0.947	0	-0.388	0	0	-0.589	0	0	-0.750	0	0
Na ⁺	-0.952	-0.085	-0.529	-2.38	-2.6:-4.0	-0.821	-3.92	-3.0:-3.3	-0.916	-2.81	-0.5:-2.7
NH ₄ ⁺	-0.942	0.085	-0.483	-1.61	-1.3:-1.9	-0.715	-2.13	-2.0:-2.1	-0.851	-1.71	-0.6:-4.1
Li ⁺	-0.957	-0.17	-0.866	-8.08	-3.1:-4.0	-0.916	-5.53	-3.4:-3.8	-1.002	-4.26	-3.9
Mg ²⁺	-		-0.972	-9.87	-3.0:-6.3	-0.896	-5.19	-3.8:-5.0	-0.937	-3.16	-2.5:-3.1
Ca ²⁺	-		-0.932	-9.19	-2.9:-5.9	-0.831	-4.09	-4.0:-4.5	-0.906	-2.64	-2.6:-3.7

3.4. Concentration dependency

One of the effects characteristic for ion transfer experiments in a three-phase electrode setup is the linear correlation between ion concentration and peak potential with the slope 59 mV per decade for one-electron redox reaction. The same relation is observed for the

electromotive force of potentiometric ion selective electrodes when single charged ions are measured [38].

To confirm that the pencil-based system responds according to the Eq. 2, concentration dependence was investigated in the presence of Valinomycin, Ionophore III and DB18C6 (Fig. 5). Experiments were performed for K^+ since, as was shown in the previous section, all chosen ionophores exhibit the highest selectivity towards this cation.

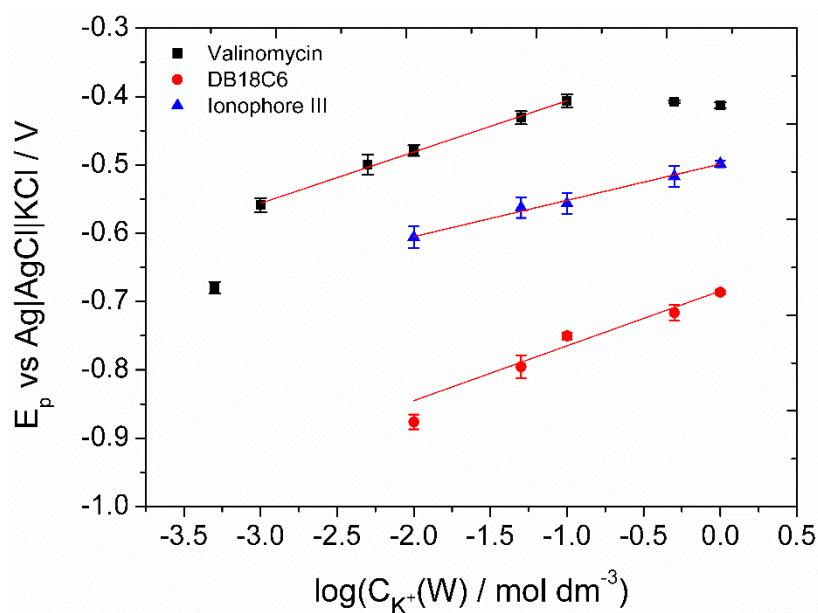


Fig. 5 Correlation between peak potential and concentration of K^+ in the presence of 1 mM Valinomycin (black squares), Ionophore III (blue triangles) or DB18C6 (red circles). The slope of the fitted line and R^2 value are 74 ± 5 mV and $R^2=0.99$, 53 ± 2 mV and $R^2=0.995$, 80 ± 10 mV and $R^2=0.94$ for Valinomycin, Ionophore III and DB18C6, respectively.

In Fig. 5 we see that a linear correlation between ion concentration and C_{60} reduction potential was achieved for all carriers, however only for Ionophore III the slope is close to Nernstian. In fact, those results are not surprising, since Eq. 2 was derived for non-facilitated ion transfer and it does not include any components related to the facilitated process e.g. concentration of the ionophore. Those parameters have a significant influence on the transfer process and their absence in the equation can be the explanation for non-ideal slope values.

Moreover, it was already shown that stoichiometry of the complex between the ionophore and the target ion is a crucial factor in this relation. A lot of research has been performed to explain super-Nernstian responses of ionophore-based ISEs as an effect of simultaneous formation of complexes of different stoichiometries of ions and ionophores [7,38–40]. It has been shown that small cations like K^+ can form 1:1 as well as 1:2 complexes with Valinomycin and DB18C6[41] resulting in deviations from the Nernstian response. This might also be a factor in our measurements [39].

It is worth to note the wider concentration range presented for Valinomycin as compared with the other two ionophores. Voltammograms recorded for 0.005, 0.001 and 0.0005 M K^+ in the presence of Ionophore III or DB18C6 did not show any signs of ion transfer.

The detection limit of electrochemically driven ion transfer is influenced by the concentration ratio between the redox probe and the ion of interest [17]. Therefore it could be possible to lower the detection limit for Potassium Ionophore III and DB18C6, however in our case change of the ratio is restricted by poor solubility of fullerene C_{60} in DCB. Although a low detection limit is a valid aim in the case of sensing applications, the main goal here is a tool for easy estimation of selectivity of ionophores.

3.5. Transfer of organic cations

Detection of inorganic cations is one of the main applications of ion-selective electrodes. Investigation of transfer of organic compounds is interesting since that can enable determination of biologically important molecules like proteins or enzymes.

We measured square-wave voltammetry for direct and facilitated transfer of 3 quaternary ammonium salts: TMA^+ , TEA^+ and TPA^+ (Fig. 6). We see that the longer the aliphatic chain the less negative potential is needed to simultaneously transfer the cation and reduce C_{60} to C_{60}^- . This is expected, since the ions with longer chains are more lipophilic than the ones with shorter chains. It is known that the large diffuse charge of the longer chained quaternary

ammonium cations interact with C_{60}^- to stabilize its charge [39]. As a result, C_{60}^- itself can act as an ionophore for these large molecules. This effect increases with the length of the aliphatic chain. It is also responsible for the lower potential value related to the second reduction. In this case C_{60}^{2-} is stabilized more effectively than C_{60}^- in the presence of TPA^+ compared with the other two cations. Therefore, the influence of an additional ionophore is much smaller for large TPA^+ cation than for TEA^+ or TMA^+ . Since size of TMA^+ is relatively small the impact of the ionophore on its transfer is most visible.

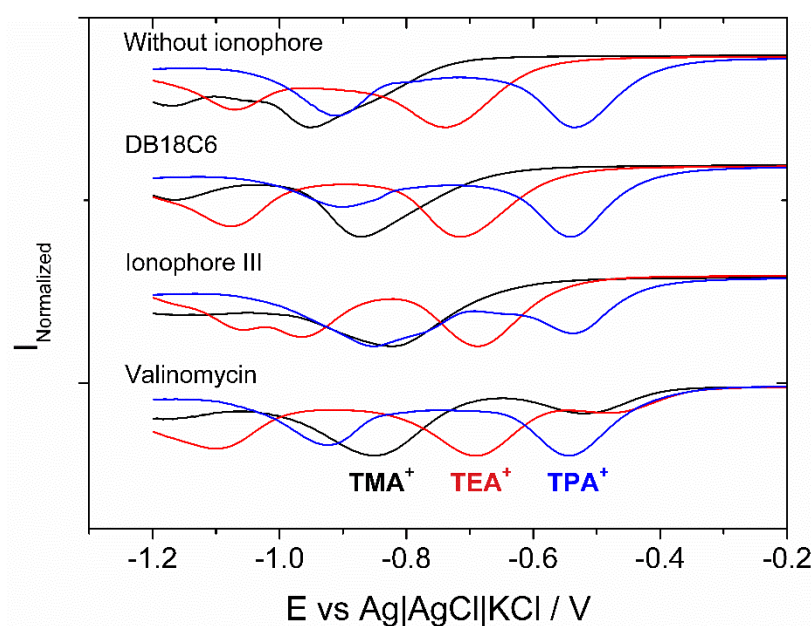


Fig. 6 Comparison of SWV plots obtained for transfer of organic salts without deliberately added ionophore and in the presence of 1 mM DB18C6, Ionophore III or Valinomycin in the organic phase. Concentration of all organic salts was 0.1 M.

The mechanism of facilitated organic cation transfer seems to be much more complicated than in case of simple, alkali metal cations. It is very possible that peak shift observed in the presence of ionophore is related to the electrostatic interaction between diffuse charge of the quaternary ammonium cation and ionophore molecule. However a detailed explanation of this effect requires further in-depth analysis, which is beyond the focus of this study.

4. Conclusions

We show that the fullerene C_{60} is an efficient redox system for the study of cation transfer at a DCB|aqueous electrolyte|electrode three-phase junction. A simple setup with a pencil graphite working electrode was developed for providing easier and more reproducible three-phase electrodes than traditional droplet-based TPEs, and this allowed us to investigate both non-facilitated and facilitated ion transfer.

The addition of ionophores to the organic phase significantly shifts the transfer potential inorganic cations. The three-phase system allows us to directly compare the selectivity of several ionophores without immobilization in a membrane, which eliminates the influence of other membrane components.

We measured the transfer of six different inorganic cations facilitated by the ionophores Valinomycin, Ionophore III, or DB18C6. All three were most efficient in carrying potassium ions, with Valinomycin showing by far the largest change in transfer potential compared to the un-facilitated measurements. However, the Ionophore III was showed somewhat better selectivity against sodium and ammonium ions, which could make it an interesting alternative to Valinomycin in some applications. Voltammetric selectivity coefficients calculated using the data from the three-phase electrode experiments are consistent with data from the literature.

The successful experiments using pencil-based as well as paper-based devices confirm that low-cost and simple ion-transfer voltammetry assemblies can be a convenient alternative to ion-selective electrodes. They enable not only fast and easy comparison of selectivity of ionophores but also allow investigation of both facilitated and non-facilitated cation transfer processes.

The authors declare no competing financial or personal interest.

Acknowledgements

This work was supported by the National Science Centre, Poland through grant NCN 2015/18/E/ST4/00319.

Appendix A. Supplementary information

Supplementary data related to this article can be found at

References

- [1] P. Bühlmann, E. Pretsch, E. Bakker, Carrier-based ion-selective electrodes and bulk optodes. 2. Ionophores for potentiometric and optical sensors, *Chem. Rev.* 98 (1998) 1593–1688. doi:cr970113+ [pii].
- [2] J. Zhang, A.R. Harris, R.W. Cattrall, A.M. Bond, Voltammetric ion-selective electrodes for the selective determination of cations and anions, *Anal. Chem.* 82 (2010) 1624–1633. doi:10.1021/ac902296r.
- [3] R.D. Johnson, L.G. Bachas, Ionophore-based ion-selective potentiometric and optical sensors, *Anal. Bioanal. Chem.* 376 (2003) 328–341. doi:10.1007/s00216-003-1931-0.
- [4] P. Bühlmann, L.D. Chen, Ion-Selective Electrodes With Ionophore-Doped Sensing Membranes, 2012. doi:10.1002/9780470661345.smc097.
- [5] H.K. Frensdorff, Salt Complexes of Cyclic Polyethers. Distribution Equilibria, *J. Am. Chem. Soc.* 93 (1971) 4684–4688. doi:10.1021/ja00748a006.
- [6] Y. Mi, E. Bakker, Determination of complex formation constants of lipophilic neutral ionophores in solvent polymeric membranes with segmented sandwich membranes, *Anal. Chem.* 71 (1999) 5279–5287. doi:10.1021/ac9905930.
- [7] P.J. Greenawalt, M.B. Garada, S. Amemiya, Voltammetric Characterization of Ion-Ionophore Complexation Using Thin Polymeric Membranes: Asymmetric Thin-Layer Responses, *Anal. Chem.* 87 (2015) 8564–8572. doi:10.1021/acs.analchem.5b02355.
- [8] A. Sabela, J. Koryta, O. Valent, Ion carrier properties of nigericin studied by

- voltammetry at the interface of two immiscible electrolyte solutions, *J. Electroanal. Chem.* 204 (1986) 267–272. doi:10.1016/0022-0728(86)80524-1.
- [9] J. Koryta, Electrochemical polarization phenomena at the interface of two immiscible electrolyte solutions, *Electrochim. Acta.* 24 (1979) 293–300.
- [10] F. Reymond, P.A. Carrupt, H.H. Girault, Facilitated ion transfer reactions across oil|water interfaces. Part I. Algebraic development and calculation of cyclic voltammetry experiments for successive complex formation, *J. Electroanal. Chem.* 449 (1998) 49–65. doi:10.1016/S0022-0728(97)00430-0.
- [11] F. Quentel, V. Mirčeski, M. L’Her, Kinetics of anion transfer across the liquid | liquid interface of a thin organic film modified electrode, studied by means of square-wave voltammetry., *Anal. Chem.* 77 (2005) 1940–1949. doi:10.1021/ac049117m.
- [12] M. Donten, Z. Stojek, F. Scholz, Electron transfer–ion insertion electrochemistry at an immobilised droplet: probing the three-phase electrode-reaction zone with a Pt disk microelectrode, *Electrochem. Commun.* 4 (2002) 324–329. doi:10.1016/S1388-2481(02)00302-8.
- [13] F. Scholz, Š. Komorsky-Lovrić, M. Lovrić, A new access to Gibbs energies of transfer of ions across liquid|liquid interfaces and a new method to study electrochemical processes at well-defined three-phase junctions, *Electrochem. Commun.* 2 (2000) 112–118. doi:10.1016/S1388-2481(99)00156-3.
- [14] E. Bak, M. Donten, Z. Stojek, Three-phase electrochemistry with a cylindrical microelectrode, *Electrochem. Commun.* 7 (2005) 483–489. doi:10.1016/j.elecom.2005.03.005.
- [15] Á. Torrinha, C.G. Amorim, M.C.B.S.M. Montenegro, A.N. Araújo, Biosensing based on pencil graphite electrodes, *Talanta.* 190 (2018) 235–247. doi:10.1016/j.talanta.2018.07.086.

- [16] E. Witkowska Nery, M. Kundys-Siedlecka, Y. Furuya, M. Jönsson-Niedziółka, Pencil Lead as a Material for Microfluidic 3D-Electrode Assemblies, *Sensors*. 18 (2018) 4037. doi:10.3390/s18114037.
- [17] M. Podrazka, E.W. Nery, A. Pacowska, D.W.M. Arrigan, M. Jönsson-Niedziółka, Paper-Based System for Ion Transfer Across the Liquid-Liquid Interface, *Anal. Chem.* 90 (2018) 8727–8731. doi:10.1021/acs.analchem.8b02695.
- [18] F. Scholz, R. Gulaboski, K. Caban, The determination of standard Gibbs energies of transfer of cations across the nitrobenzene|water interface using a three-phase electrode, *Electrochem. Commun.* 5 (2003) 929–934. doi:10.1016/j.elecom.2003.09.005.
- [19] F. Quentel, V. Mirčeski, M. L'Her, Lutetium bis(tetra-tert-butylphthalocyaninato): A superior redox probe to study the transfer of anions and cations across the water|nitrobenzene interface by means of square-wave voltammetry at the three-phase electrode, *J. Phys. Chem. B*. 109 (2005) 1262–1267. doi:10.1021/jp045914c.
- [20] S. Wu, B. Su, 7,7',8,8'-Tetracyanoquinodimethane as a redox probe for studying cation transfer across the water/2-nitrophenyl octyl ether interface at three-phase junctions supported by carbon ink screen-printed electrodes, *J. Electroanal. Chem.* 656 (2011) 237–242. doi:10.1016/j.jelechem.2010.11.004.
- [21] J. Zhang, P.R. Unwin, Investigation of the kinetics of electron transfer processes involving fullerenes at liquid–liquid interfaces using scanning electrochemical microscopy: evidence for Marcus inverted region behaviour, *J. Chem. Soc. Perkin Trans. 2*. (2001) 1608–1612. doi:10.1039/b102734b.
- [22] W. Adamiak, G. Shul, M. Opallo, Anion sensitive voltammetry of fullerene C60 dissolved in 1,2-dichlorobenzene deposit in contact with aqueous electrolyte, *Electrochem. Commun.* 11 (2009) 149–152. doi:10.1016/j.elecom.2008.10.048.

- [23] F. Quentel, V. Mirčeski, M. L'Her, K. Stankoska, Assisted Ion Transfer at Organic Film-Modified Electrodes, *J. Phys. Chem. C.* 116 (2012) 22885–22892.
doi:10.1021/jp3067603.
- [24] A.G. Volkov, *Liquid Interfaces in Chemical, Biological and Pharmaceutical Applications*, 1st ed., Marcel Dekker, New York, 2001.
- [25] C. Min Choi, J. Heo, N. Joon Kim, Binding selectivity of dibenzo-18-crown-6 for alkali metal cations in aqueous solution: A density functional theory study using a continuum solvation model, *Chem. Cent. J.* 6 (2012) 426. doi:10.1186/1752-153X-6-84.
- [26] B. Su, S. Zhang, Y. Yuan, J. Guo, L. Gan, Y. Shao, Investigation of ion transfer across the micro-water/nitrobenzene interface facilitated by a fullerene derivative, *Anal. Chem.* 74 (2002) 373–378. doi:10.1021/ac0155658.
- [27] F. Scholz, R. Gulaboski, Determining the Gibbs energy of ion transfer across water-organic liquid interfaces with three-phase electrodes., *Chemphyschem.* 6 (2005) 16–28. doi:10.1002/cphc.200400248.
- [28] B. Hundhammer, C. Müller, T. Solomon, H. Alemu, H. Hassen, Ion transfer across the water-o-dichlorobenzene interface, *J. Electroanal. Chem. Interfacial Electrochem.* 319 (1991) 125–135. doi:10.1016/0022-0728(91)87072-C.
- [29] S. Amemiya, Voltammetric Ion Selectivity of Thin Ionophore-Based Polymeric Membranes: Kinetic Effect of Ion Hydrophilicity, *Anal. Chem.* 88 (2016) 8893–8901. doi:10.1021/acs.analchem.6b02551.
- [30] R. Gulaboski, V. Mirčeski, C.M. Pereira, M.N.D.S. Cordeiro, A.F. Silva, F. Quentel, M. L'Her, M. Lovrić, A comparative study of the anion transfer kinetics across a water/nitrobenzene interface by means of electrochemical impedance spectroscopy and square-wave voltammetry at thin organic film-modified electrodes., *Langmuir.* 22

- (2006) 3404–3412. doi:10.1021/la052565k.
- [31] W. Simon, Neutral-carrier-based ion-selective electrodes, 180 (1986).
- [32] E. Witkowska Nery, E. Jastrzębska, K. Żukowski, W. Wróblewski, M. Chudy, P. Ciosek, Flow-through sensor array applied to cytotoxicity assessment in cell cultures for drug-testing purposes, *Biosens. Bioelectron.* 51 (2014) 55–61. doi:10.1016/j.bios.2013.07.023.
- [33] P.C. Hauser, D.W.L. Chiang, G.A. Wright, A potassium-ion selective electrode with valinomycin based poly(vinyl chloride) membrane and a poly(vinyl ferrocene) solid contact, *Anal. Chim. Acta.* 302 (1995) 241–248. doi:10.1016/0003-2670(94)00472-X.
- [34] E. Lindner, K. Tóth, J. Jeney, M. Horváth, E. Pungor, I. Bitter, B. Ágai, L. Töke, Novel bis (crown ether)-based potassium sensor for biological applications, *Mikrochim. Acta.* 100 (1990) 157–168. doi:10.1007/BF01244839.
- [35] J. Tarcali, G. Nagy, K. Tóth, E. Pungor, G. Juhász, T. Kukorelli, In vivo measurements with a potassium ion-selective microelectrode based on a new bis(crown ether), *Anal. Chim. Acta.* 178 (1985) 231–237. doi:10.1016/S0003-2670(00)86273-X.
- [36] K. Taheri, T. Afsari, Fabrication of Potassium Ion-selective Membrane Electrode as Immobilized Liquid based on Dibenzo-18- Crown-6, *Anal. Bioanal. Electrochem.* 6 (2014) 475–484.
- [37] M.M. Zareh, M.A. Akl, A.K. Gohneim, M.H. Abd El-Aziz, PVC-membrane electrodes based on 18-crown-6 and dibenzo-18-crown-6 ethers for determination of silver, *Turkish J. Chem.* 31 (2007) 449–456.
- [38] E. Bakker, P. Bühlmann, E. Pretsch, The phase-boundary potential model, *Talanta.* 62 (2004) 843–860. doi:10.1016/j.talanta.2003.10.006.
- [39] D. Dubois, G. Moninot, W. Kutner, M.T. Jones, K.M. Kadish, Electroreduction of Buckminsterfullerene, C₆₀, in aprotic solvents. Solvent, supporting electrolyte, and

temperature effects, *J. Phys. Chem.* 96 (1992) 7137–7145. doi:10.1021/j100196a056.