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Improvement of solid-contact lithium ion-selective electrodes: Super-Nernstian sensitivity and calibration-free approaches for decentralized analysis

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Bachelor's Thesis

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Summary

This bachelor's thesis is centered on the characterization and improvement of an analytical measurement procedure with potentiometric detection using paper-based sensors. A lithium ion-selective membrane (ISM) will be optimized and characterized. Moreover, a potassium ISM will be used as a model membrane for the experiments.

Ion-selective electrodes (ISEs) used in potentiometry have many advantages but also some limitations. The most important limitations are the fix sensitivity depending on the charge of the ion (59.2 mV/decade for monovalent ions, 29.6 mV/decade for divalent ions, etc.) and the need for calibration before every analysis due to the variability on the standard potential (E^0). There are some approaches on the literature to overcome these limitations; for example, an array of ISEs can generate a super-Nernstian sensitivity and the integration of redox buffer species on the membrane can improve the reproducibility of the standard potential. This work studies the improvement of these limitations using some of these approaches.

Objectives

The general objective of this thesis is to improve the ISEs performance, the bachelor's thesis is focused on different approaches to improve two important parameters: sensitivity and standard potential reproducibility.

Specific objectives are:

- Optimize and characterize the lithium ion-selective membrane.
- Determine and optimize the conditions for the super-Nernstian sensitivity.
- Optimize the membrane composition to improve the E^0 reproducibility.
- Integrate the ISE with our reference electrode (previously optimized on work placement).
- Apply all the improvements for the detection of lithium in real samples.

1. Introduction

Centralized model is the paradigm of the XXI century and the responsible of collapsing systems, therefore decentralized generation of information is the key solution to this problem. Analytical chemistry has an important role in the decentralization of the information allowing the generation of information in any part of the world without the necessity of a laboratory, infrastructure, personnel, etc. In the medical field, analytical sensors provide information about patients in real time to the doctor and in this way connect them remotely. This helps the health care system to have a better management of the information generated in the primary care centre or in the patient's home.

The sensors studied in this bachelor's thesis use potentiometry as a detection technique. This technique is based on the measurement of the potential generated between two electrodes (reference and working electrodes) in a closed circuit, which can be related to the concentration of the target analyte. The potential is recorded with a high impedance voltmeter that ensures that the current flow is negligible. Since there is no net current, there are not electrochemical reactions involved, which means that the system is in equilibrium. The reference electrode potential must be constant independently of the solution composition, whereas the potential of the working electrode changes depending on the analyte concentration. See the experimental set up in Figure 1.

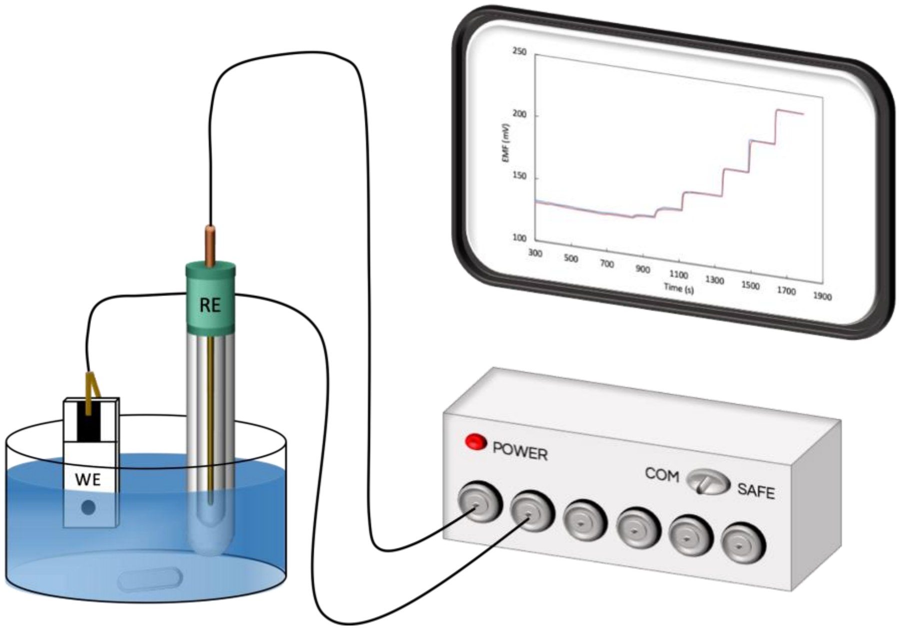

Figure 1. Experimental set-up for potentiometric sensors. Working electrode (WE) and reference electrode (RE).

Phase-boundary potential model[1] is used to explain the potentiometric response of ISEs. This model is based in two assumptions:

1. Potential of the membrane/solution interface (aqueous/organic) is the most significant. Electroneutrality principle is accomplished in the membrane face, therefore the diffusion potential is neglected.
2. There is a local chemical equilibrium between the membrane/solution interface.

The phase-boundary potential follows the next expression:

$$E_{PB} = \frac{RT}{z_i F} \ln k_I + \frac{RT}{z_i F} \ln \frac{a_I\,(aq)}{a_I\,(org)} \qquad \text{Equation 1}$$

Equation 1 is formed by several variables: R, T and F are the universal gas constant (8.314 J/K·mol), the absolute temperature (in K) and the Faraday constant (96485 C/mol) ; z_i is the charge of the primary ion; a_I(aq) is the activity of the uncomplexed primary ion in the aqueous sample; a_I(org) is the activity of the primary ion in contact with the organic phase boundary and k_I is the function of the relative free energies of solvation (where μ_I^0(aq) and μ_I^0(org) are the chemical standard potentials of the ion I^{zI+} in the respective phase):

$$k_I = e^{\frac{\mu_I^0(aq) - \mu_I^0(org)}{RT}} \qquad \text{Equation 1.1}$$

The first term of the equation 1 is the standard potential (E^0) which is constant for a given ion but varies from ion to ion. Therefore, E^0 changes for each experiment and it does not have a fix value. Activity coefficients are assumed to have a constant value in the membrane phase. Thus, equation 1 can be simplified mathematically to equation 2, most known as Nernst-equation:

$$E_I = E_I^0 + \frac{RT}{z_i F} \ln a_I \qquad \text{Equation 2}$$

$$\ln a_I = \frac{\log a_I}{\log(2.718)} \qquad \text{Equation 3}$$

At room temperature (25°C), RT/F is approximately 25.693 mV in equation 2. Applying the mathematical equation 3, Nernstian sensitivity adopts the value of 59.2 mV/log a_I for monovalent ions.

Solid-state technology introduced in potentiometric decentralized analysis has become an important field to be studied due to its characteristics combining simple methodology, powerful detection abilities, low-power consumption and easy maintenance. This opens the door to the opportunity of developing a low-cost diagnostic dispositive based on paper-based substrates using potentiometric tools[2].

Although potentiometry is a simple technique, it has some important limitations that makes decentralized analysis more complex than with other techniques. Therefore, the improvement of these parameters to make the analysis simpler has captivated researchers among the last years.

Lithium. A key parameter in the medical field

Lithium has been the selected analyte in this thesis because of its interesting medical applicability. Lithium salts are used as a mood stabilizer drug for patients with manic and bipolar disorders and recurrent depression. However, the use of lithium is not as simple as with other drugs due to the narrow effective therapeutic range. Concentration of lithium must be adjusted to levels between 0.5 and 1.5 mM in blood to obtain a positive effect in patients. Moreover, concentration of lithium below 0.5 mM in blood does not have any significant positive effect, while a concentration above 1.5 mM can have toxic effects. Long-term treatments can lead to liver, brain or kidney damage due to chronic toxicity, being a problem that cannot be ignored. In the worst situation, lithium concentration around 5 mM in blood can lead to permanent damage or even death.[3]

For that reason, as precise lithium concentration must be known to determine the effectivity of the treatment and avoid long-term illnesses, determination of this analyte becomes more challenging. The determination of lithium in routine analysis is usually performed by flame emission photometry, atomic absorption spectroscopy or ISEs. However, this analysis requires a laboratory, infrastructure, personnel, instrumentation, etc. This means complex and slow procedures to obtain lithium concentration in blood. The necessity of having simpler and faster ways to do this determination has motivated the development of several decentralized approaches[4].

Lithium ISM is reported in the literature having good performance in real samples prediction[4]. However, during this thesis it has not been possible to reproduce the same results with the reported lithium ISM composition. For that reason, membrane composition has been optimized and characterized in order to obtain the higher sensitivity and selectivity for sample prediction.

In this bachelor's thesis, the improvement of the most important limitations in ISEs has also been studied. These limitations are the fix sensitivity depending on the charge of the ion (59.2 mV/decade for monovalent ions, 29.6 mV/decade for divalent ions, etc.) and the need for calibration before every analysis due to the variability on the standard potential (E^0). Sensitivity of ISEs is not a high limitation in the determination of species having a wide concentration range in blood or serum. However, for some analytes having a narrow effective therapeutic range -such as lithium-, sensitivity of ISE is crucial to reach a better accuracy on the results. In order to improve the sensitivity, an array of 10 sensors has been connected to generate a super-Nernstian sensitivity. This is based on the hypothesis that sensitivity is multiplied by the number of sensors connected and, in this way, more accurate and precise predictions could be achieved compared to the use of a single sensor.

To solve the need of calibration before each analysis, which is one of the highest limitations in ISEs, the integration of a redox buffer specie that can improve the reproducibility of the standard potential is studied. Table 1 shows a scheme with the most studied calibration-free strategies.

Table 1. Calibration-free most important strategies.[1]

CONDUCTING POLYMERS ($E^0 \approx \pm 3$ mV)	Polypyrrole (PPy) Polyaniline (PANI) Poly(octylthiophene) (POT) Poly(ethylenedioxythiophene) (PEDOT)
MOLECULAR REDOX BUFFER ($E^0 \approx \pm 1$ mV)	Co (II) and Co (III) complexes

INORGANIC REDOX BUFFERS ($E^0 \approx \pm 1$ mV)	Gold nanoparticles Ag/AgCl Intercalation materials
CAPACITIVE TRANSDUCERS E^0 variability depends on the material	Porous carbon materials Carbon nanotubes (CNTs) Graphene oxide Carbon black Noble metal

These strategies consist in the addition of an intermediate layer containing these species between the substrate or solid electron conductor and the ISM, or the integration of these species in the membrane composition. Depending on its characteristics, these species can reduce the irreproducibility of the standard potential and improve the stability of the measurements controlling the potential at the interface. However, there is still not a well-defined strategy that fulfils all the requirements for a good calibration- free electrode. Among all the approaches found in the literature[5], some of the articles[6,7] have been reproduced to evaluate the best and simpler strategy to use in the lithium ISM for building calibration-free electrodes.

The first approach[6] uses two PVC-based sandwiched membranes. The first membrane contains a suspension of halogen salts of silver and alkaline metals, as well as silver nanoparticles placed on a silver substrate or a conducting substrate covered with silver. This membrane acts as a solid contact ion-to-electron transducer, controlling the redox reactions and promoting a stable interface potential providing a reproducible E^0 of the ISEs when covered with a second PVC membrane sensitive to ions. In this way, potassium ISEs are characterized by a very stable and reproducible standard potentials with a reported electrode-to-electrode standard deviation of the E^0 of ± 2 mV for bromide-based membranes and ± 3 mV for chloride-based membranes.

PVC based membranes absorb 0.1 - 0.4 wt % of water when in contact with solutions. Considering the amount of salts and possible water content inside the PVC membrane, a solution saturated by soluble salts and soluble complexes of silver can be formed inside the membrane phase after some conditioning. This pseudo "internal solution" is made by soluble salt and silver complexes filling the membrane pores, determining the potential drop on the membrane/Ag substrate phase-boundary.[6]

The second approach[7] is based on the introduction of a lipophilic redox buffer prepared from tetrakis(pentafluorophenyl)borate ($TPFPB^-$) salts of cobalt(II)tris(1,10-phenanthroline) ($[Co(phen)_3]^{2+}$) and cobalt(III)tris(1,10-phenanrheoline) ($[Co(phen)_3]^{3+}$). The two complexes are introduced into a potassium polymeric membrane and drop casted onto gold or glassy carbon electrodes. The electrode-to-electrode standard deviation reported in this study is lower than 0.7 mV after short conditioning. The $TPFPB^-$ salts of $[Co(phen)_3]^{3+}$ and $[Co(phen)_3]^{2+}$ (Figure 2) were selected because of their electrochemical stabilities and their high solubility in plasticized polymeric membranes.

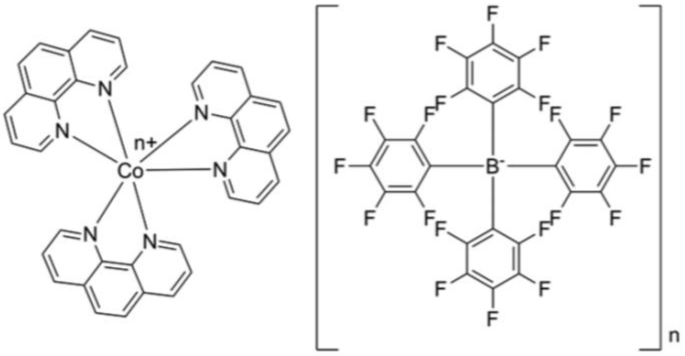

Figure 2. Cobalt(II/III)tris(1,10-phenanthroline) and tetrakis(pentafluorophenyl) borate ($TPFPB^-$).

The improvement of sensitivity and reproducibility of ion-selective electrodes and the optimization of the lithium ion-selective membrane could trigger the opportunity for the development of a decentralized platform for the determination of lithium.

2. Experimental part

2.1. Reagents

For the ISMs, the following ionophores and ion exchangers have been used:

Ionophores. Valinomycin is used for potassium ISM and 6,6-Dibenzyl-14-crown-4 is used for the lithium ISM.

Ion exchanger. Potassium tetrakis(pentafluorophenyl) borate (KTPFPhB) with >98 % purity is used for the potassium ISM and lithium tetrakis(pentafluorophenyl)borate ethyl etherate (LiTPFPhB) is used for the lithium ISM.

Other reagents used are: High molecular weight polyvinyl chloride (PVC), Bis(2-ethylhexyl)sebacate (DOS) with > 97 % purity, 2- nitrophenyl octyl ether (o-NPOE) with > 99 % purity, trioctylphosphine oxide (TOPO), tetrahydrofuran (THF), methanol, silver nitrate, silver chloride, silver bromide, polyvinyl butyral (PVB, B-98), $Co(II)(phen)_3(TPFPB)_2$, $Co(III)(phen)_3(TPFPB)_3$, ionic liquid (1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate) and “Compound R”[1].

Commercial carbon ink (122-49) was purchased from Creative Materials Inc.

Silver and Ag/AgCl paste was purchased from Gwent group.

Prat dumas filter paper 150 mm was purchased from Labox.

Analytical-grade chloride salts of sodium, potassium, lithium, magnesium, ammonium and calcium and bromide salt of sodium were also used. Sodium carbonate and sodium hydrogen phosphate were also used for the preparation of artificial serum.

Solutions were prepared with deionized water (WATR-00P-25K) from labkem.

Doble-junction Ag/AgCl electrode has been used as reference electrode in the potentiometric measurements.

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[1] This compound cannot be revealed due to confidentiality terms.

Composition of artificial serum in mM is shown in Table 2. Potassium chloride is not added when potassium calibrations in artificial serum are performed.

Table 2. Artificial Serum mM composition.

NaCl	111
$NaHCO_3$	29
Na_2HPO_4	2.2
$MgCl_2 \cdot 6\ H_2O$	0.8
KCl	4

Toxic reagents and corresponding manipulation are indicated in Table *3*.

Table 3. Toxicity of the reagents and manipulation.

Reagent	Hazardous	Manipulation
THF	Flammable, irritant and health hazard	Gloves, glasses, lab coat and fume hood
TOPO	Corrosive	Gloves, glasses and lab coat
Silver nitrate	Corrosive and environmentally damaging	Gloves, glasses and lab coat

2.2. Membranes

During the experimental part, different membranes have been prepared. The composition of these membranes can be seen in Tables 4 and 5.

Table 4. Reference membranes composition.

PVC membrane	PVB membrane
27 mg PVC	80 mg PVB
53 mg ionic liquid	50 mg NaCl
1 mL THF	1 mL methanol

Table 5. Potassium ion-selective membrane composition

K^+ ISM
2 mg K^+ ionophore
0.5 mg KTPFPhB
65.6 mg PVC
129.4 mg DOS
1 mL THF

2.3. Sensor's fabrication

For the fabrication of the paper ion-selective electrodes, a conductive paper substrate (filter paper painted with the conductive carbon ink) is sandwiched between two plastic masks. One of the plastic masks has a circular orifice of 2.8 mm to drop cast the ion-selective membrane, as it can be seen in Figure 3. Finally, the two plastic masks are glued together to avoid any direct contact between the paper substrate and the solution. The substrate is exposed at the top of the electrode in order to be electrically connected with the instrument.

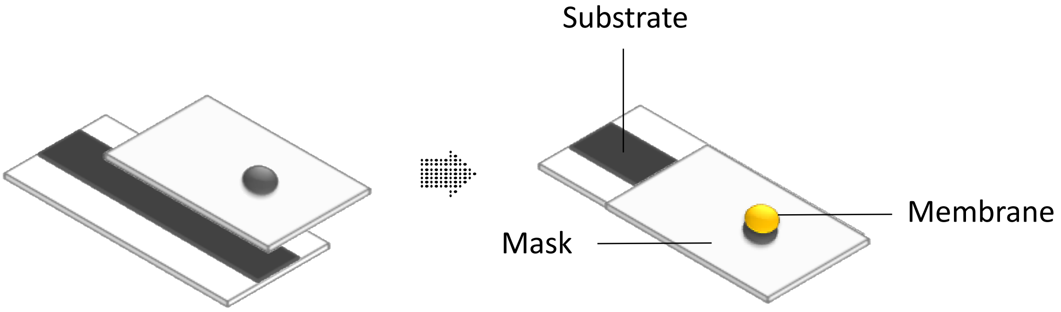

Figure 3. Handmade solid-contact paper-based ion-selective electrodes fabrication procedure.

2.4. Lithium ion-selective membrane

Starting with the most used lithium ISM (Table 6), its composition has been optimized to obtain the best performance.

Table 6. Standard Li^+ ISM composition[4].

Li^+ ISM
1.5 mg Li^+ ionophore
0.5 mg KTPFPhB
28 mg PVC
68.7 mg o-NPOE
1.5 mg TOPO
1 mL THF

Lithium ISM without the addition of TOPO shows a Nernstian slope of 59.7 mV/decade[8], unfortunately it has a low selectivity coefficient of lithium towards sodium. The addition of TOPO increases significatively this selectivity, but additionally causes a decrease in sensitivity. For that reason, the amount of TOPO added is critical for the membrane performance. The variability on the membrane composition due to the irreproducibility of weights makes it difficult to have the exact components ratio for the best membrane performance. Different membrane compositions have been compared to obtain the best sensitivity (the closest to Nernstian) and selectivity ($K_{Li,Na} < -2.46$). Therefore, five parameters of the lithium ISM have been optimized:

1) **Polymer/plasticiser ratio**. Two PVC/o-NPOE amounts have been tested; 28 mg PVC with 68.7 mg o-NPOE and 56 mg PVC with 137.4 mg o-NPOE, maintaining the rest of the components as indicated in Table 6. A volume of 6 µL of membrane in successively amounts of 3 µL is drop casted onto the carbon substrate paper-based electrodes.

2) **Ion exchanger type**. The most known lithium ISM composition is based in KTPFPhB ion exchanger. However, same standard membrane composition varying the ion exchanger by LiTPFPhB has been tested. A volume of 6 µL of membrane in successively amounts of 3 µL is drop casted onto the carbon substrate paper-based electrode.

3) **Membrane volume**. After the previous optimizations, the membrane volume is optimized. Three different membrane volumes are tested: 6, 9 and 12 µL of membrane are drop casted in successively amounts of 3 µL onto the carbon substrate paper-based electrode.

4) **Ion exchanger quantity**. Following the literature[9], different ion exchanger amounts in the lithium ISM are compared. Three different ion exchanger - ionophore molar ratios have been tested (14.6, 29.2 and 70.5%), maintaining the same composition for the rest of the components. See the composition in Table 7. A volume of 9 µL of membrane in successively amounts of 3 µL is drop casted onto the carbon substrate paper-based electrodes.

Table 7. Ion exchanger - ionophore molar ratios.

Ion exchanger (mg)	Ionophore (mg)	Molar ratio (%)
0.5	1.5	14.6
1	1.5	29.2
2.4	1.5	70.5

5) **TOPO amount**. After lithium ISM optimization, TOPO amount has been increased to 2 mg to study if higher selectivity is obtained without a significant decrease of sensitivity. A volume of 9 µL of membrane in successively amounts of 3 µL is drop casted onto the carbon substrate paper-based electrodes.

The membranes compositions have been studied with lithium calibrations in both water and artificial serum from 10^{-7} M to 10^{-2} M of Li^+, comparing sensitivity (slope), linear range (LR), limit of detection (LOD) and the selectivity coefficient of the most significant interferent ($K_{Li,Na}$). All the experiments are done with three replicates of the electrodes. The optimized lithium ISM membrane has been characterized by doing lithium calibrations in water and artificial serum. The selectivity coefficients of the most significant interferents (Ca^{2+}, Mg^{2+}, K^+, NH_4^+ and Na^+, from more to less interferent) have been calculated.

Selectivity coefficients (K_{IJ}) are calculated though the FIM (fixed interferent method)[10], following the next expression:

$$\log K_{IJ} = \log a_I\,(LOD) / a_J\,(BG)^{zI/zJ} \qquad \text{Equation 3}$$

Concentration is used instead of activity due to mathematics simplification. All the interferents are added to a constant concentration of 0.01 M, corresponding to the background (BG) value. The minimum selectivity coefficients are calculated having into account the maximum value of interferent in real sample. Table 8 displays the normal concentration range of interferent in blood. The minimum selectivity coefficients required for real samples measurements are shown in Table 9 and the way to calculate them is shown in Equation 4.

Table 8. Normal concentration of ions in blood.

Analyte	**Concentration (mM)**
Mg^{2+}	0.62 – 0.99
Ca^{2+}	2.2 – 2.6
K^+	3.5 - 5
NH_4^+	23 - 47
Na^+	136 - 145

Equation 4. Calculation of the minimum selectivity coefficient required in Li^+ ISM for real samples measurements.

$$\text{min. } K_{Li,Na} = \log\,[\text{min. } Li^+ \text{ normal value}] / \log\,[\text{max. } Na^+ \text{ normal value}]$$

$$\text{min. } K_{Li,Na} = \log 0.0005 / \log 0.145$$

$$\text{min. } K_{Li,Na} = -\,2.46$$

Table 9. Required selectivity coefficients in Li^+ ISM for real samples measurements.

Analyte	**Minimum $K_{Li,interf.}$**
Mg^{2+}	- 0.30
Ca^{2+}	- 0.72
K^+	- 1.00
NH_4^+	- 1.97
Na^+	- 2.46

One of the objectives of this thesis is the prediction of lithium in real samples with a complete paper-based sensor; integrating the characterized lithium ISM and the PVB reference electrode optimized during the work placement. However, the obtention of blood samples from people undergoing lithium treatment during the bachelor's thesis period was not possible due to the small availability from hospitals. Artificial serum samples measurements have been the alternative to study the performance of lithium ISM in samples prediction.

Preparation of the paper potentiometric cell can be seen in Figure 3, although some slight differences must be considered. In this case, the sensor integrates the working electrode (WE) and the reference electrode (RE). For the WE, a volume of 9 µL of lithium ISM in successively amounts of 3 µL is drop casted onto the carbon substrate electrode. For the RE a volume of 2.7 µL from a 360 mg/mL NaCl solution is drop casted onto the Ag/AgCl substrate and allowed to dry at least for 2 hours. The salt is covered with a volume of 6 µL of PVB membrane, drop casted in successively amounts of 3 µL and allowed to dry. Before the use of RE, some conditioning in 1 mM LiCl solution is needed to moisten the membrane.

Two lithium standards of 0.32 and 3.2 mM are used to obtain the calibration curve used in the prediction. Standards concentrations are based on the therapeutic range lithium values (0.5 to 1.1 mM). After the calibration, artificial serum samples with lithium concentrations inside the therapeutic range are measured.

2.5. Super-Nernstian sensitivity

Hypothetically, an array of sensors connected between them can multiply the sensitivity by the number of sensors in the system. This hypothesis has been experimentally tested with an array of 10 sensors, connecting the RE of the first sensor to the reference channel of the potentiometer, then the WE is connected to the RE of the second sensor and so on; the WE of the last sensor is connected to the working channel of the potentiometer. Moreover, WEs of the 1st, 3rd, 5th and 7th sensors have been connected to the potentiometer in order to compare the sensitivity depending on the number of electrodes connected. The experimental setup can be observed in Figure 4.

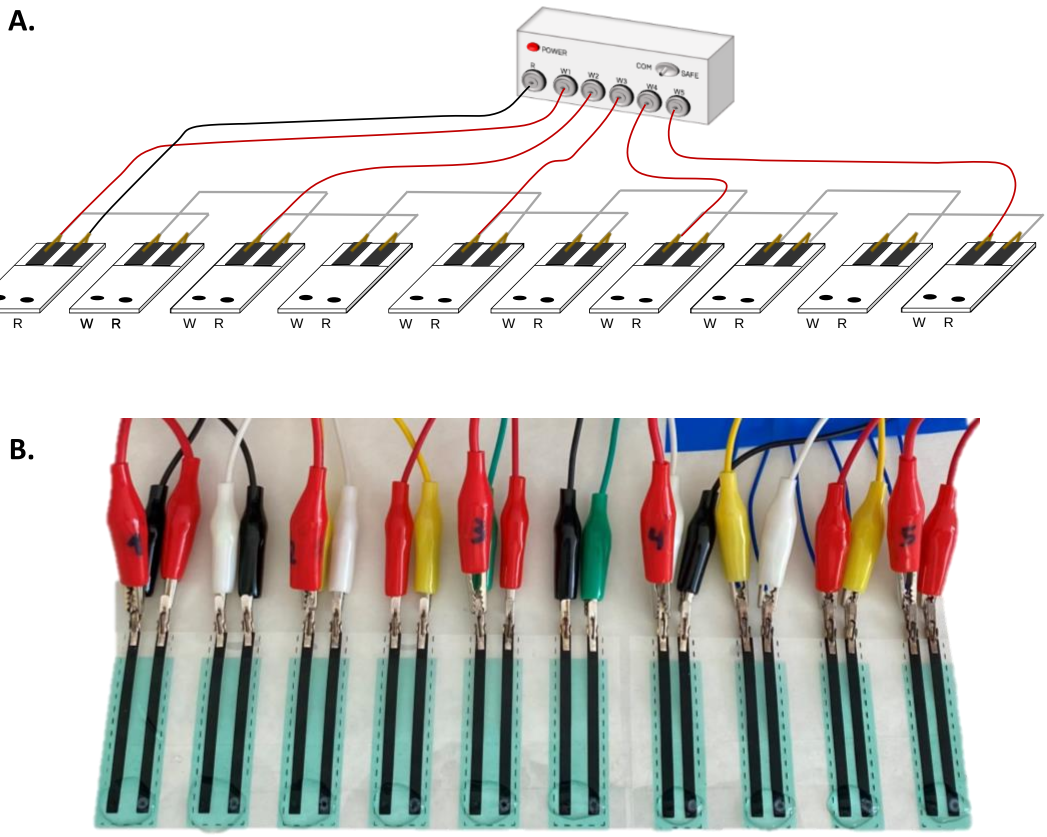

Figure 4. A) Super-Nernstian experimental shceme and B) Super-Nernstian experimental setup.

The first approach has been performed with sensors based on a PVC RE, because this RE does not need previous conditioning. A volume of 6 µL of PVC membrane in successively amounts of 3 µL is drop casted onto the carbon substrate. K^+ ISM is used for the WE instead of the Li^+ IMS (since the Li^+ ISM was not already optimized). A volume of 9 µL of K^+ ISM in successively amounts of 3 µL is drop casted onto the carbon substrate. Potassium calibration in artificial serum has been performed to see the response of the number of electrodes connected, and thus demonstrate that the sensitivity is multiplied by the number of sensors in the network. Moreover, predictions of unknown artificial samples have been carried out to study if precision improves in a system with several sensors connected.

Prediction measurements are done with a different sensor system. K^+ ISM has been used in the fabrication of the WE. However, the RE used is based on a PVB reference membrane, because it shows better performance as a RE, therefore more precise and accurate predictions are obtained. Fabrication of PVB reference electrode is indicated in the previous section.

2.6. Calibration-free electrodes

The need of sensors calibration in potentiometry is a barrier to extend potentiometric detection to home-care devices. To simplify the use of these sensors, some approaches to make calibration-free electrodes have been studied during the last years. In this section, two main approaches[6,7] already reported by different articles have been reproduced to obtain electrodes with similar standard potential and evaluate how to integrate these systems in the paper-based potentiometric Li^+ sensors.

Silver halide-based membranes

Following the literature[6,11] some calibration-free reference electrodes based in a redox buffer membrane of silver halide and the corresponding salt of the halide have been prepared. Membrane compositions can be seen in Table 10.

Table 10. Silver halide-based membranes compositions.

Components	REF-Cl-photo	REF-Br-photo
KCl	161.8 mg	-
NaBr	-	222.7 mg
AgCl	62.2 mg	-
AgBr	-	81.3 mg
DOS	123.6 mL	123.6 mL
PVC	74.4 mg	74.4 mg
THF	1.5 mL	1.5 mL

To prepare these membranes, the silver halide and the respective salt are mixed and exposed to light for 24 hours to produce photochemical decomposition of the silver halide forming solid silver, needed to have the redox buffer. A solution with DOS and PVC in THF is prepared and mixed with the AgX/salt decomposed solid.

The literature refers to glassy carbon (GC) substrate covered with electrodeposited silver. However, Ag/AgCl substrate, silver substrate and electrodeposited silver onto carbon substrate are also tested in paper-based electrodes. Electrodeposition of silver on GC electrodes and on paper-based carbon substrate is done in 0.05 M $AgNO_3$, using a silver chloride electrode Ag/AgCl/sat. KCl with a salt bridge filled with 1 M KNO_3 as a RE. Deposition potential was adjusted to a current density of - 0.21 mA with a deposition time of 1000 s. Before the electrodeposition, GC are polished with aluminium oxide and sonicated in water. After electrodeposition, a volume of 50 µL of the membrane is drop casted onto the GC surface and allowed to dry for 24 hours. A volume of 15 µL of membrane in successively amounts of 3 µL is drop casted onto paper-based electrodes. To measure the potential reproducibility between electrodes, potential over time is recorded for triplicates of each electrode during the conditioning on 2M solution of the corresponding salt.

Reproducible standard potential of ISM can be obtained with the previous addition of the silver-halide based membrane sandwiched between the substrate and the ISM. To study the reproducibility of the potassium ISM, 25 µL of REF-Br-photo membrane is drop casted onto silver covered GC or 6 µL in successively amounts of 3 µL in silver covered paper-based carbon substrate electrodes and allowed to dry for at least 24 hours. To prepare solid contact ISEs, dry REF-Br-photo membrane is covered with 50 µL K^+ ISM in GC or 9 µL of K^+ ISM in successively amounts of 3 µL in paper-based electrodes. Potassium calibrations from 10^{-7} to 10^{-2} M KCl in water are carried out and the reproducibility and performance of the ISE was analysed.

Co(II) / Co(III) redox buffer complex

Following an already reported approach[7], $Co(phen)_3(TPFPB)_2$ / $Co(phen)_3(TPFPB)_3$ redox buffer complexes are added to potassium ISM to decrease the E^0 irreproducibility. The resulting membrane composition is shown in Table 11.

Table 11. Co(II)/Co(III) redox buffer K^+ ISM composition.

Co(II)/Co(III) redox buffer K^+ ISM
1.05 mg valinomycin
0.35 mg KTPFPB
6.76 mg $Co(phen)_3(TPFPB)_2$
9.20 mg $Co(phen)_3(TPFPB)_3$
32.97 mg PVC
66.06 mg o-NPOE
0.5 mL THF

A volume of 30 µL of membrane in successively amounts of 5 µL is drop casted onto a carbon substrate paper-based electrode. Conditioning in 1 mM KCl for 1 hour is needed prior to analysis. Potassium calibration from 10^{-7} to 10^{-2} M KCl in water is done to evaluate the performance and the standard deviation of the E^0 of the new potassium ISM composition. After one month of the membrane drop casting, the electrodes are measured for a second time to study if the standard potential value is maintained.

3. Results and discussion

3.1. Lithium ion-selective membrane

For the fabrication of lithium decentralized sensors, the simplification of the industrial fabrication process is really important. The reported[4] lithium ISM is drop casted onto carbon nanotubes (CNTs) substrate, which implies a more complex process. For that reason, the same membrane is used in carbon substrate to simplify the fabrication process of the sensors.

Table 12 shows the performance parameters of the standard lithium ISM (see the composition in Table 6) compared with the reported ones.

Table 12. Experimental *vs*. reported performance parameters of lithium ISM. Reported results are obtained using paper-based CNTs substrate. Experimental results are obtained using carbon substrate.

		Reported[4]	**Experimental**
H_2O	**Sensitivity (mV/decade a_{Li+})**	60 ± 1.4	49.2 ± 0.5
	LR (M)	3.6 x 10^{-5} - 10^{-1}	$10^{-4.5}$ - $10^{-2.0}$
	LOD (M)	1.1 × 10^{-5}	10^{-5}
Artificial Serum	**Sensitivity (mV/decade a_{Li+})**	57.3 ± 0.8	49 ± 1.2
	LR (M)	10^{-4} - 10^{-1}	$10^{-3.5}$ - $10^{-2.0}$
	LOD (M)	5.7 x 10^{-5}	5 x 10^{-5}
	Coefficient (log $K_{Li,Na}$)	-2.7 ± 0.4	-2.70 ± 0.01

As it can be seen in Table 12, worst performance of the lithium ISM is obtained when carbon substrate is used instead of CNTs. An optimization of the membrane composition is carried out to obtain good performance of the membrane without the use of CNTs.

3.1.1. Optimization

Polymer and plasticiser

Having into account that potassium ISM has the double amount of PVC and o-NPOE compared with the lithium one, a modification of the quantity of PVC and o-NPOE in the lithium ISM has been tested to evaluate how this affects the performance.

Table 13. Different polymer/plasticiser amount in membrane compositions.

		28 mg PVC 68.7 mg o-NPOE	56 mg PVC 137.4 mg o-NPOE
H_2O	**Sensitivity (mV/decade a_{Li+})**	50 ± 3	52 ± 1
	LR (M)	$10^{-4.5}$ - $10^{-2.0}$	10^{-4} - 10^{-2}
	LOD (M)	1.6×10^{-5}	3.2×10^{-5}
Artificial Serum	**Sensitivity (mV/decade a_{Li+})**	52 ± 3	55.4 ± 0.6
	LR (M)	$10^{-3.5}$ - $10^{-2.0}$	$10^{-3.5}$ - $10^{-2.0}$
	LOD (M)	10^{-4}	1.6×10^{-4}
	Coefficient (log $K_{Li,Na}$)	-2.4 ± 0.4	-2.2 ± 0.2

Table 13 displays the performance of the membrane depending on the proportion of the polymer and the plasticiser, maintaining the rest of the parameters with the original composition. The addition of extra amount of polymer and plasticiser do not imply a significant improvement on the performance. Notably, the selectivity coefficient of lithium towards sodium is fairly decreased, which is probably explained by the lower proportion of TOPO in this membrane. For that reason, the quantity of PVC and o-NPOE will not be modified.

Ion exchanger

Although lithium is the target analyte, the most widely used lithium ISM is based on a potassium-ion exchanger, for that reason the use of lithium-ion exchanger is compared with the previous one. As it is shown in Table 14, the performance of the membrane does not critically depend on the type of ion exchanger. However, the electrodes containing LiTPFPhB as ion exchanger shows better stability than with KTPFPhB, which must be previously conditioned until reaching stable potentials. This is because the membrane containing LiTPFPhB ion exchanger has already Li^+ ions in the cavities, meanwhile the KTPFPhB initially contains K^+ ions and needs some conditioning time to exchange the K^+ by Li^+ ions. For that reason, LiTPFPhB has been selected as ion exchanger.

Table 14. KTPFPhB *vs*. LiTPFPhB in Li^+ ISM.

		KTPFPhB	**LiTPFPhB**
H_2O	**Sensitivity (mV/decade a_{Li+})**	50 ± 3	50.8 ± 0.8
	LR (M)	$10^{-4.5}$ - $10^{-2.0}$	$10^{-4.5}$ - $10^{-2.0}$
	LOD (M)	1.6×10^{-5}	2×10^{-5}
Artificial Serum	**Sensitivity (mV/decade a_{Li+})**	52 ± 3	53.4 ± 0.5
	LR (M)	$10^{-3.5}$ - $10^{-2.0}$	$10^{-3.5}$ - $10^{-2.0}$
	LOD (M)	10^{-4}	7.9×10^{-5}
	Coefficient (log $K_{Li,Na}$)	-2.4 ± 0.4	-2.3 ± 0.1

Membrane volume

During the ion exchanger amount optimization (next section), some sensors with smaller hole diameter (2 mm) were also used and exhibited better performance. This suggests a thicker membrane leads to a better performance. All previous experiments were done with 6 µL membrane volume, therefore 9 µL membrane volume drop casted in successive amount of 3 µL was also tested with normal hole diameter (2.8 mm).

Table 15. Membrane volume comparison for 29.2 % ion exchanger – ionophore molar ratio lithium ISM (membrane composition studied in the next section).

		6 µL	**9 µL**
H_2O	**Sensitivity (mV/decade a_{Li+})**	50.9 ± 0.5	52.5 ± 0.1
	LR (M)	$10^{-4.5}$ - $10^{-2.0}$	$10^{-4.5}$ - $10^{-2.0}$
	LOD (M)	5×10^{-5}	1.3×10^{-5}
Artificial Serum	**Sensitivity (mV/decade a_{Li+})**	46.3 ± 0.2	52.3 ± 0.3
	LR (M)	$10^{-3.5}$ - $10^{-2.0}$	$10^{-3.5}$ - $10^{-2.0}$
	LOD (M)	4×10^{-4}	1.6×10^{-4}
	Coefficient (log $K_{Li,Na}$)	-2.06 ± 0.05	-2.50 ± 0.01

A higher membrane volume and therefore a thicker membrane gives a better performance of the membrane, with higher sensitivity and selectivity as indicated in Table 15. Once the lithium ISM has been optimized, a membrane volume of 12 µL is tested to see if even thicker membrane improves the performance, as it has been demonstrated previously. See the experimental results in Table 16.

Table 16. Membrane volume comparison for 70.5 % ion exchanger – ionophore molar ratio lithium ISM (membrane composition studied in the next section).

		9 µL	**12 µL**
H_2O	**Sensitivity (mV/decade a_{Li+})**	58.7 ± 0.6	55.7 ± 0.1
	LR (M)	$10^{-4.5}$ - $10^{-2.0}$	$10^{-4.5}$ - $10^{-2.0}$
	LOD (M)	10^{-5}	1.6×10^{-5}
Artificial Serum	**Sensitivity (mV/decade a_{Li+})**	51.3 ± 0.5	53 ± 2.5
	LR (M)	$10^{-3.5}$ - $10^{-2.0}$	$10^{-3.5}$ - $10^{-2.0}$
	LOD (M)	10^{-4}	10^{-4}
	Coefficient (log $K_{Li,Na}$)	-2.80 ± 0.03	-2.50 ± 0.03

When the volume is increased to 12 µL, selectivity and sensitivity shows slightly worst results. For that reason, a volume of 9 µL drop casted in successive amounts of 3 µL is selected as the optimized membrane volume.

Ion exchanger amount

Following the literature[9], it has been demonstrated that different ionophore-primary ions complexes are formed with different stoichiometries depending on the membrane composition. The increase of the ion exchanger amount in the membrane composition favours 1:1 analyte – ionophore interactions, limiting 1:2 interactions and leading to a higher sensitivity. Different ion exchanger amounts are tested to see how it affects in the membrane performance. The optimized Li^+ ISM with LiTPFPhB ion exchanger and 28 mg PVC / 68.7 mg o-NPOE has been selected. Three different ion exchanger – ionophore molar ratios have been tested (14.6, 29.2 and 70.5%). See the amounts in Table 7. As it can be seen in Table 17, the increment of ion exchanger improves the performance of the lithium ISM. Using a 70.5% of ion exchanger – ionophore molar ratio, the optimum results are obtained; sensitivity close to ideal Nernstian and the higher selectivity coefficient compared with all the previous membrane variations.

Table 17. Li^+ ISM performance varying ion exchanger – ionophore ratio.

		14.6 %	**29.2 %**	**70.5 %**
H_2O	**Sensitivity (mV/decade a_{Li+})**	50.8 ± 0.8	51.8 ± 0.8	58.7 ± 0.6
	LR (M)	$10^{-4.5}$ - $10^{-2.0}$	$10^{-4.5}$ - $10^{-2.0}$	$10^{-4.5}$ - $10^{-2.0}$
	LOD (M)	2×10^{-5}	1.6×10^{-5}	10^{-5}
Artificial Serum	**Sensitivity (mV/decade a_{Li+})**	53.4 ± 0.5	53.1 ± 0.8	51.3 ± 0.5
	LR (M)	$10^{-3.5}$ - $10^{-2.0}$	$10^{-3.5}$ - $10^{-2.0}$	$10^{-3.5}$ - $10^{-2.0}$
	LOD (M)	7.9×10^{-5}	1.3×10^{-4}	10^{-4}
	Coefficient (log $K_{Li,Na}$)	-2.3 ± 0.10	-2.50 ± 0.04	-2.80 ± 0.03

TOPO amount

The amount of TOPO in the already optimized lithium ISM has been increased to study whether higher selectivity is obtained without a critical decrease of sensitivity. Table 18 displays all the performance parameters comparing the use of 1.5 and 2 mg of TOPO.

Table 18. Performance of Li^+ ISM with different TOPO amounts.

		1.5 mg TOPO	**2 mg TOPO**
H_2O	**Sensitivity (mV/decade a_{Li+})**	58.7 ± 0.6	59 ± 2.2
	LR (M)	$10^{-4.5}$ - $10^{-2.0}$	$10^{-4.5}$ - $10^{-2.0}$
	LOD (M)	10^{-5}	10^{-5}
Artificial Serum	**Sensitivity (mV/decade a_{Li+})**	51.3 ± 0.5	52 ± 1.3
	LR (M)	$10^{-3.5}$ - $10^{-2.0}$	$10^{-3.5}$ - $10^{-2.0}$
	LOD (M)	10^{-4}	10^{-4}
	Coefficient (log $K_{Li,Na}$)	-2.80 ± 0.03	-3.0 ± 0.2

The use of a higher amount of TOPO (2 mg) implies a better selectivity coefficient of lithium towards sodium. Moreover, sensitivity is maintained contrary to the expected decrease of it, as reported in the literature[11]. For that reason, the previous optimized lithium ISM with 2 mg of TOPO is selected as the composition giving the best performance. Next section includes the characterization of this membrane.

3.1.2. Characterization

The optimized Li^+ ISM (see composition in Table 19) is characterized as described in the experimental part. Details of time-trace response of lithium-paper electrode and the corresponding calibration plot can be seen in Figure 5. All the performance parameters are indicated in Table 20.

Table 19. Li^+ optimized ISM.

Li^+ ISM
1.5 mg Li^+ ionophore
2.4 mg LiTPFPhB
28 mg PVC
68.7 mg o-NPOE
2 mg TOPO
1 mL THF

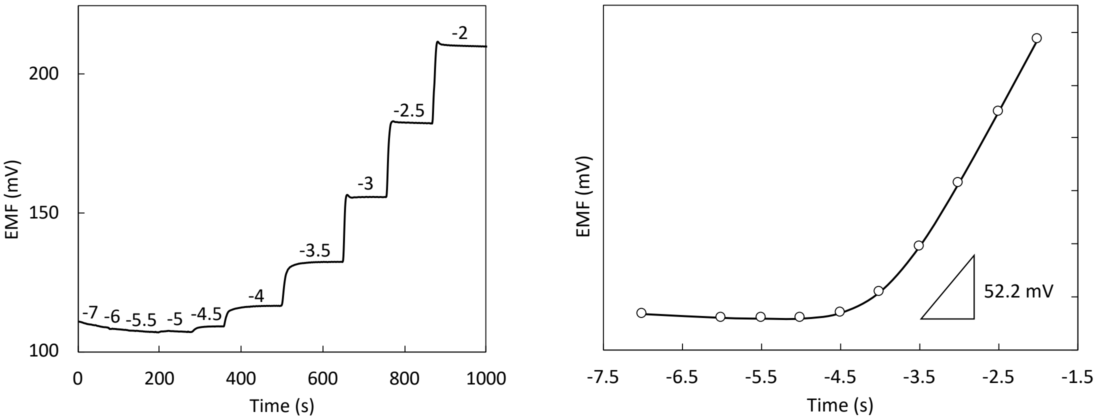

Figure 5. Potentiometric time trace graph at the left and the corresponding lithium calibration curve in artificial serum at the right for lithium ISE.

The new membrane composition shows the highest sensitivity, 59 mV/decade in water and 52 mV/decade in artificial serum, and the best selectivity coefficient of lithium towards sodium. Moreover, all the rest of the coefficients are smaller than the minimum required to be selective enough to predict lithium in real samples.

Table 20. Li^+ optimized ISM characterization. Minimum required selectivity coefficients are indicated in brakets.

		H_2O	**Artificial serum**
Sensitivity (mV/decade a_{Li+})		59 ± 2.2	52 ± 1.3
LR (M)		$10^{-4.5}$ - $10^{-2.0}$	$10^{-3.5}$ - $10^{-2.0}$
LOD (M)		10^{-5}	10^{-4}
Selectivity coefficients	**$K_{Li,Ca}$ (-0.72)**		-3.6 ± 0.1
	$K_{Li,Mg}$ (-0.30)		-3.7 ± 0.1
	$K_{Li,K}$ (-1.00)		-2.7 ± 0.1
	K_{Li,NH_4} (-1.97)		-2.7 ± 0.1
	$K_{Li,Na}$ (-2.46)		-3.0 ± 0.2

3.1.3. Real samples measurements

Figure 6 displays the prediction of artificial serum samples using the optimized lithium ISM as WE and the PVB reference membrane as RE. Sensors are both precise and accurate enough for the prediction of artificial serum samples. In a graph representing measured *vs.* theoretical lithium values, the slope closer to 1 and intercept closer to 0 indicates the best predictions. In this case, values of slope and intercept near to the previous mentioned are obtained.

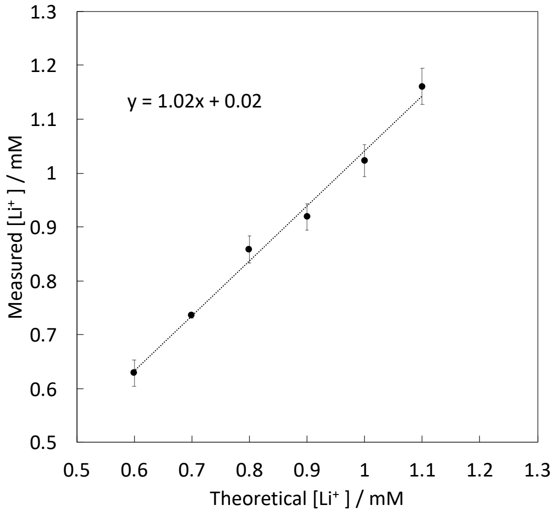

Figure 6. Prediction of lithium concentrations (mM) in artificial serum samples using the optimized lithium ISM.

Further steps include the integration of the PVB reference membrane and lithium ISM in an already fabricated screen-printed base for real samples predictions. This sensor is adapted to be measured in the "CKDSens device" (Figure 7). A preliminary potentiometric device to be commercialised in the future. This will be used by patients with therapeutic lithium treatments, to facilitate the lithium analysis at the patient's home.

Figure 7. CKDSens device, standards and sensor.

"CKDSens device" performs the potentiometric determination of lithium in real samples following a simple methodology. This device has a screen where all the steps are indicated. Two standards of 0.32 mM and 3.2 mM are used to make the calibration curve, then a drop of blood is placed on the sensor to obtain the predicted value. This device will be validated using real lithium samples.

3.2. Super-Nernstian sensitivity

Potassium calibration in artificial serum with a set of sensors connected can be seen in Figure 8. Sensitivity is multiplied by the number of sensors connected, without decreasing the linear range (from 10^{-4} to 10^{-2} M) and the limit of detection (2.2×10^{-5} M). Sensitivity for the different number of sensors and the individual sensitivity for each of them can be seen in Table 21. Calibrations of potassium in the presence of different interferents are also performed, demonstrating that selectivity coefficients do not vary with the number of sensors connected, as shown in Table 22.

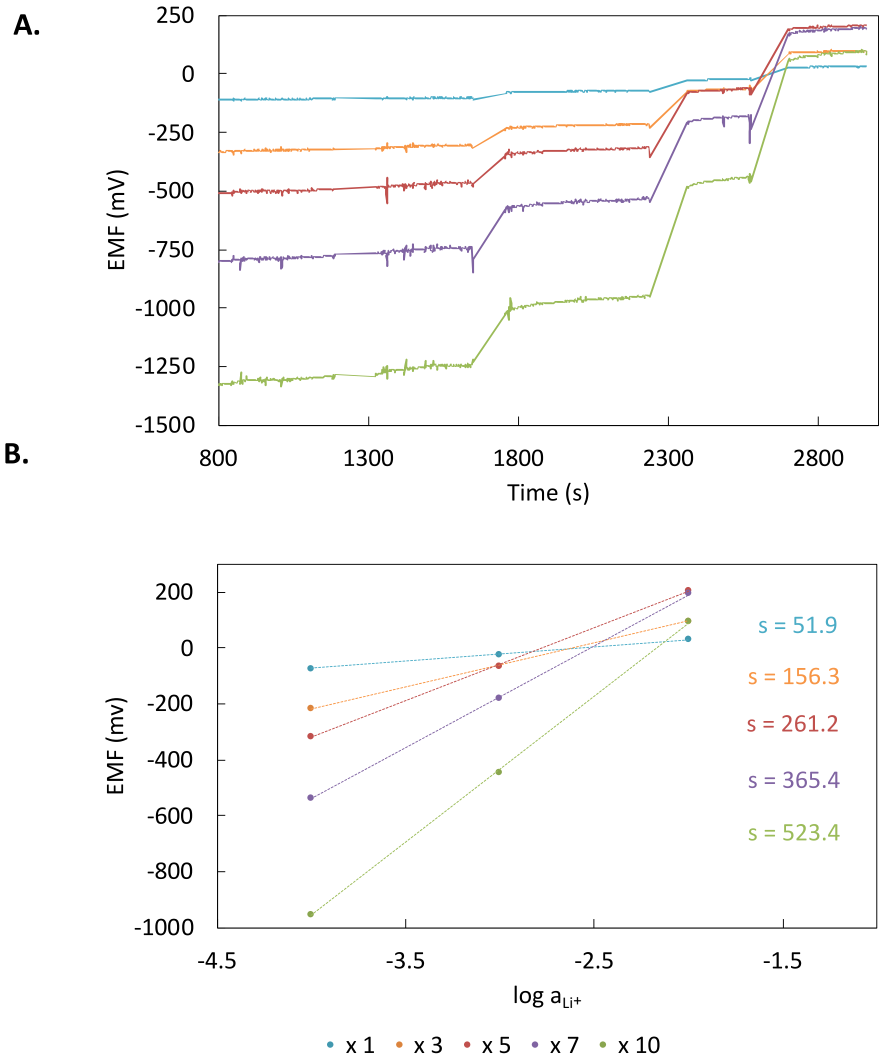

Figure 8. A) Time-trace graph of potassium calibration in artificial serum and B) Potassium calibration curve within the linear range (s corresponds to sensitivity, mV/decade). In both figures the different responses for 1, 3, 5, 7 and 10 sensors connected are shown.

Table 21. Sensitivity for different number of electrodes connected.

Sensors connected	**Sensitivity (mV/decade)**	**Individual sensitivity (mV/decade)**
1	52.1	52.1
3	156.7	52.2
5	262.0	52.4
7	366.4	52.3
10	524.5	52.4

Table 22. Mean selectivity coefficients of 1, 5 and 10 sensors connected.

$K_{K,Na}$	K_{K,NH_4}	$K_{K,Ca}$	$K_{K,Mg}$	$K_{K,Li}$
-2.65 ± 0.03	-2.26 ± 0.03	-4.73 ± 0.05	-4.89 ± 0.03	-3.82 ± 0.07

Predictions of unknown artificial serum samples are done with an array of 6 sensors connected. 1 mM and 10 mM K^+ standards are used for the construction of the calibration curve. Then, artificial serum sample is measured to predict the K^+ value. Results are shown in Figure 9.

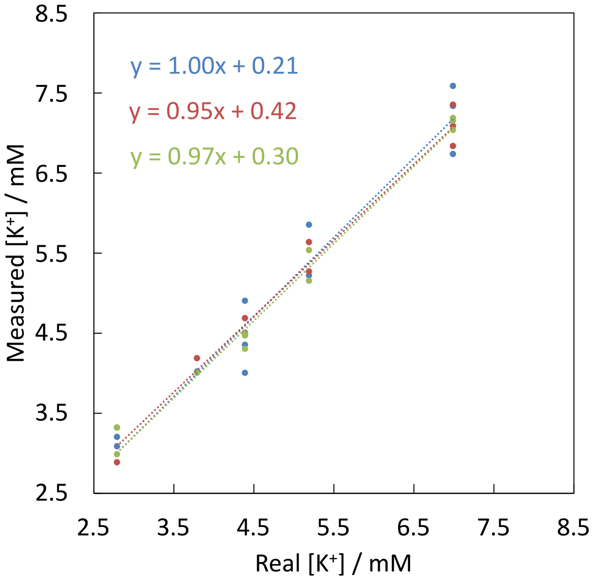

Figure 9. Theoretical *vs*. measured K^+ concentration in unknown artificial serum samples with 1 (blue), 3 (red) and 6 (green) sensors connected.

Although sensitivity is multiplied by the number of sensors connected in the array, the prediction error is also multiplied. Slopes closer to 1 and intercepts closer to 0 reflects the best results. In this case, the best predictions are obtained with a single sensor. Increasing the number of sensors increases the global standard error because the individual error of all connected sensors is added in the final prediction. Random accuracy and precision are obtained with this method, depending on both the individual sensor performance and the connection order. If the first sensor of the array has a lower error than the others (as in this case), increasing the number of sensors in the array moves away from 1 the slope, as it can be observed in Figure 9.

Hypothetically, if the first sensor has a worst performance, the prediction will be slightly improved with the increase of the number of sensors in the array, since the mean error of the overall network will be lower than the individual error of the first sensor. Therefore, in this mentioned case, accuracy and precision will improve with higher number of sensors decreasing the mean prediction error.

Concluding, even though sensitivity is increased using more than one sensor in the array, this methodology is not efficient to improve the precision in real samples measurements. In the literature[12], super-Nernstian sensitivity is achieved with the use of multiple WE and a single RE modifying the electronics. However, in this case, the use of more than one sensor, implies the increase of sensitivity and error at same proportion. Deeply research must be done in order to solve this limitation.

3.3. Calibration-free electrodes

Different approaches found in the literature are reproduced to evaluate which strategy can be used in the construction of lithium calibration-free electrodes. During this section, the integration of some redox buffer species has been studied to be introduced in the potassium ISM and once obtained the best methodology in the lithium ISM.

Silver halide-based membranes

The integration of a redox buffer membrane containing silver halide and the corresponding salt below the potassium ISM, leads to the possibility of obtaining calibration-free ISEs with the same standard potential.

Different substrate variations of the reference calibration-free electrodes reported[6] are measured. Electrodeposited (ED) silver in GC and in paper-based carbon substrate is studied. Moreover, Ag and Ag/AgCl paper-based substrates are also tested using the reference membranes. Standard deviation of the potentiometric response between electrodes after short conditioning in 2M of the corresponding salt (KCl or NaBr) can be seen in Figure 10. As it can be observed, the calibration-free reference electrodes displaying higher potential reproducibility are the ones composed by the REF-Br-photo membrane drop casted onto GC with electrodeposited silver, showing a standard deviation of 1.2 mV between a batch of three electrodes. When the same membrane is measured in Ag/AgCl paper-based substrate the standard deviation between electrodes increases considerably. In addition, when Ag paper-based substrate is used instead of an electrodeposited layer of silver onto GC, higher irreproducibility of the response of electrodes is also observed.

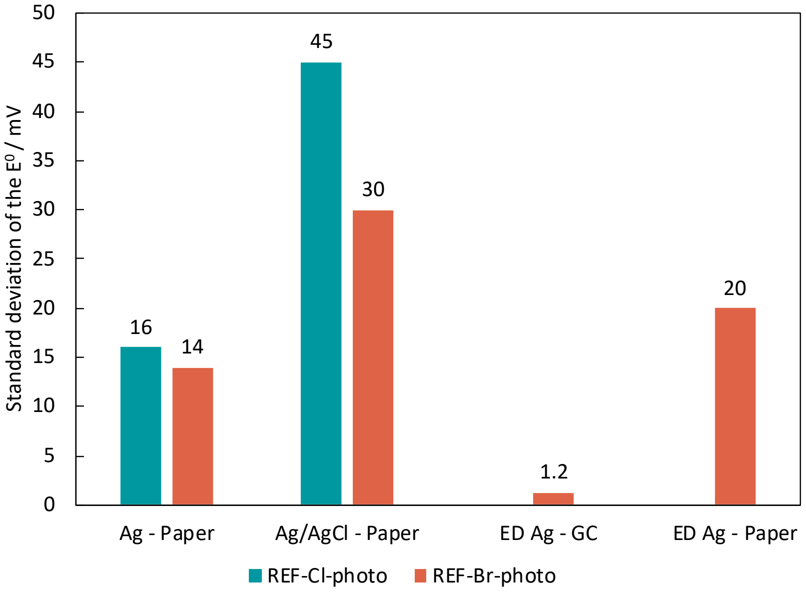

Figure 10. Standard deviation of potentiometric response between three electrodes. At the bottom it is indicated the substrate used for the construction of the electrodes. The colours indicate the different membranes variation.

REF-Br-photo membranes drop casted onto GC with electrodeposited silver, covered with potassium ISM imply even higher standard deviation of the standard potential than for normal potassium ISM. Moreover, irreproducible and smaller values of sensitivity compared with the normal potassium ISM are obtained for potassium calibration in water, as shown in Figure 11.

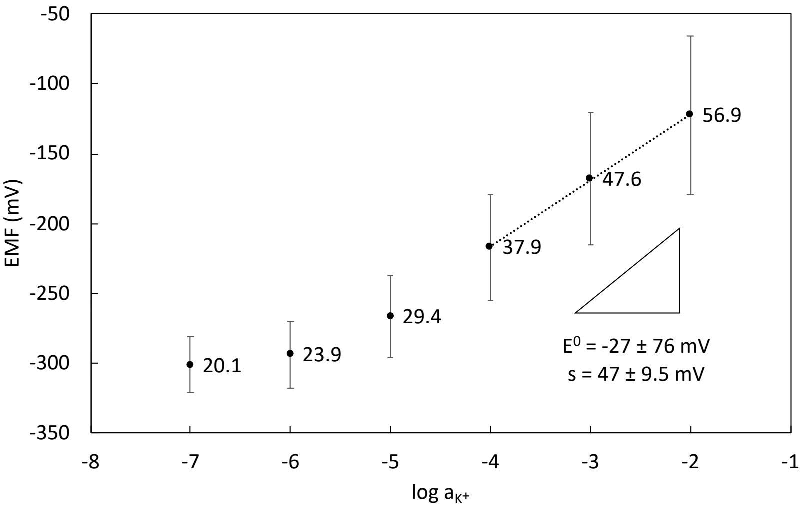

Figure 11. K^+ calibration in water of REF-Br-photo membrane covered with K^+ ISM in GC with electrodeposited Ag. Values shown represent the standard deviation between the three electrodes for each concentration. E^0 corresponds to the standard potential and “s” to the slope.

Silver has also been electrodeposited on carbon paper-based substrate and high standard deviation among electrodes (± 20 mV approximately) has been obtained for both REF-Br-photo membrane and for REF-Br-photo membrane covered with K^+ ISM. Concluding, reference calibration-free electrodes results have been reproduced. However, when the ion-selective membrane is added to the system, the performance changes drastically, with high irreproducibility of both sensitivity and standard potential.

Co(II) / Co(III) redox buffer complex

The addition of a lipophilic redox buffer specie in the ISM leads to the possibility of obtaining the same E^0 without variating the membrane performance. The $TPFPB^-$ salts of $[Co(phen)_3]^{3+}/[Co(phen)_3]^{2+}$ are selected because of their high electrochemical stability. Therefore, they are introduced in the potassium ISM to study the reproducibility of E^0.

Conditioning is needed to obtain similar potential value between electrodes. After 1 hour of conditioning in 1 mM KCl, electrodes exhibit a standard deviation of ± 1.7 mV. When potassium calibration is performed in water, the electrodes exhibit an E^0 of 311 ± 2.1 mV. The sensitivity of this new membrane is 56.0 ± 0.2 mV/decade, a value slightly smaller than for the normal potassium ISM composition.

When electrodes are allowed to dry and measured after one-month without conditioning, the standard deviation between electrodes increases to ± 5 mV approximately. However, after 1 hour conditioning, they show the same behaviour as previous experiments with a different standard potential value of 347 ± 2.6 mV. Potassium calibration in water is shown in Figure 12.

After the second potassium calibration, the electrodes are not dried and instead, are left in 1 mM potassium solution to study the potential stability. After 72 hours of conditioning, electrodes show an EMF of 20 mV more than the previous measurement. However, the standard deviation between electrodes is maintained in ± 1.7 mV.

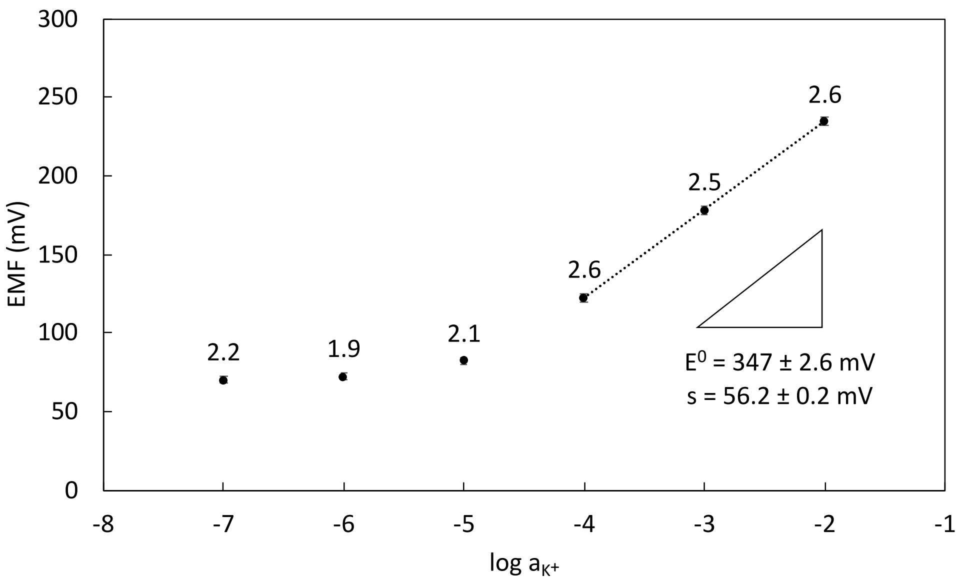

Figure 12. Potassium calibration in water of 3 K^+ ISM with the redox buffer complex. At the top of each bar, the standard deviation between electrodes for each concentration is displayed. E^0 corresponds to the standard potential and "s" to the slope.

"Compound R" for calibration-free electrodes

The two approaches previously mentioned lead to calibration-free electrodes with small standard deviation of the E^0. However, the silver-halide based membrane has only succeed as a reference membrane and cobalt salt redox buffer is really difficult to synthesize. For that reason, a deep bibliographic research has been done though all species used in calibration-free electrodes and a new specie called "compound R" has been selected to be used in this section for the obtention of calibration-free electrodes.

"Compound R" is an organic compound characterized by its high lipophilicity and polarity, usually used as stabilizing agent. Presumably, this compound will be axially coordinated with the ionophore stabilising the crown ether leading to calibration-free electrodes. To test this hypothesis, "Compound R" is firstly added to the standard potassium ISM composition (Table 5). Potassium calibration in water is carried out to study the reproducibility of the standard potential (Figure 13). The addition of "Compound R" in the potassium ISM implies an improvement in the reproducibility of the standard potential value between electrodes constructed in the same day. An E^0 of 431 ± 1.5 mV is obtained with a sensitivity of 52 mV/decade, high reproducible results compared with the normal potassium ISM, providing an E^0 with a standard deviation of approximately ± 41 mV. However, a decrease in sensitivity is produced (having a sensitivity of approximately 58 mV/decade in normal potassium ISM).

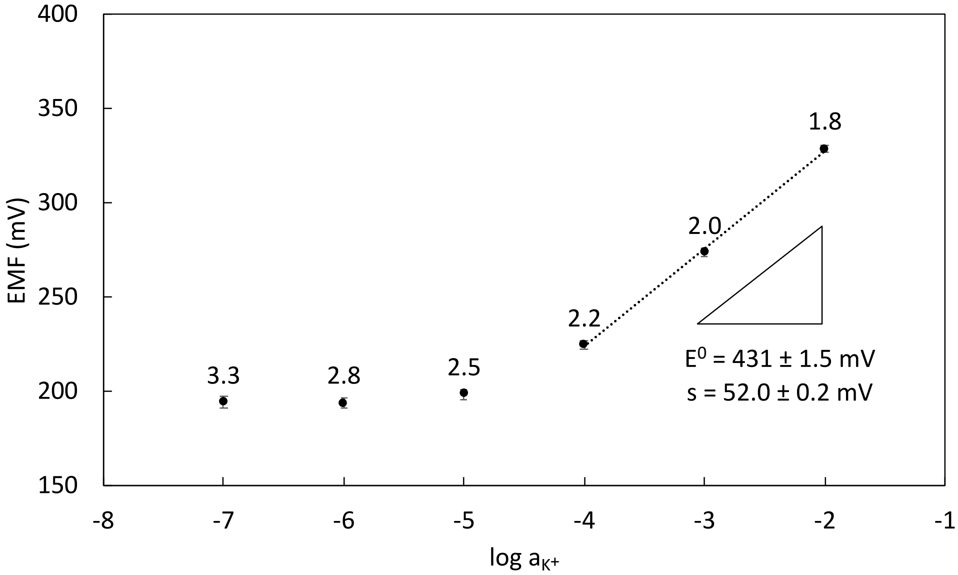

Figure 13. Potassium calibration in water of 3 electrodes with the addition of “Compound R” in the potassium ISM. At the top of each bar, the standard deviation between electrodes for each concentration is displayed. E^0 corresponds to the standard potential and “s” to the slope.

Once “Compound R” is verified to work properly, this specie has been integrated in the optimized lithium ISM. Lithium ISM containing “Compound R” shows the same behaviours as the potassium one. Table 23 displays the standard potential of three baches of electrodes fabricated on different days. Electrodes built on different days cannot be compared between them because higher standard deviation of E^0 are obtained.

Table 23. Standard deviation of E^0 for three different batches of Li^+ ISEs containing “Compound R”

	Batch 1	**Batch 2**	**Batch 3**
E^0	350 ± 1.6	361 ± 1.2	334.3 ± 0.5

Comparing the three different batches of electrodes previously mentioned, a E^0 of 348 ± 13.3 mV is obtained, a value far from the expected one for calibration-free electrodes. However, the fabrication of these sensors at large scale will only involve the pre-calibration of some of the electrodes built on the same day, to know the standard potential of each batch of sensors fabricated.

Moreover, a preliminary observation shows that the addition of higher amount of “Compound R” involves that the standard potential between different days constructed electrodes is maintained. 13 electrodes fabricated on 6 different days display a E^0 of 312 ± 5.5 mV. Although standard deviation is higher than for a batch of electrodes fabricated on the same day, it is an interesting approach to continue studying. All seems to indicate that the amount of “Compound R” can have some effect in the high reproducibility of the standard potential.

Conclusions

The principal objectives of this thesis have been achieved. A lithium ISM has been developed with the optimum sensitivity and selectivity to obtain good predictions of artificial serum samples. During the lithium ISM optimization, it is observed that the amount of polymer and plasticiser do not have a high influence in the membrane performance. However, the ion exchanger type and quantity have an important effect. LiTPFPhB has been selected as ion exchanger and the best performance is obtained with the highest ion exchanger – ionophore ratio, obtaining higher selectivity and sensitivity compared with smaller ratios compositions. Theoretically 1:1 analyte – ionophore interactions are favoured increasing the ion exchanger amount, limiting 1:2 interactions and therefore better sensitivity is obtained. The increment of TOPO amount implies better selectivity with the same sensitivity, although critical decrease of sensitivity was expected.

Super-Nernstian sensitivity has been obtained successfully. As observed in previous experiments, connecting an array of sensors multiplies the sensitivity by the number of sensors connected. However, it does not always imply an improvement in samples predictions as expected. Meanwhile sensitivities are multiplied, the prediction error of sensors is also increased, having a higher prediction error with higher number of sensors connected. When the first sensor has a smaller prediction error than the rest, the predicted value is even worst for higher number of sensors in the array, compared with the prediction for only one sensor. However, when the first sensor has a high prediction error, the predicted value is slightly better for a higher number of sensors, because the mean error of the array becomes smaller than the error of the first one. For that reason, as prediction error cannot be controlled, it is not worth to have an array of sensors to have more precise predictions.

All the approaches of calibration-free electrodes tested provide partially good results. Silver halide-based membranes only work as reference membrane using electrodeposited silver onto GC, as reported. However, when Ag or Ag/AgCl substrates are tested to simplify the electrodes fabrication process, calibration-free electrodes are not obtained. This could be explained hypothetically due to the high control of the layer's deposition during the electrodeposition of silver in GC, having the exact amount of silver in the substrate with a controlled thickness. However, when Ag or Ag/AgCl ink is used in a paper filter as a substrate, the amount of silver in each electrode cannot be controlled, producing a high deviation in the electrode's potential. The same occurs when silver is electrodeposited in carbon paper-based electrodes. Carbon surface in paper-based electrode is not as regular and flat as GC, therefore irregular electrodeposition may be occurring, avoiding a homogeneous silver electrodeposition onto the carbon substrate as occurs in the GC. The cobalt redox buffer complex has also provided good results, but the synthesis of these salts is really complex.

Moreover, during the bibliographic research of a new specie to construct calibration-free electrodes, "Compound R" has been selected and tested. The first approach seems to indicate that "Compound R" can have some influence in the high reproducibility of the electrodes. The addition of this compound to the potassium and lithium ISM provides high reproducible standard potentials. Hypothetically, it could be explained because the stabilization through axial coordination of the ionophore with "Compound R" that implies a stable interphase boundary potential, implying a decrease of the standard deviation in the E^0 of intra-day fabricated electrodes. Moreover, the addition of higher amount of this specie implies the stability of the E^0 with a value of 312 ± 5.5 mV between different day fabricated sensors. There are no studies that demonstrate that theory, but this could be an interesting topic to study and understand better the membrane behaviour. This opens the door for the development of a new and simpler system of calibration-free ion-selective electrodes.

Future experiments will involve the addition of "Compound R" to the PVB reference membrane to test if potential reproducibility improves, since both WE and RE integrated in the sensor need to have high reproducible potential to avoid calibration in real samples predictions. However, if the theory of "Compound R" – ionophore interaction is accomplished, this specie is not going to have any positive effect in the reproducibility of the potential in PVB membrane since no ionophore is included in this membrane. Moreover, lithium real samples will be available in the next months and real samples predictions will be performed with the "CKDSens device".

Bibliography

(1) Bakker, E.; Bühlmann, P.; Pretsch, E. The Phase-Boundary Potential Model. *Talanta* **2004**, *63* (1), 3–20.

(2) Bartoszewicz, B.; Dąbrowska, S.; Lewenstam, A.; Migdalski, J. Calibration Free Solid Contact Electrodes with Two PVC Based Membranes. *Sensors and Actuators, B: Chemical* **2018**, *274* (April), 268–273.

(3) Duh, S.; Cook, J. D.; Laboratory Reference Range Volues. **2004**, *1* (November 2003), 96–112.

(4) Novell, M.; Guinovart, T.; Blondeau, P.; Rius, F. X.; Andrade, F. J. A Paper-Based Potentiometric Cell for Decentralized Monitoring of Li Levels in Whole Blood. *Lab on a Chip* **2014**, *14* (7), 1308–1314.

(5) Liu, M.; Marshall, A.; Mccolgan, P. Ur Na L Pr Oo F. *Journal of Multinational Financial Management* **2020**, 100663.

(6) Bartoszewicz, B.; Sylwia, D.; Lewenstam, A.; Migdalski, J. Sensors and Actuators B : Chemical Calibration Free Solid Contact Electrodes with Two PVC Based Membranes. **2018**, *274* (July), 268–273.

(7) Zou, X. U.; Cheong, J. H.; Taitt, B. J.; Bu, P. Solid Contact Ion-Selective Electrodes with a Well-Controlled Co(II)/ Co(III) Redox Bu Ff Er Layer. **2013**, 85, 9350-9355.

(8) Kang, Y. R.; Lee, K. M.; Nam, H.; Cha, G. S.; Jung, S. O.; Kim, J. S. Lithium Ion-Selective Electrodes Employing Tetrahydrofuran-Based 16-Crown-4 Derivatives as Neutral Carriers. *Analyst* **1997**, *122* (11), 1445–1450.

(9) Miyake, M.; Chen, L. D.; Pozzi, G.; Bühlmann, P. Ion-Selective Electrodes with Unusual Response Functions: Simultaneous Formation of Ionophore-Primary Ion Complexes with Different Stoichiometries. *Analytical Chemistry* **2012**, *84* (2),

(10) Bakker, E.; Pretsch, E.; Bühlmann, P. Selectivity of Potentiometric Ion Sensors. *Analytical Chemistry* **2000**, *72* (6), 1127–1133.

(11) Coldur, F.; Andac, M. All-Solid-State Polyvinyl Chloride Membrane Lithium-Selective Electrode with Improved Selectivity and Its Application in Serum Lithium Assay. *Sensor Letters* **2011**, *9* (5), 1738–1744.

(12) Wang, C.; Yuan, H.; Duan, Z.; Xiao, D. Integrated Multi-ISE Arrays with Improved Sensitivity, Accuracy and Precision. *Scientific Reports* **2017**, *7* (February), 1–10.

APPENDICES

Appendix 1. Abbreviations

a_I (DL) – Activity of the primary ion in the detection limit

a_J (BG) – Activity of the interfering ion in the background solution

CNTs – Carbon nanotubes

E^0 – Standard potential

FIM – Fixed interference method

GC – Glassy carbon

ISE – Ion-selective electrode

ISM – Ion-selective membrane

$K_{I,J}$ – Selectivity coefficient of the I-ISE towards the interferent J

KTClPB – Potassium tetrakis (4-chlorophenyl) borate

LOD – Limit of detection

NPOE – 2-nitrophenyl octyl ether

PVC – Polyvinyl chloride

RE – Reference electrode

THF – Tetrahydrofuran

TOPO – Trioctylphosphine oxide

WE – Working electrode

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