Solid-State Reference and Ion-Selective Electrodes

– Towards Portable Potentiometric Sensing

Ulriika Vanamo (née Mattinen)

Laboratory of Analytical Chemistry
Process Chemistry Centre
Department of Natural Sciences
Åbo Akademi University
Åbo, Finland
2015

Ulriika Vanamo (née Mattinen)

Born 1983 in Åbo, Finland
Obtained her M.Sc. degree at
the Department of Natural Sciences
in Åbo Akademi University in 2008.
Joined the Laboratory of
Analytical Chemistry in 2007.

Solid-State Reference and Ion-Selective Electrodes

- Towards Portable Potentiometric Sensing

Ulriika Vanamo (née Mattinen)

Åbo Akademi
University

Laboratory of Analytical Chemistry
Process Chemistry Centre
Department of Natural Sciences
Åbo Akademi University
Åbo, Finland
2015

Supervised by

Professor Johan Bobacka
Laboratory of Analytical Chemistry
Åbo Akademi University
Åbo, Finland

and

Professor Andrzej Lewenstam
Laboratory of Analytical Chemistry
Åbo Akademi University
Åbo, Finland

Reviewer

Professor Konstantin N. Mikhelson
St. Petersburg State University
St. Petersburg, Russia

Reviewer and Opponent

Professor Róbert E. Gyurcsányi
Budapest University of Technology and Economics
Budapest, Hungary

ISBN 978-952-12-3189-6
Painosalama Oy – Åbo, Finland 2015

To my mum and dad

Preface

This work was done at the Laboratory of Analytical Chemistry at Åbo Akademi University, as a part of the activities of the Process Chemistry Centre. Papers I and II were done within the MATERA ERA-NET project "Miniaturized all-solid-state sensors for trace analysis of substances relevant to health and welfare (MASTRA)" with collaborators from Dublin City University (DCU), Adaptive Sensors Group, National Centre of Sensor Research, and AGH University of Science and Technology in Krakow, Poland. The collaborators from these groups are gratefully acknowledged. The Graduate School of Chemical Sensors and Microanalytical Systems (CHEMSEM), Finnish Funding Agency for Technology and Innovations (TEKES, MASTRA 2084/31/06, FiDiPro – Finnish Distinguished Professor Programme Dnro 2920/31/08), Åbo Akademi University, Stiftelsen för Åbo Akademi, Waldemar von Frenckells Stiftelse, Oskar Öflunds Stiftelse, Magnus Ehrnrooths Stiftelse and Stiftelsen för teknikens främjande are gratefully acknowledged for financial support .

I always thought that when this moment would arrive, I would have so much to say, but now I feel surprisingly speechless. There is so much to be thankful for and so many people who have helped me in so many ways.

I will start by thanking my respected supervisors. When meeting new people in conferences, I feel so very proud to be able to say that I come from your group. Professor Johan Bobacka, thank you giving me the opportunity to work in the Laboratory of Analytical chemistry and letting me do research with your great ideas, giving me responsibility and trusting me. Thank you for keeping your door open and always being so calm and supporting.

Professor Andrzej Lewenstam, thank you for taking me on board to do my master thesis and the beginning of my PhD with the very exciting project on solid-state reference electrode. Thank you for the scientific inspiration and guidance, and for always encouraging me to do my best and no less. Thank you for taking me to church in Krakow and to my first conference, for all the very nice discussions on many topics and for widening my perspective on life.

I want to thank Professor Konstantin Mikhelson for reviewing my thesis and for giving valuable comments on it, and also for the good scientific discussions from which I have learned a lot. Thank you Professor Róbert Gyurcsányi for reviewing

my thesis, for accepting the assignment to be my opponent and especially for organizing the very best Matrafüred-conferences!

I also wish to thank M.Sc. Sylwia Strzałkowska for our collaboration and FM Sara Suominen for proofreading the manuscript.

I have been so fortunate to work in such an amazing atmosphere we share at the Analyten. We have been taking care of each other and sharing many things throughout the years. All the international visitors from all over the world we have had have been such richness in our group! Thank you ALL for the coffee breaks, Friday nights out and for the many memorable trips together. I sincerely want to thank all who have had the patience to speak Swedish with me!

Thank you professor Ari Ivaska for the supporting atmosphere and the wisdom you have spread in the group, professor Carita Kvarnström for the excellent teaching and kind support and professor Kalle Levon for the chance to work with new exciting topics.

Special thanks to my wonderful longtime office roommates, FD Anna Österholm and TD Maija Blomquist. You have set such an example for me. Thank you Jesus for being so caring and considerate person! Thank you Docent Tom Lindfors for being a Supertyyppi for all these years, Greg and Michal for being the seal-team, Lotta for appreciating Iron the Red Atom Molecule and Yasuhito for being the exquisite Santa Claus. Thank you Ning for being my life-advisor and coach and Masha for the lovely time together in and out of the lab. Thank you Zekra for all your kindness and Marceline for the joy you brought to the lab. Thank you Lassi, Sten and Paul for fixing all the things I broke, always accompanied by happy laughter.

These years of PhD have been significant in my life also outside science. I cannot believe how lucky I am to have so wonderful friends and extended family. Thank you Raikastajat for keeping the spirits high and supporting me even if I think I have failed to explain what I do at work. Thank you Girls, Matami Tatami and Princess Madeleine’s tiara that can even be used as a necklace, you are just *perfect*. Thank you Henna and Hans for the special time I spent in your attic in Ermelo writing my first draft. I wish to personally thank every member of the Fjällräven-group for all the fun! Thank you girls of my Solu for sharing the past years, you are treasures! Selina, Mikko, Eino and Tikkis, thank you for being such wonderful persons and for always setting me an extra plate in your table.

Each of you, thank you for being in my life!

My deepest gratitude goes to my mum and dad. Thank you for the solid foundation, support, love and imagination you have given me, you are the best parents one could possibly have.

Finally I would like to thank my husband and best friend Akseli. Thank you for suffering the damage when something with my experiments went wrong, for providing me with topics to make friends with in conferences (opera and football) making me lunch and for telling me what I do is important. Thank you for the diversity, depth, music and richness you bring to my life, I love you.

In Åbo, on a windy and sunny day in April

Ulriika Vanamo

Try not. Do. Or do not. There is no try.
- Master Yoda

Abstract

Potentiometric sensors are very attractive tools for chemical analysis because of their simplicity, low power consumption and low cost. They are extensively used in clinical diagnostics and in environmental monitoring. Modern applications of both fields require improvements in the conventional construction and in the performance of the potentiometric sensors, as the trends are towards portable, on-site diagnostics and autonomous sensing in remote locations.

The aim of this PhD work was to improve some of the sensor properties that currently hamper the implementation of the potentiometric sensors in modern applications. The first part of the work was concentrated on the development of a solid-state reference electrode (RE) compatible with already existing solid-contact ion-selective electrodes (ISE), both of which are needed for all-solid-state potentiometric sensing systems. A poly(vinyl chloride) membrane doped with a moderately lipophilic salt, tetrabutylammonium-tetrabutylborate (TBA-TBB), was found to show a satisfactory stability of potential in sample solutions with different concentrations. Its response time was nevertheless slow, as it required several minutes to reach the equilibrium. The TBA-TBB membrane RE worked well together with solid-state ISEs in several different situations and on different substrates enabling a miniature design.

Solid contacts (SC) that mediate the ion-to-electron transduction are crucial components of well-functioning potentiometric sensors. This transduction process converting the ionic conduction of an ion-selective membrane to the electronic conduction in the circuit was studied with the help of electrochemical impedance spectroscopy (EIS). The solid contacts studied were (i) the conducting polymer (CP) poly(3,4-ethylienedioxythiophene) (PEDOT) and (ii) a carbon cloth having a high surface area. The PEDOT films were doped with a large immobile anion poly(styrene sulfonate) (PSS^-) or with a small mobile anion Cl^-. As could be expected, the studied PEDOT solid-contact mediated the ion-to-electron transduction more efficiently than the bare glassy carbon substrate, onto which they were electropolymerized, while the impedance of the PEDOT films depended on the mobility of the doping ion and on the ions in the electrolyte. The carbon cloth was found to be an even more effective ion-to-electron transducer than the PEDOT films and it also proved to work as a combined electrical conductor and solid contact when covered with an ion-selective membrane or with a TBA-TBB-based reference membrane.

The last part of the work was focused on improving the reproducibility and the potential stability of the SC-ISEs, a problem that culminates to the stability of the standard potential E°. It was proven that the E° of a SC-ISE with a conducting polymer as a solid contact could be adjusted by reducing or oxidizing the CP solid contact by applying current pulses or a potential to it, as the redox state of the CP solid-contact influences the overall potential of the ISE. The slope and thus the analytical performance of the SC-ISEs were retained despite the adjustment of the E°. The short-circuiting of the SC-ISE with a conventional large-capacitance RE was found to be a feasible instrument-free method to control the E°. With this method, the driving force for the oxidation/reduction of the CP was the potential difference between the RE and the SC-ISE, and the position of the adjusted potential could be controlled by choosing a suitable concentration for the short-circuiting electrolyte. The piece-to-piece reproducibility of the adjusted potential was promising, and the day-to-day reproducibility for a specific sensor was excellent. The instrument-free approach to control the E° is very attractive considering practical applications.

Referat

Potentiometriska sensorer är mycket attraktiva verktyg för kemisk analys p.g.a. att de är enkla, billiga och kräver bara lite ström. De används rutinmässigt inom klinisk analys samt inom miljöundersökning. Moderna tillämpningar inom dessa branscher kräver förbättringar i sensor-design och i deras prestationsförmåga, med tanke på att utvecklingen går mot bärbar diagnostik och självständiga mätstationer på avlägsna platser.

Målet för detta arbete var att utveckla vissa sensoregenskaper som för tillfället hindrar potentiometriska sensorer från att tas i bruk i de moderna tillämpningarna. Första delen av arbetet koncentrerade på att utveckla en fastfas-referenselektrod (RE) som kunde kombineras med redan existerande fastfas-jonselektiva elektroder till en miniatyriserbar potentiometrisk sensor. En polyvinylklorid (PVC) membran innehållande ett måttligt lipofilt salt, tetrabutylammonium-tetrabutylborat (TBA-TBB), uppvisade tillfredställande stabilitet i potential då provlösningens koncentration varierades. Det tog dock några minuter för denna referensmembran att nå stabilitet, vilket kan anses vara långsamt. Referensmembranen med TBA-TBB fungerade bra tillsammans med olika fastfas-jonselektiva elektroder i olika situationer och på olika substrater.

En fast kontakt som förmedlar jon-till-elektron-överföringen är en ytterst viktig komponent av en välfungerande fastfas-potentiometrisk sensor. Överföringsprocessen konverterar den joniska ledningsförmågan hos den jonselektiva membranen till en elektronisk ledningsförmåga hos den elektriska ledaren i mätkretsen. Processen undersöktes med hjälp av elektrokemisk impedans spektroskopi för olika fasta kontakter. En ledande polymer, poly(3,4-ethylienedioxythiophene) (PEDOT) med en stor, immobil motjon poly(styrensulfonat) (PSS^-) eller en liten mobil motjon Cl^- samt koltyg med stor ytarea inkluderades i denna undersökning. Alla undersökta fasta kontakter kunde förmedla jon-till-elektron-överföringen effektivare än en bar vitröst kolyta, på vilken PEDOT(PSS) samt PEDOT(Cl) elektropolymeriserades. Impedansen för fasta kontakter av ledande polymer berodde på samverkan av

mobiliteten av motjonen och jonen i provlösningen. Koltyget visade sig vara en ännu effektivare jon-till-elektron-överförare än PEDOT-filmerna, och det fungerade också bra som elektrodsubstrat på vilken både jonselektiva membran och TBA-TBB referensmembran kunde appliceras.

Sista delen av arbetet koncentrerade sig på at förbättra reproducerbarheten och stabiliteten av fastfas-jonselektiva elektroder, som påverkas mest av variationer i standardpotentialen E°. Det kunde bevisas, att E° av fastfas-jonselektiva elektroder med ledade polymer som fast kontakt kunde justeras genom att reducera eller oxidera den ledande polymeren med strömpulser eller applicerad potential. Orsaken till detta är att redox-tillståndet hos den ledande polymeren bidrar till helhetspotentialen av den jonselektiva elektroden. Lutningen av kalibreringskurvan, dvs. analytiska prestandan hos den jonselektiva elektroden stördes inte av E° justeringen. Det upptäcktes också, att E° av en fastfas jonselektiv elektrod kunde nollställas genom att kortsluta den med en konventionell referenselektrod med inre lösning och stor kapacitans. I detta fall drivs redox-reaktionen med hjälp av potentialskillnaden mellan den jonselektiva elektroden och referenselektroden, vars potential inte påverkas p.g.a. dens stora kapacitans. Kortslutningsmetoden för justering av E° är instrument-fri, vilket är en stor fördel med tanke på praktiska tillämpningar.

List of publications

I. Ulriika Mattinen, Johan Bobacka and Andrzej Lewenstam, **Solid-Contact Reference Electrodes Based on Lipophilic Salts**, *Electroanalysis 2009, 21, No. 17-18, 1955 – 196*

II. Salzitsa Anastasova, Ulriika Mattinen, Aleksandar Radu, Johan Bobacka, Andrzej Lewestam, Jan Migdaski, Marek Danielewski and Dermot Diamond, **Development of miniature all-solid contact potentiometric sensing system**, *Sensors and Actuators B (2010) B146(1) , 199-205*

III. Ulriika Mattinen, Sylwia Rabiej, Andrzej Lewenstam and Johan Bobacka, **Impedance study of the ion-to-electron transduction process for carbon cloth as solid-contact material in potentiometric ion sensors**, *Electrochimica Acta (2011), 56(28), 10683-10687*

IV. Ulriika Vanamo, Johan Bobacka, **Electrochemical control of the standard potential of solid-contact ion-selective electrodes having a conducting polymer as ion-to-electron transducer,** *Electrochimica Acta 112 (2014) 316-321*

V. Ulriika Vanamo, Johan Bobacka, **Instrument-Free Control of the Standard Potential of Potentiometric Solid-Contact Ion-Selective Electrodes by Short-Circuiting with a Conventional Reference Electrode,** *Analytical Chemistry (2014) 86, 10540−10545*

Contributions of the author

Paper I The author did the experimental work, participated in planning of experiments, evaluated all the results, wrote the first draft of the manuscript and finalized it together with the co-authors.

Paper II The author did the experimental work together with one of the co-authors, contributed to the planning of the experiments and to the writing of the first draft and finalized the paper together with the co-authors

Paper III The author contributed to the planning of the experiments and realization of the set-up and electrode design, did the experimental work together with one of the co-authors, wrote the first draft of the manuscript and finalized it together with the co-authors.

Paper IV The author did all the experimental work, evaluated the results and wrote the first version of the manuscript. Planning of the experiments and finalizing of the paper was done together with the co-author.

Paper V The author did all the experimental work, evaluated the results and wrote the first version of the manuscript. Planning of the experiments and finalizing of the paper was done together with the co-author.

Supporting publications

Dimitrije Cicmil, Salzitsa Anastasova, Andrew Kavanagh, Dermot Diamond, Ulriika Mattinen, Johan Bobacka, Andrzej Lewenstam, Aleksandar Radu, **Ionic Liquid-Based, Liquid-Junction-Free Reference Electrode**, *Electroanalysis 2011, 23, No. 8, 1881 – 1890*

Petri Ihalainen, Anni Määttänen, Ulriika Mattinen, Milena Stepien, Roger Bollström, Martti Toivakka, Johan Bobacka and Jouko Peltonen, **Electrodeposition of PEDOT-Cl film on a fully printed Ag/polyaniline electrode,** *Thin Solid Films, volume 519, issue 7, 31 January 2011, Pages 2172-2175*

Salzitsa Anastasova, Aleksandar Radu, Giusy Matzeu, Claudio Zuliani, Ulriika Mattinen, Johan Bobacka, Dermot Diamond, **Disposable solid-contact ion-selective electrodes for environmental monitoring of lead with ppb limit-of-detection** *Electrochimica Acta 73 (2012) 93– 97*

Justyna Kupis, Ulriika Mattinen, Anna Kisiel, Teresa Błaż, Jan Migdalski and Andrzej Lewenstam, **Biomimetic membranes made of conducting polymers doped with adenosine diphosphate (ADP)**, *Electrochimica Acta 77 (2012) 23-28*

Anni Määttänen, Ulriika Vanamo, Petri Ihalainen, Petri Pulkkinen, Heikki Tenhu, Johan Bobacka, Jouko Peltonen, **A low-cost paper-based inkjet-printed platform for electrochemical analyses**, *Sensors and Actuators B 177 (2013) 153– 162*

Table of contents

PREFACE II

ABSTRACT V

REFERAT VII

LIST OF PUBLICATIONS IX

- *Contributions of the author* .... *ix*
- *Supporting publications* .... *x*

TABLE OF CONTENTS XI

SYMBOLS XIII

ABBREVIATIONS XIV

1. INTRODUCTION 1
    - *1.1 Applications and current trends* *2*

2 THEORY 5

- *2.1 Electrochemical cell* *5*
- *2.2 Liquid junction potential* ***10***
- *2.3 Ion selective electrodes (ISEs)* ***12***
    - 2.3.1 Glass membranes 13
    - 2.3.2 Solid-state membranes 14
    - 2.3.3 Liquid and polymer membranes 14
    - 2.3.4 Ion-to-electron transduction 19
    - 2.3.5 Solid-contact ion selective electrodes 20
- *2.4 Reference electrodes* ***22***
    - 2.4.1 Conventional reference electrodes 22
    - 2.4.2 Solid-state reference electrodes (ssRE) 24

3. TOWARDS PORTABLE AND REMOTE POTENTIOMETRIC SENSING 28
    - 3.1 The Stability and the reproducibility of the standard potential E° in potentiometric sensors 29

4. SOLID CONTACT MATERIALS IN ION SELECTIVE ELECTRODES 31

- *4.1 Conducting polymers* ***32***
    - 4.1.1 Introduction 32
    - 4.1.2 Synthesis of conducting polymers 35
    - 4.1.3 Conducting polymers in potentiometric sensors 36
    - 4.1.4 Poly(3,4-ethylenedioxythiophene) PEDOT 38
- *4.2 Nanostructured carbon materials as solid contacts* ***39***

5. CHARACTERIZATION TECHNIQUES 40

- *5.1 Chronoamperometry and chronopotentiometry* ***41***
- *5.2 Electrochemical impedance spectroscopy (EIS)* ***42***

6. RESULTS AND DISCUSSION 46

6.1 Solid-contact reference electrodes based on lipohilic salts *46*
6.1.1 Preparation of studied reference membrane candidates 46
6.1.2 Comparison of the different membranes 47
6.1.3 TBA-TBB reference membrane 49
6.2 All-solid-state potentiometric sensors with a TBA-TBB reference membrane...*50*
6.2.1 All-solid-state multielectrode 51
6.2.2 All-solid-state sensor based on carbon cloth 53
6.3 Impedance study of the ion-to-electron transduction process for carbon cloth and PEDOT as a solid-contacts...*55*
6.4 Electrochemical control of the standard potential E° of a solid state ISE with a CP as a solid contact *59*
6.4.1 Control of the E° by applying current or potential 60
6.4.2 An instrument-free method to control the E° of a SC-ISE by short-circuiting it with a conventional RE.. 62
7. CONCLUSIONS AND CLOSING REMARKS...66
8. REFERENCES 68

Symbols

a_i° = activity of species i in the standard concentration
a_i = activity of species i
C_{dl} = double layer capacitance
f = frequency ([f]=Hz=s^{-1})
ω = angular frequency
Φ = phase shift
emf = electromotive force
E_{cell} = cell potential
E_1, E_2 = theoretical electrode potentials
E_{PB} = phase boundary potential
E_j = Liquid junction potential
E° = standard potential
E°′ = formal potential
F = Faraday constant
G = Gibbs free energy
$[i^z]_{memb}$ = concentration of ion i in the membrane phase
K_{ij} = selectivity coefficient for primary ion i over interfering ion j
k_i = the phase transfer energy for ion i
μ_i = chemical potential of species i
$\bar{\mu}_i$= electrochemical potential of species i
ϕ = inner potential of a phase
Δϕ= galvani potential difference
R = gas constant (8.314 J/(mol*K))
R_s = solution resistance
R_{ct} = charge transfer resistance
T = absolute temperature (0 K, -273.15 °C)
γ = activity coefficient
u_i = mobility of ion i
Z_w = Warburg impedance
z = charge number

Abbreviations

CC = carbon cloth
CE = counter electrode
CP = conducting polymer
CWE = coated wire electrode
DOS = bis(2-ehtylhexyl)sebacate
ETH-500 = tetradodecylammonium tetrakis(4-chlorophenyl)borate
GC = glassy carbon
ISE = ion-selective electrode
ISM = ion-selective membrane
LiOAc = lithium acetate
o-NPOE = o-nitrophenyl octyl ether
PEDOT = poly(3,4 ethylenedioxythiophene)
POC = point-of-care
POT = poly(3-octylthiophene)
PPy = polypyrrole
PSS = poly(styrenesulfonate)
QRE = quasi reference electrode
RE = reference electrode
SC = solid contact
SC-ISE = solid-contact ion-selective electrode
TBA-TBB = tetrabutylammonium-tetrabutylborate
TBA-TPhB = tetrabutylammonium-tetraphenylborate
T(hept)A-TPhB = tetraheptylammonium-tetraphenylborate
TDMACl = tridodecylmethylammoniumchloride
KTpClPB = potassium tetrakis(4-chlorophenyl)borate

1. Introduction

Chemical sensors provide information about the presence and concentration of specific chemical species of interest in our natural surroundings or in a sample. Chemical sensors are in a way an extension to human senses [1,2]. Sensors play a major role in medicine, environmental monitoring and industrial process control. Electrochemical sensors are an especially important group of chemical sensors [3].

Chemical sensing consists of two major steps, the molecular recognition and the transduction of the signal [1]. A sensor reacts to an input, a property that is wished to be quantified, and produces an output signal that can then be registered, as shown schematically in Figure 1 [4]. While the recognition layer should show chemical selectivity towards the analyte, when coming into contact with the sample, the transducer layer is not usually selective or sensitive, and does not have a dynamic range. It rather reacts to a change in the recognition layer, and transforms it to something measurable [5] such as an electrical signal in the case of electrochemical sensors [6].

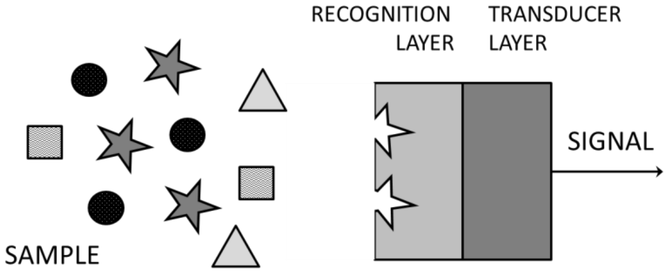


Figure 1. *A schematic representation of chemical sensing consisting of chemical recognition and signal transduction*

Ion selective electrodes (ISEs), essentially potentiometric sensors, comprise an important and a very successful, widely used class of electrochemical sensors [2,4,7]. The attraction of potentiometry as a measurement technique lies in its simplicity, low cost, low power consumption, small size, portability and fast measurements [1,6,8]. ISEs are essentially passive electrochemical devices [9], as potentiometry is a zero-current technique where the potential difference between two electrodes in a solution, forming a galvanic cell, is measured [4,10,11].

Ideally, potentiometric sensors respond linearly to the logarithm of the activity of the analyte in the sample. Actually, a potentiometric measurement with ISEs is one of the few techniques available that enable study of activity instead of the total concentration [4,12,13]. The logarithmic dependency has its positive and negative aspects. A positive aspect is that usually a wide range of activities can be measured, but in the exchange, the precision of the measurement suffers [13] as small changes of the concentration in the sample translate into extremely small changes in the measured potential [11,14]. This can nevertheless be useful in potentiometric determination of the formation constants between a receptor (ionophore) and its analyte [7,8]. Thus, the limits of the technique are often set by the analytical performance of the sensor itself [1].

1.1 Applications and current trends

The main application fields of potentiometric sensing are clinical, environmental and industrial monitoring and analysis and the study of biological systems [4,15]. Of these, clinical analysis is perhaps the most important, as the true interest and the driving force in the development of ISEs has been towards the physically relevant blood electrolytes, K^+, Na^+, Li^+, Ca^{2+}, pH, HCO_3^- and Mg^{2+} ions [16-18]. Traditionally, large high-throughput clinical analyzers in central laboratories are used. More recently, portable point-of-care (POC) devices are emerging in the healthcare applications too [17] as near-patient testing is a clear trend in the research for all modern health-related chemical sensing [7,19-21]. The non-profit organization Diagnostics for All, that aims for low-cost, easy-to-use, point-of-care diagnostic devices designed specifically for the developing world [22], is a good example of such development in the field. In addition to good sensors, portable potentiometry requires also compact portable analytical instruments, which have also been developed rapidly in recent years [23].

Remote sensing and wireless sensor networks are very exciting directions in environmental analysis at the moment [14,23,24]. Miniaturization that would allow mass-fabrication is an important topic in the current research with the ISEs, as industrial-scale production would reduce the costs and enable for example sensor networks [23,24]. Miniaturization requires preparation of the sensors in solid state, without the conventional liquid components [2,8,25]. The sensors developed for remote locations must be rather robust to be able to operate for long times without maintenance. Thus many of the remote sensing stations at the moment consist of physical sensors (for example for temperature and pressure) that are not worn down by contact with sample [23]. The ion-selective electrodes are good candidates for remote chemical sensing as they require no sample preparation and consume very little power [14], though there are some challenges related to them that will be discussed later in the text.

Microbiologically polluted aqueous sites pose a challenge for potentiometric sensing [26]. Also clinical samples, such as blood, serum and urine are complicated matrices [27]. Biofouling may be a problem as proteins can deposit on the membrane surface. In clinical applications this requires frequent recalibration [28,29]. Biofouling can also be a problem in long term potentiometric measurements in biological samples [24], though to a lesser extent than for many other types electrochemical sensors. If the adsorbed contaminants do not cover the ion selective membrane completely, the ISE is expected to work properly [2]. Solid-contact ISEs have actually proven to be surprisingly robust compared to the traditional liquid filled ISEs when exposed to physical damage [26]. Biofouling and clogging of the electrode surface are nevertheless serious practical problems in environmental measurements [14] considering that autonomous operation for at least one year for a remotely located sensor is desirable [23].

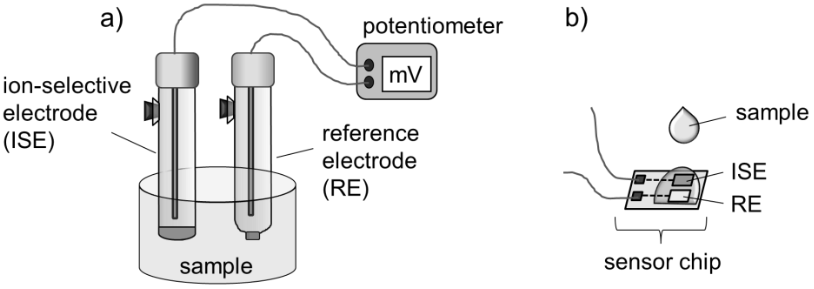


Figure 2. *A schematic picture of a) a conventional potentiometric measurement and b) a modern planar sensor chip*

Other current trends in the ISE research are the further lowering of the detection limit [30,31], improving the lifetime and robustness of the sensors and reducing or even eliminating the need for frequent calibrations [2,31].

Calibration is a critical aspect of any analysis, especially one involving sensors. Understanding the sensor signal and the choice of a proper calibration technique is very important [32]. Regarding potentiometric sensors, the measured signal must be related to the activity of the sample by determining a calibration curve in a series of standard solutions with known compositions. The calibration curve should ideally be a straight line, from which the calibration parameters, the slope (S) and the standard potential (E°) of the ISE can be determined. The stability of the calibration curve is very important for reliable analysis results. The slope of the calibration curve shows typically better stability and reproducibility than the standard potential [4] but instability of the E° can be compensated with frequent calibrations [23] as is done with automated clinical analyzers [4,18].

The development of the sensors and their utilization in situations where they could not have been used earlier creates new challenges and issues to deal with. Many of the new applications, such as point-of-care and home diagnostics, require simple and easy-to-use devices as the end user does not necessarily have the required skills or time to perform a good calibration, thus compromising the reliability of the analysis [26,33]. In remote, autonomous sensing, calibration is especially problematic as it increases the complexity and costs of the sensor system [23]. The problems related to the calibration with portable potentiometry, and means to circumvent those problems with potentiometric sensors, belong to the central themes of this work.

2 Theory

2.1 Electrochemical cell

An electrochemical cell in its simplest form consists of two electrodes (electric conductors) separated by an electrolyte (ionic conductor). A chemical reaction that occurs spontaneously in an electrochemical cell is called the cell reaction and the driving force (sometimes called the electro motive force, emf) for this reaction is the potential difference across the cell, E_{cell}, as shown in Equation 1, where E_1 and E_2 represent the half-cell potentials. [13,34,35]

$$E_{cell} = E_2 - E_1 \qquad (1)$$

By connecting the electrodes to a voltmeter, as shown in Figure 2a, the potential of the cell can be measured. Potentiometric measurements are based on the thermodynamic relation of the potential of a galvanic cell (formed by an ion-selective electrode and a reference elelctrode) to the activity of the analyte in the sample solution. Only the potential differences, not the potentials of the individual electrodes, can be measured experimentally. The processes occurring at the individual electrodes may though be theoretically treated separately and combined then together to obtain the complete cell response [3,4,13,15,36,37].

When an electrode is immersed in an electrolyte solution and a thermodynamically reversible chemical equilibrium is formed at the interface with respect to a species i, the electrochemical potential $\bar{\mu}_i$ must be equal in both phases. This is true for all the equilibrated species between phases α and β, for example an electrode and an electrolyte phase [38-40], as shown in Equation 2

$$\bar{\mu}_i^{\alpha} = \bar{\mu}_i^{\beta} \qquad (2)$$

The electrochemical potential of the species *i* is the sum of chemical and electrical terms

$$\bar{\mu}_i = \mu_i + z_i F \varphi \qquad (3)$$

where μ_i is the chemical potential of the species i, z_i is the charge number, F is the Faraday constant and φ is the (inner) electrical potential of the phase containing the ion i. For uncharged species that are not affected by any electric field, the second term in Equation 3 is zero and thus, at the equilibrium, the chemical potentials in the phases α and β are equal [3,10,36,41].

The chemical potential is a thermodynamic function with SI dimensions energy per mole [J/mol]. For a species i distributed in two phases, it is defined as the work performed against chemical forces when changing the number of these particles in the system, divided by the number of added or removed particles

$$\mu_i = \left(\frac{\partial G}{\partial n_i}\right)_{p,T,n_{j\neq i}} \tag{4}$$

where ∂G is the change in the Gibbs free energy as a consequence of a change of ∂n moles of species i, p is the pressure and T is the absolute temperature. The chemical potential of species i is influenced by the activity of that species in the solution, a_i, according to Equation 5, where μ_i^0 is the standard chemical potential of species i and R is the gas constant [10,37]

$$\mu_i = \mu_i^\circ + RT \ln a_i \tag{5}$$

The activity of a component can be thought as the effective concentration that is influenced by the real chemical environment. The use of the activities instead of the concentrations acknowledges a deviation from ideality, which is stronger for charged species than for uncharged ones. A solvated ion is surrounded by a cloud of ions with an opposite charge. Before the central ion can react, it must free itself from this cloud, resulting in a lower free energy than in ideality. The relation of the activity and the concentration of an ion in a solution is given by Equation 6, where γ is the activity coefficient describing the deviation from the standard state and c is the concentration [3,34,41]

$$a_i = \gamma_i c_i \tag{6}$$

Activity coefficients for solutions with known compositions can be estimated with the Debye-Hückel theory [37]. The difference in the inner potentials between the two phases $\Delta\phi$ is called the Galvani potential and by combining Equations 2 and 3 we obtain the boundary potential for phases α and β at the equilibrium [37]

$$\Delta\varphi_{\alpha,\beta} = \varphi_\alpha - \varphi_\beta = -\frac{\left(\mu_i^\alpha - \mu_i^\beta\right)}{z_i F} \tag{7}$$

And the insertion of Equation 5 to Equation 7 gives

$$\begin{aligned}\Delta\varphi_{\alpha,\beta} &= \varphi_\alpha - \varphi_\beta \\ &= \frac{\left(\mu_i^{\circ,\beta} + RT\ln a_i^\beta\right) - \left(\mu_i^{\circ,\alpha} + RT\ln a_i^\alpha\right)}{z_iF} \\ &= \frac{\left(\mu_i^{\circ,\beta} - \mu_i^{\circ,\alpha}\right)}{z_iF} + \frac{RT}{z_iF}\left(\ln a_i^\beta - \ln a_i^\alpha\right) \\ &= \frac{\left(\mu_i^{\circ,\beta} - \mu_i^{\circ,\alpha}\right)}{z_iF} + \frac{RT}{z_iF}\left(\ln\frac{a_i^\beta}{a_i^\alpha}\right) \qquad (8)\end{aligned}$$

The activity of the ion i in the phase α (ion selective membrane) can be made constant with help of an ionophore that complexes selectively most of the ion i present in the membrane phase, and a lipophilic ion with opposite charge to that of i, to balance the charge of the complex, rendering the concentration of the free primary ion very low. Then, the first term in the final version of Equation 8 can be inserted into the constant potential term $\varphi°$, and it can be written as

$$\Delta\varphi_{\alpha,\beta} = \varphi° + \frac{RT}{z_iF}\ln a_i^\beta \qquad (9)$$

which is the Nernst equation that is the basis for the potentiometric sensing [4,10]. If we now consider a cell with two metal electrodes in an electrolyte solution, Equation 1 can be written as

$$\begin{aligned}E_{cell} &= E_2 - E_1 = \Delta\varphi_2 - \Delta\varphi_1 \\ &= \left(\varphi_{2,electrode} - \varphi_{2,solution}\right) - \left(\varphi_{1,electrode} - \varphi_{1,solution}\right) \qquad (10)\end{aligned}$$

And the complete cell response is given by

$$E_{cell} = \left(\varphi^\circ_{halfcell,2} + \frac{RT}{zF}\ln a_i\right) - \left(\varphi^\circ_{halfcell,1} + \frac{RT}{zF}\ln a_j\right) \qquad (11)$$

In the case of a potentiometric cell with an ion-selective electrode and a reference electrode, the half-cell potential of the reference electrode is constant. This constant and the constant potential term for the ion-selective electrode can be included in a constant standard potential $E°$ for the whole cell. Since ion selective electrodes ideally respond to only one ion of interest, Equation 10 can be simplified to [3,10,41]

$$E_{cell} = E_{ISE} - E_{RE} = E_{cell}^{\circ} + \frac{RT}{z_i F} \ln a_i \tag{12}$$

The standard potential $E°$ can be derived directly from the thermodynamic data and standard Gibbs free energies or obtained experimentally by extrapolating the measured potential to the unit activity, where $\ln(a_i) = 0$. In many cases, the activities are unknown and it can be practical to replace the standard potential with a formal potential $E^{\circ\prime}$ that incorporates the standard potential and the activity coefficients and assumes unit concentrations rather than unit activities. The formal potential takes into account the overall composition of the solution [37,40].

Changing the natural logarithm to a 10-based logarithm and calculating the constants gives the familiar form of the Nernst equation

$$E_{cell} = E_{cell}^{\circ} + \frac{0.0592}{z_i} \log a_i \tag{13}$$

from which it follows that the Nernstian calibration slope for an electrode responding to a monovalent ion at 25°C is 59.2 mV / dec and for a divalent ion 29.6 mV / dec and so on. As a consequence of this relation, the sensitivity of an ISE decreases dramatically with an increasing z_i [11].

Any electrochemical cell can be seen as a collection of phases and interfaces where all phases have their own inner potential ϕ. Across each interface, there is a galvani potential difference $\Delta\phi$. The transition in the electrical potential is sharp when crossing from one conducting phase to another, and the change usually occurs almost entirely at the interface. The measured potential is the added potential differences of all the phases in the system consisting of an ISE, a sample and a RE, as shown schematically in Figure 3 where a potassium selective ISE is used as an example [2,10,11,36,40].

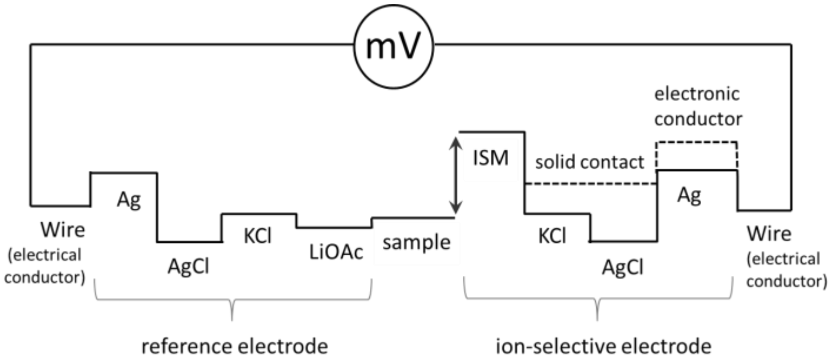


Figure 3. *A schematic presentation of the potentials across an electrochemical cell with a potassium selective ISE and a RE. Dashed lines represent an ISE-arrangement in a solid state.*

The response of a normal ion selective electrode is often interpreted with a phase boundary (PB) model, which is a somewhat idealized model assuming a total equilibrium [8]. Nevertheless, it is sufficient in many cases of practical significance [16] as in the context of this work. The signal is formed at the ion-selective membrane (ISM) ǀ sample solution interface, and the measured phase boundary potential, E_{PB}, governed by the primary ion, is the difference of the inner potentials of the membrane and the sample solution. Equation 9 is modified further to

$$E_{PB} = \varphi_{membrane} - \varphi_{sample} = E^{\circ}_{PB,i} + \frac{RT}{z_i F} \ln \frac{a_i^{sample}}{a_i^{membrane}} \tag{14}$$

where $E^{\circ}_{PB,i}$ contains chemical standard potentials from both phases [8,39]. Considering now a complete electrochemical cell, when all constant components, including the reference electrode, are included in the E°, we can write

$$E_{cell} = E^{\circ}_{cell} + \frac{0.0592}{z_i} \log \frac{a_{i(sample)}}{a_{i(membrane)}} \tag{15}$$

The ion selective membrane is designed in such a way that the activity of the primary ion in the membrane phase is constant. Thus the activity of the primary ion in the sample phase determines the signal which means that Equation 13 is valid for the ion selective electrodes [2,7].

2.2 Liquid junction potential

A liquid junction is a solution contact that physically separates but also connects two solutions. In the potentiometric measurements, a liquid junction typically appears between the internal filling solution of the reference electrode and the sample solution. A potential difference is formed at the junction due to the different diffusion mobilities of the ions across the nonselective interface. The ions diffuse from a solution with a higher activity to a solution with a lower activity. If the diffusing ions with opposite charges have different mobilities, so that one diffuses faster than the other, a charge separation arises, creating an electric field that actually works against the further separation of the diffusing ions. This results in a coupled diffusion/migration, where both of the ions move at the same velocity [4,10,15,37,41].

The potential difference caused by the charge separation of the ions is the troublesome liquid junction potential E_j that must always be taken into account as it is a substantial component of the cell potential [10,15,36,40] and Equation 1 should thus be written as

$$E_{cell} = E_{ISE} - E_{RE} + E_j \qquad (16)$$

Liquid junction potential is of a diffusional origin, and thus also referred to as diffusion potential. Unfortunately, direct measurement of the liquid junction potential is not possible. It can be estimated with the Henderson equation (Equation 17) when the experimental conditions are set up to allow some simplifications by using dilute solutions of the same salt where u is the ionic mobility ($cm^2s^{-1}V^{-1}$) and z is the charge of the ion and C_{i1} and C_{i2} are the concentrations of the solutions containing the ion i [10,15]

$$E_j = \frac{RT}{F}\frac{\Sigma(|z_i|/z_i)u_i(C_{i2} - C_{i1})}{\Sigma|z_i|u_i(C_{i2} - C_{i1})} ln\frac{\Sigma|z_i|u_iC_{i1}}{\Sigma|z_i|u_iC_{i2}} \qquad (17)$$

The liquid junction potential cannot be eliminated, but it can be minimized and stabilized by the use of a salt bridge. As shown in Figure 4a, in the salt bridge two liquid junctions in series connect two solution compartments such as the reference and the sample solution [10,15,36,37,40,41].

The inner filling solution of the bridge is chosen so that it dominates the junction potential. It should have a high concentration of an equitransferent salt where the anion and the cation have similar mobilities as in the case of KCl, lithium acetate (LiOAc) or NH_4NO_3 [4,10,15,40]. In cases, where the sample composition is relatively constant and of a known composition, an additional salt in the bridge electrolyte can help to minimize the diffusion potential [42].

If the E_j can be made stable and reproducible, it can be included in the fixed standard potential of the cell which is naturally desirable. To realize this, a constant, substantial flow rate is probably the most critical factor, as the massive amount of salt flowing slowly but continuously through the junction controls and stabilizes the junction potential E_j [15,36,40].

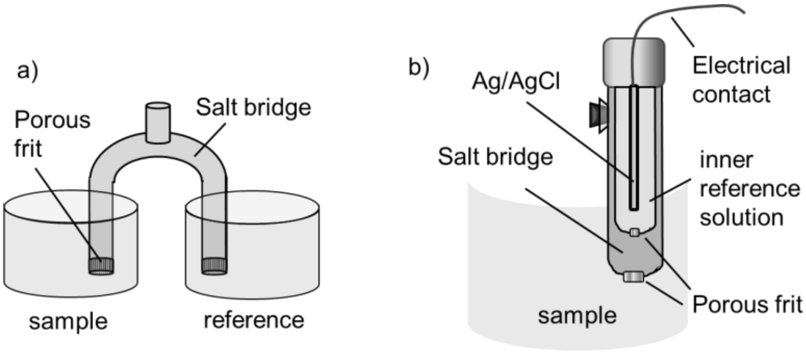


Figure 4. *A schematic picuter of a) a salt bridge connecting two solutions and b) a salt bridge integrated in the reference half-cell design.*

A typical design for a macroscopic reference electrode is shown in Figure 4b. A Ag/AgCl element is in direct contact with an inner filling solution that has a high concentration of Cl^- -ions, (typically 3-4 M KCl) that determine the potential of the Ag/AgCl/Cl^- -system. This reference half-cell is connected to the next solution via a junction, typically a porous frit [15]. There are different types of junctions, but most commercial electrodes have a junction based on a constant, continuous impeded flow. The flow rate of the ions in the bridge solution through the junction should be high enough to govern the junction potential and to prevent clogging of the junction, one of the common sources of measurement errors. At the same time, the flow rate should be low enough to protect the sample from a contamination by the bridge filling solution [4,15].

In a single junction reference electrode (SJ RE), the liquid junction connects the sample and the internal filling solution of the reference electrode. To avoid contamination, the filling solution of the reference electrode should be chosen so that it does not contain ions that are being measured. A way to circumvent this problem is to use a double junction reference electrode (DJ RE), as shown in

Figure 4b, where the salt bridge is integrated into the reference electrode design. The outer compartment may be filled with an equitransferent solution chosen so that it does not interfere with the measurement. Often lithium aceteate (LiOAc) or NH_4NO_3 are used. The concentration of the outer bridge solution should be at least ten-fold compared to the concentration of the sample solution in order for it to govern the junction potential over the ions in the sample solution [4,10,15].

2.3 Ion selective electrodes (ISEs)

An ideal ion-selective electrode (ISE) responds to the target ion (primary ion) according to the Nernst law shown in Equation 13, and this response is the potential difference formed at the ion selective membrane | sample solution interface [10]. Even if this phase boundary potential has its origin in the interfacial charge separation, a charge neutrality must prevail in the bulk of both the membrane and the sample phase and thus the thickness of the charge separation layer (electrical double layer) is only in the order of nanometers [2,4,12].

An ion-selective membrane (ISM) is the key component of all potentiometric ion sensors, and it dictates the response of the sensor [7,10,12,17,43]. In a conventional model (shown in Figure 5a), the ion selective membrane is sandwiched between a sample solution and an internal filling solution with a constant activity of the primary ion. An internal reference electrode responsible for the ion-to-electron transduction is needed to close the circuit [13,37].

As potentials of all other components of the circuit are constant, the potential at the outer side of the membrane (ISM|sample) is determined by the activity of the primary ion in the sample solution as already discussed in the theory section, and as shown schematically in Figure 3. The two main requirements for an ion selective electrode are that the concentration of the primary ion in the membrane-phase must be kept constant and that all the factors contributing to the measured potential, except for the potential at the membrane | sample interface, are sample independent and constant [2,40].

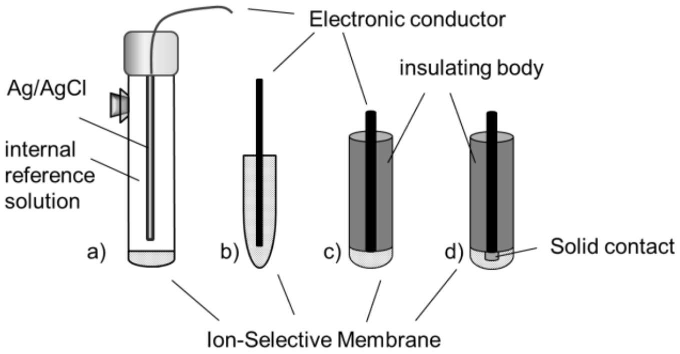


Figure 5. *A schematic picture of a) a conventional ISE with an internal reference electrode and a filling solution, b-c) coated-wire arrangements where an ion selective membrane is placed directly on an electronic conductor, (wire b or disk c) and d) a solid contact arrangement where an ion-to-electron trandsucer is placed between an electric conductor and an ion-selective membrane*

Ion-selective electrodes can be categorized in several ways. A typical approach used for example by Bard and Faulkner [40] is to divide membranes to solid-state membranes and to liquid and polymer (or plastic) membranes [40]. Solid-state membranes are often still separated to glass membranes and crystalline membranes as suggested by Mikhelson [4]. Ion-selective membranes can also be sorted by the type of the ion binding sites integrated in the membrane matrix: fixed ionic sites (like for example in glass and crystalline membranes), mobile ion exchangers or neutral carriers as pointed out by Janata [10].

2.3.1 Glass membranes

The oldest and the best-known ISE is the pH glass electrode, responding to hydronium ions (H^+) [2,4,13,37]. The pH-electrode has a glass membrane consisting of a disordered network of tetravalent silicon and divalent oxygen forming Si-O-Si-O-Si –chains. Defects in this SiO_2 matrix create anionic sites and thus cationic vacancies, as part of the chains are terminated with negatively charged oxygen. The charge is balanced with Ca^{2+} ions and with slightly mobile Na^+ ions inducing a weak conductivity on the glass [10,13,40]. When the electrode comes in contact with the aqueous sample solution, a thin surface layer of some 20 nm is formed in the glass, and H^+ and Na^+ ions compete for the negative sites at the hydrated | unhydrated glass interface [13] Interactions between the sample solution and the glass membrane i.e. the ion exchange and the diffusion of all participating ions, occur exclusively at the hydrated zone of the glass, and are facilitated kinetically by a swelling that accompanies the hydration [10,40]. The hydrated layer of the glass membrane becomes highly selective for H^+ ions. The glass membrane of the pH-electrode belongs to the group of membranes with fixed ionic sites [10].

By altering the composition of the glass membrane, it can be made responsive also for other cations such as Na^+, Li^+, Ag^+, K^+ and NH_4^+, though selectivities of these electrodes remain modest. The potentiometric Na^+ glass membrane electrode suffers from an interference by H^+ and Ag^+ ions, but it competes with other measurement techniques with its simplicity and speed. In addition, glass electrodes sensitive for univalent cations have found some practical value [2,3,40].

2.3.2 Solid-state membranes

After the development of the glass membranes during the first half of the 1900s, the introduction of the crystalline materials as sensing membranes increased the number of ions that could be detected with ISEs. They were used in the form of single crystals or compressed disks placed between the sample solution and the internal reference solution (as shown schematically in Figure 5a). LaF_3 for fluoride and mixtures of low-soluble silver salts for the determination of anions such as Cl^- and Br^- are examples of typical crystalline membranes. The conductivity and thus also the response time of the crystalline electrodes can be improved by introducing dopants to the structure [2-4,10]. Designing crystalline materials with a selectivity towards a specific compound was found challenging and thus the introduction of the ion-binding receptors (ionophores or ion carriers) as components for the ISEs was most welcome to boost the research in the field of potentiometric ion sensors [2].

2.3.3 Liquid and polymer membranes

Liquid membranes are hydrophobic sensing layers containing mobile or fixed binding sites [10]. The chelating agents with selectivity towards the ion of interest are dissolved in the membrane matrix providing thus a mechanism for a selective charge transport across the membrane [40]. Liquid membranes can be stabilized physically in a porous lipophilic diaphragm, whose edges contact reservoirs holding this liquid, [2,40] or more typically nowadays, the liquid ion-exchanger is immobilized in a hydrophobic polymer matrix, like plasticized poly(vinyl chloride). Such electrodes are often called polymer or plastic membrane ISEs [4,40]. The discussion below will be focused on plastic membranes.

2.3.3.1 Selectivity

Selectivity is one of the most important characteristics of a potentiometric sensor, and it describes the ability of the ISE to discriminate between the primary ion and the interfering ions [4,43,44]. The membrane selectivity is achieved by introducing a selective binder molecule, an ionophore, to the membrane [12]. Ionophores are charged or neutral receptor molecules that bind the analyte ion selectively and reversibly [2,4,7,28]. Also the strength of the binding is of importance. A strong binding between the primary ion and the ionophore is a prerequisite for good selectivity, whereas too strong binding can lead to the co-extraction of the primary ion and an ion of the opposite charge from the aqueous phase [7], causing loss of permselectivity, the so called Donnan failure [4].

At present, there are ionophores with good selectivity for many important cations such as H^+, Na^+, K^+, Ca^{2+} and Ag^+. Receptors with adequate selectivity towards Mg^{2+} and NH_4^+ ions are nevertheless still missing when demands for real applications are considered, and even more work remains to be done with ionophores for anions [2]. The best known ionophore molecule is the neutral carrier antibiotic valinomycin that is highly selective for K^+ [2,10]. A valinomycin containing PVC membrane is often chosen as model membrane for experimental work. Bobacka used it to study the influence of the capacitance of a conducting polymer solid-contact to the potential stability of a K-ISEs [45]. Mousavi et al [46] used valinomycin based K-selective membrane for testing poly(3,4-ethylenedioxythiophene) films doped with carbon nanotubes as solid contacts. Novell et al. [48] demonstrated functionality of their new carbon nanotube impregnated paper as an ISE substrate with help of the valinomycin membrane. It has also been used to present other solid contacts such as tetrakis(4-chlorophenyl)borate (TB−) anion doped nanocluster films [49], three-dimensionally ordered macroporous carbon [50] and gold nanoparticles [51]. Gyurcsányi et al. [52] have used the valinomycin membrane to compare performance of hydrogel and potassium hexacyanoferrate(II)/(III) doped polypyrrole (PPy/FeCN) inner contacts in a poteassium selective ISE. Such a membrane is also used in Papers III, IV and V in this work.

The selectivity of the real-world ISEs is not ideal and a potential bias induced by interfering ions must be acknowledged. Selectivity coefficients are used to describe and quantify the preference of the membrane for the primary ion over the interfering ions. Selectivity is related to the binding constants (or complex formation constant) of the ionophore and the analyte, and ionophore and interfering ions respectively [7]. In general, selectivity depends on the relative magnitude of the exchange current density of the primary ion relative to the interfering ions in a way that the higher the ratio, the more selective membrane [4,10].

In addition to the ionophore, also lipophilic ion exchangers contribute to the selectivity of an ISE. The selectivity of the membranes containing only an ion-exchanger and no ionophore is given by the Hofmeister series that lists cations and anions according to their affinity to the aqueous phase, i.e. according to their free hydration energy. There are different models and equations for the quantification and the determination of the selectivity and the selectivity coefficients (K_{ij}) of ISEs, depending for example on the composition of the membrane and on the valency of the primary and the interfering ion [4,44,53].

Selectivity can be determined by calibrating the ISE for the primary ion at a constant background concentration of the interfering ion (fixed interference method, FIM) or by calibrating the ISE for the interfering ion at a constant concentration of the primary ions (fixed primary ion method, FPIM), i.e. in mixed solutions. Another possibility is to use pure solutions containing only primary ions and only interfering ions respectively (separate solution method, SSM) [43], which is the predominating experimental method at the moment [4]. When performed carefully, all of these methods should give the same value for K_{ij} [2], though an inconsistency in the measurement conditions and protocols have led to varying selectivity coefficients in the literature [4,54]. One reason for the variation is the leaching of the primary ion to the vicinity of the membrane when determining selectivity coefficients for interfering ions. This can be avoided by using a protocol initially proposed by Hulanicki et al. [55] to determine "the true selectivity", or with a protocol later reported by Bakker to determine the so called "unbiased selectivity coefficients", in which the membrane is first conditioned in and calibrated with interfering ions, and only then with primary ions [4,44,56].

A typical approach for evaluating the selectivity is to use the Nikolskii-Eisenman equation, where K_{ij} is the potentiometric selectivity coefficient for a primary ion i in the presence of an interfering ion j [2,10,12,43] as shown in Equation 18:

$$E_{cell} = E^{\circ}_{cell} + \frac{RT}{z_i F}\left[\ln\left(a_i + \sum_j K_{ij} a_j^{z_i/z_j}\right)\right] \tag{18}$$

It should be taken into account that the Nikolskii-Eisenman equation is only valid when the valencies of the interfering ion and the primary ion are the same (z_i=z_j). An extended version of the equation should be used when the valencies differ (z_i≠z_j) [16]. Problems related to the determination of selectivity with different methods, covering even the time domain, are discussed by Lingenfelter et al [53].

2.3.3.2 Sensitivity and detection limits

The sensitivity of an ISE can be defined as the ratio between the change in the signal and in the analyte activity. Thus from Equation 13 it is evident that the sensitivity of a potentiometric sensor decreases with an increasing valency of the primary ion [16]. An ISE can be used in the dynamic range located between its upper and lower detection limits [43]. For the conventional ISEs with an internal filling solution, the lower detection limit is typically around 10^{-6} or 10^{-7} M, which is dictated mainly by the leaching of the ions from the internal filling solution to the sample phase. The leakage, and thus the detection limit can be lowered by adjusting the concentration of the primary ion in the internal filling solution to a lower level with the help of a complexing agent [30,40]. This finding of Sokalski et al. [30] was a milestone in the ISE research staring a new boost in efforts to lower the detection limits of the potentiometric ion sensors[4,57-59]. Even the detection limits of the solid-contact ISEs were found to be affected by the fluxes of the primary ions from the membrane phase. In analogy with the liquid filled ISEs, suppressing these fluxes for example by galvanostatic polarization [60], by introducing a primary-ion complexing ligand into the conducting polymer solid contact [61] or by using suitable hydrophobic solid-contact materials with hydrophobic polymer matrix [62,63] led to improvements in the lower detection limits also for the solid-contact ISEs.

2.3.3.3 Ionic additives

In order for the ISE response to be dependent only on the activity of the primary ion in the sample phase, the activity of that same ion in the membrane phase must be kept constant. This is achieved by introducing a hydrophobic ion with an opposite charge than that of the primary ion into the membrane. The added ion exchanger sites are lipophilic salts that dissociate in the membrane phase. Due to the macroscopic electroneutrality demand, these charged ion-exchanger sites prevent the co-extraction of the co-ions (ions whose charge is opposite to the charge of the analyte ion) from sample into the membrane, thus maintaining the permselectivity and the Donnan exclusion [2,4,10]. Such added ionic sites are crucial for the proper functioning of the ion-selective membranes with electrically neutral ionophores and improve even the selectivity of the membranes with charged ionophores. Even if the charged ionophores induce some ion-exchange capacity to the membrane themselves, a presence of ionic sites with an opposite charge is required for an optimal response. The early works with the ion-selective membranes containing only a neutral carrier were found to function simply because of the presence of some ion-exchanging impurities [2,4,28].

2.3.3.4 Polymer matrix

The thickness of the membrane is not decisive, as long as it is over the critical thickness, for which the trans-membrane fluxes worsening the detection limits of the conventional ISEs with filling solution are suppressed [2]. The interior of the membrane should be electrically neutral, indicating that the minimum thickness should be larger than the combined thicknesses of the space charges extending on both sides of the membrane. In practice, the minimum thickness of the polymeric membranes is around 50 μm, a demand easily met by conventional macroscopic membranes having typically rather thick membranes around 200 μm [10,28]. The minimum thickness might though be an issue for the miniaturized solid-state sensors that should be compatible with the integrated circuit fabrication technology [10].

The matrix material in polymer membranes should establish a rubber-like, homogenous and hydrophobic medium, in which the membrane components can move freely, as they would do in a water-immiscible organic solvent [2,10]. Poly(vinylchloride) PVC is still the most common membrane matrix used for the liquid membrane ISEs [2,4,10] and, it is therefore chosen as the matrix for all the ISEs prepared in this work. Other typical matrix-materials are for example silicone rubbers, polyurethanes and polyacrylates [2,4,64-66].

The matrix polymer can be mixed with a plasticizer, [10] a liquid that is fully miscible with the polymer, but has a lower molecular weight and a low vapor pressure at room temperature. In this way the glass transition temperature of the membrane can be shifted below the room temperature [2]. Typical plasticizers used in the PVC membranes are bis(2-ehtylhexyl) sebacate (DOS) for monovalent ions and 2-nitrophenyloctyl ether (oNPOE) for divalent ions [4].

A typical plasticized PVC membrane contains ca. 30-33% (wt) PVC, 60-66 % (wt) plasticizer and 0.5-2% (wt) ionophore [4, 10]. The molar ratio of ionic sites to ionophore should be optimized carefully depending on the charge of the analyte and the interfering ion. so that there is an excess of free ionophore with respect to the primary ion [2, 28]. All compounds are dissolved in a volatile organic solvent, typically tetrahydrofuran (THF), and this membrane solution is referred to as a membrane cocktail. In the case of solid-state ISEs, a suitable aliquot of the membrane cocktail is drop-cast directly on a chosen substrate and let to evaporate, followed by appropriate conditioning procedures [4].

2.3.4 Ion-to-electron transduction

The membrane (and the internal solution in the case of conventional models), are ionic conductors, while the metal wire or the carbon substrate are electronic conductors [4]. Since ions cannot enter the electronic components of the measurement device, a reversible ion-to-electron transduction is a necessary process in all potentiometric electrodes, in conventional ISEs with an internal fillings solution, in solid-state arrangements as well as in reference electrodes [4,8].

Typically, the transduction is realized via a reversible redox reaction, as in the conventional Ag/AgCl/Cl^- system (Equation 21) used as an internal reference with the conventional ISEs with a liquid filling solution (as in Figure 5a). The principle of this ion-to-electron transduction is comparable to that of other electroactive materials [8] that will be discussed later. Electrodes containing a finite amount of a redox-active material have a finite redox-capacitance C_{redox}, which has a time-dependent influence to the measured potential according to Equation 19,

$$\frac{\Delta E_C}{\Delta t} = \frac{i}{C} \qquad (19)$$

It follows that a large redox capacitance is a beneficial for obtaining a stable potential [45], as is the neglibly small magnitude of the net-current that is allowed to flow through the potentiometric measurement system. The latter is guaranteed by a very high input impedance of the instrument, typically in the range of 10^{14} Ω [35].

In the absence of a redox-active ion-to-electron transducer system or layer, as in the case of coated-wire ISEs with blocked interface (fig. 5 b-c), the capacitance is a double-layer capacitance, C_{dl}, whose magnitude depends on the contact area. In this case, the capacitance of the solid contact and thus the potential stability can be improved by increasing the contact area, which is the basis for use of many high-surface area carbon materials as solid contacts [8,47,67-70].

2.3.5 Solid-contact ion selective electrodes

Having a liquid component in the sensor construction, like the internal filling solution in the conventional ISE (and reference electrodes) is not ideal in all cases. A working position that ensures a contact between the liquid component or liquid contact and the sample is required. Liquid components have also a poor tolerance towards high pressure, which is problematic for example in deep sea environmental measurements or in applications requiring sterilization. Evaporation of the inner filling solution in miniaturized electrodes is problematic and there is also a risk of contaminating the sample with the leaching filling solution [4,17,25,71-73]. Even in macroscopic electrodes the maintenance of the inner filling solution can be found time consuming and irritating. The conventional construction with filling solution is not compatible with the modern fabrication technologies in planar form, and thus elimination of the liquid component from the construction design is highly desirable [4,72]. The absence of an internal reference system results nevertheless in insufficient long-term stability and poor piece-to-piece reproducibility in liquid membrane-based sensors. The reproducibility and stability of the standard potential has been found to be especially problematic [4,74,75].

In the early 1970's, Cattrall and Freiser introduced the so called "coated-wire arrangement" (fig. 5b), where a metallic wire is dip-coated with an ion selective membrane, allowing thus fabrication of much smaller and simpler ISEs [76]. This was a major leap forward in the ISE research, but later it was found that the long term stability of this configuration was not satisfactory due to the blocked interface [77] between the purely ionic conductor (the membrane) and purely electronic conductor (the metal wire) [8,71].

Obtaining stable and reproducible readings calls for a stable electrical potential and a reversible electrode processes for current passage at the interface between the ionically conducting ion selective membrane and the electrically conducting substrate [4,71].

Thus, the ionic conductivity of the ISM must be converted to an electronic conductivity of the substrate in a reversible manner. The stability of ISEs was improved by the introduction of electroactive materials with mixed ionic and electronic conductivity, such as AgF [78], polypyrrole doped with tetrafluoroborates [79] or poly(vinyl ferrocene) [25] that were able to function as ion-to-electron transducers changing from one charge carrier type to another. The switch from ionic to electronic conductivity is often accomplished with a fast redox reaction, for which both phases are buffered [71,73]. In the absence of a suitable redox-reaction, the interface is blocked, and no current can pass it forming thus a capacitor. Then, the electrode behaves like an ideally polarizable electrode, and passage of even small charges can cause a considerable change in the potential [4,73]. In solid-state ISEs the conversion of the current transport mode is accomplished with help of ion-to-electron transducers called solid contacts [8].

2.4 Reference electrodes

Half of the signal in potentiometry, no more and no less, comes from the reference system [10]. The main requirement for the reference electrode is to provide a reliable, stable and fixed potential that is independent of the sample solution and does not vary during the experiment. It should be reproducible from day to day and reversible i.e. return quickly to the equilibrium value after passage of small currents or some other accidental perturbation [10,15,34,80]. The chemistry of the reference electrode should be well-defined and understood [34]. The reversibility-condition is easily fulfilled by choosing a fast electrode reaction with a high exchange current density though in potentiometric measurements the net current density is practically zero [4,10,80].

2.4.1 Conventional reference electrodes

Since the potential of any individual electrode cannot be measured, a universal reference point is used to enable the comparison of potentials. The electrode potential of reaction

$$2H^{+}_{(aq)} + 2e^{-} \leftrightarrow H_{2(g)} \qquad (20)$$

in standard conditions is by convention zero, and the reference electrode based on this reaction is called the standard hydrogen electrode (SHE) [3,34,41,81]. Standard potentials of other electrodes and redox-reactions (at 25°C) are compared and tabulated against this electrode [3,37]. Use of the standard hydrogen electrode in practice is troublesome, as the solution, in which it is immersed to, and the surface of the platinum electrode at which the reaction occurs, must be saturated with gaseous H_2 [34,82]. Thus the SHE is very seldom used in the laboratory conditions, though it may still be used to determine standard potentials of other reference electrodes [82].

One of the most common reference electrodes used in the everyday lab work is the Ag/AgCl/Cl^- -electrode, where a silver wire is coated with a thin layer of silver chloride and then immersed into a solution with a constant Cl^- concentration [41]. It has a well-defined, fast electron transfer process (Equation 21) with a high exchange current density [10,13,41]

$$Ag_{(s)} + Cl^{-}_{(aq)} \rightleftharpoons AgCl_{(s)} + e^{-} \qquad (21)$$

This silver-silver chloride reference electrode belongs to electrodes of the second kind, where a metal is coated with its sparingly soluble salt [10,15], and the potential is determined by the activity of the anion of the sparingly soluble salt in the contacting solution. When such an electrode is used as a reference electrode, the activity of the anion of the sparingly soluble salt should be kept constant in order to obtain stable potential, as the electrode responds to it in a Nernstian manner [10]. The dependency of the potential on the anion activity for electrodes of the 2nd kind fails to fulfill the demand for the potential independency on the sample. The bare Ag/AgCl –element is thus often called a quasi-reference electrode (QRE) or a pseudo reference electrode, even if it may be the best solution for example for measurements in miniaturized systems [83]. When the Ag/AgCl –element is in contact with a solution of a constant, high concentration of Cl^- in a vessel or a compartment that can be connected to the sample with a liquid junction or a salt bridge, it becomes a complete reference electrode as shown in Figure 4b [10]. The filling solution with constant anion (Cl^-) concentration should be saturated with the sparingly soluble salt (AgCl) in order to avoid dissolving and thus consuming the AgCl layer [15]. A Ag/AgCl electrode can easily be prepared in the laboratory by anodizing a piece of silver metal (for example a disk or a wire) in a chloride containing solution [15,41].

Another typical reference electrode used in practical laboratory experiments is the calomel electrode [10,34], in which mercury is in contact with a KCl-solution saturated with calomel (Hg_2Cl_2) [41]. The calomel electrode is less popular nowadays due to the toxicity of mercury and mercury salts [4,84]. Finding a good reference electrode for non-aqueous solutions can be tricky [34,40,80] and therefore different quasi-reference electrodes are often used [40,83].

As it is a liberty and a display of expertise of an electrochemist to choose a suitable reference electrode for a particular measurement [3,72], it is very important to advice precisely the reference system used in the described experiment. There is a bothersome negligence in the literature to report the applied reference electrode. Referring to a "Ag/AgCl" electrode can be especially confusing, as it may stand for a quasi-reference electrode or a conventional $Ag/AgCl/Cl^-$ electrode with a filling solution, whose concentration and /or cation can be varied, affecting the potential the reference has versus the standard hydrogen electrode.

2.4.1.1 Restrictions with conventional reference electrodes

Problems related to the conventional reference electrode with an internal filling solution are its size, leaching of the concentrated filling solution and thus contamination of the sample, poor tolerance towards pressure and a need for maintenance (replacement or refill of the solution) due to evaporation and leakage. Additionally, the liquid junction potential present in REs of the conventional design is undesirable and can even give rise to large measurement errors if clogged [14,72,73,84]. All these drawbacks limit the use of reference electrodes with liquid components in remote autonomous sensing applications [85].

2.4.2 Solid-state reference electrodes (ssRE)

It cannot be enough emphasized that half of the response of the potentiometric sensor comes from the reference half-cell. For a long time, all research under the title solid-state ISEs was really focused on ISEs, resulting in an asymmetric situation with modern, miniaturized solid-state ISEs and conventional macroscopic REs with internal filling solution. When most of the research groups working in the field of potentiometric sensors were already able to prepare solid-state ISEs, the lack of a good solid-state reference electrode was found very troublesome [86-90]. Also, it proved very difficult to prepare a well-functioning solid-state reference electrode [72,91] that was even referred to as the Holy Grail of electrochemistry [92,93].

Desirable characteristics for a solid-state reference electrode are reliability, stability, low cost, adequate life time and shelf-life (depending on the application) and reproducibility of the potential from day-to-day and electrode-to-electrode. With direct potentiometric measurement it is possible to achieve accuracy in the range of 0.1 mV (or even 0.01 mV) which translates to 0.4% (0.04%) measurement error in the activity for monovalent ions and 0.8% (0.08%) for divalent ions, though often in practice the imprecision is much larger [4,11]. Accuracy of the measurement can be improved by choosing potentiometric titration instead of direct measurement [13]. Precision required for clinical analysis of blood electrolytes is in the range of 0.05-0.1 mV [17, 28]. From a manufacturing point of view, it would be beneficial to be able to produce both the ISE and the RE component of a potentiometric sensor with similar techniques and from same or similar materials [17,66,73,89,90,94-96]. With the modern printing technologies available, planar constructions are especially attractive [51,72,88,97-99].

The early attempts to improve the RE construction were often focused on the miniaturization of the conventional Ag/AgCl/Cl^- electrode as such, or by capturing the chloride-containing filling solution into a gel-like electrolyte or some other matrix [72,89,100]. Problems arose from the limited lifetime of these designs due to the dissolution of the thin AgCl layer in high concentration KCl solutions [84,101], and on the other hand, due to the limited reservoir of Cl^- -ions [10,102] in the miniaturized, often solidified internal filling solution. The risk of contaminating the sample with ions leaching from internal filling solution still remained [72,103]. For disposable sensors, the limited lifetime of miniaturized Ag/AgCl/Cl^- system is not a problem. Mroz et al. [89] have reported planar Ag/AgCl/Cl^- construction sealed between two films. These REs responded only with few millivolts to changes in NaCl or KCl concentration, and were practically not influenced by changes in pH range from 3 to 11. Stability of their potential was reported to last at least for one day. Unfortunately no data on reproducibility of the potential from electrode to electrode is shown.

Several materials to host KCl in such constructions have been used, for example silicone rubber that shows good adhesion to the Ag/AgCl surface [104], and more typically hydrogels [95] such as agar [97,105]. Improvement was achieved by protecting the KCl-saturated solidified electrolyte with polymer coatings like chloroprene rubber [97], mixture of polyurethane and cellulose acetate [95] or double layers of PVC and cellulose nitrate [105] thus slowing down the escape of Cl^- -ions needed to stabilize the potential [102]. Liao et al [97] reported that the potential of their Ag/AgCl/KCl(agar)/chloroprene rubber referernce electrode stays within 10 mV when changing concentration of numerous electrolytes from 10^{-6} to 0.3 M. The polymer coating may be permselective for cations (for example thermally cured Nafion), in order to trap the Cl- ions inside [104,106,107], or possess some other discriminating characteristic promoting the insensitivity of the RE potential to the changes in the sample [88].

One of the first very promising approaches for improving reference electrodes was the Refex-electrode from the mid 90's, in which the Ag/AgCl/$KCl_{(aq)}$ system was shielded with a KCl doped solid vinylester resin junction [86], later used also in an all-solid-state planar configuration [108]. Also other efforts to design solid

REs by inserting KCl into strong, hardening polymers and resins have been made throughout the years [72].

After 20 years since Refex, Lewenstams group has recently developed a solid-state reference electrode, in which a Ag/AgCl element is covered with a mixture of an inorganic salt (KCl) and a polymer (polyvinyl acetate) composite forming a reservoir of Cl^- and contact to the sample solution [109]. Leakage of KCl from the composite RE to the sample is minor, but it can nevertheless be manufactured also as a "double junction electrode" by adding a layer of composite hosting lithium acetate instead of KCl. The solution is thus protected from any KCl contamination. The composite can be prepared by photo-polymerization [109] or by injection molding [110], which is an attractive approach from the mass production standpoint. This electrode is one that can truly compete with the conventional liquid-filled RE in terms of potential stability. Its potential remained within ±0.5 mV in 0.1 M KCl for more than two months. It also showed very good potential stability (±1 mV) in "multi-solution protocol" (MSP) test that was developed by Mousavi et al [109] to reveal the effect of the electrolyte concentration (ranging from de-ionized water to 3M KCl) and the type of the electrolyte on the response of the studied reference electrode. The MSP test is very demanding for any reference electrode, and is very informative on its performance. A lack of a generally accepted uniform evaluation and characterization procedure hampers comparison of many novel solid-state reference electrodes.

Other main approaches and milestones for preparing solid-state reference electrodes have been the use of PVC membranes containing lipophilic salts. For example teraphenyl borates of quaternary ammonium ions that partition slowly from the membrane to the aqueous phase were discussed by Vincze and Horvai in the late 90's [111]. This approach is studied further in Paper I. A similar concept, partitioning of a moderately hydrophobic salt between the membrane and the sample phase, creating thus local distribution equilibrium, has been used when doping membrane with ionic liquids [96,112] or using ionic liquids as a salt bridge into which AgCl is dissolved [113]. Zhang et al [112] report an excellent long term potential stability for a reference electrode with a three dimensionally ordered macroporous carbon (3DOM) solid contact, impregnated with an ionic liquid. The PVC membrane covering the macroporous carbon contains 20% (wt) of the same ionic liquid. Potential drift of this electrode is reported to be only 0.042 mV/h during 26 days. Unfortunately no data on the potential stability of the proposed reference electrode against changes in electrolyte concentration is presented.

Ionic liquids have also been added to the membrane to maintain a constant chloride concentration at the Ag/AgCl element [87]. Poly-ion sensitive membranes have been suggested [94,114], as well as outbalancing of cationic and anionic response by using conducting polymer bilayers of opposite ion-exchange characteristics [115].

The conducting polymer bilayer can be protected against redox interferences with suitable PVC [116] or other matrix polymer [66, 117] membranes possessing mixed cation/anion exchange properties. Kisiel et al [66] have use poly(n-butyl acrylate) matrix and report slopes less than 1 mV/dec when calibrating their reference electrode in several electrolytes from 10^{-1} to 10^{-5} M and minimal pH response 0.1 mV/pH unit in the pH-range from 2 to 12 .

In an early work by Nagy et al. [118] a combination of cation and anion-sensitive ISEs together as one complete RE, where cationic and anionic responses would cancel out each other, was suggested. An interesting solid-state RE based on carbon nanofiber composite, with limited ion flow through the membrane, has also been presented [85]. The authors believe that balance in acidic and basic constituents of the surface functionalities of the stacked graphene layers of the carbon nanofibers enable independency of the potential on changes in the electrolyte composition. The changes in potential are small indeed, 10 mV over pH range from 2 to 12 and 4 - 12mV when increasing concentration of different salts from 10^{-4} M to 10^{-2} M, but still not as small as those of conventional liquid-filled reference electrodes.

Blaz et al. [92] have proposed a solid state RE based on conducting polymers for clinical applications. The conducting polymer layer was doped with a pH buffering ligand making the film responsive to H^+, while keeping its potential constant within the specific pH range characteristic for the biological fluids like blood and urine. Noh et al. [98] suggested a pH-buffered polymer junction for solid-state RE in a disposable pH sensor.

In some cases, the problem of the reference electrode can be circumvented by using an electrode selective for an ion, whose activity can be kept constant, as a RE [14,103]. In a recent, very impressive performance of ISEs, in the wet chemistry lab of Phoenix Mars Scout Lander, two Li^+-ISEs were used as reference electrodes [119,120].

3. Towards portable and remote potentiometric sensing

The tremendous progress made within "The New Wave of ISEs", mainly concerning the significant improvements in the lower detection limit, fundamental theoretical understanding of the working principles of the response, and the all-solid-state configuration, holds enormous potential for the modern world sensing applications [8,9,121]. The momentum brought to the research field by these improvements is fortunate, considering that many of the major issues concerning practical implementation to new biomedical and environmental applications are still not completely solved [122]. As sensors are not ideal, emphasizing one desired feature comes often at the expense of others, and thus most important qualities for a specific application should be specified.

For vanguard-applications with simple, disposable sensors for screening of large amount of samples, from which the distinguishable ones will be sent to further analysis [123], the most important qualities are the price and easy operation rather than the long term stability or superb sensitivity [23,72]. Then again for sensors used in clinical analyzers in hospitals, where the number of samples per day is enormous and doctors need to make decisions on patient treatment based on the analysis results, the reliability, sensitivity and speed overbalance having sensors in a solid state or calibration-free [17]. Sensors operated in remote locations to perform long-term environmental measurements must then again above all be robust and stable, and if possible, calibration-free or very simple to calibrate, as maintenance of such sites is troublesome and should be minimized [14,26,75,112,122,124-126].

Many of the desired characteristics for the portable and remote potentiometric sensing are linked together: being calibration-free or very simple to calibrate is essential for disposability [33]. Stability and reproducibility of the standard potential E° are prerequisities for calibration-free measurements with ISEs [127]. Miniaturization is essential for the development of multielectrodes (the topic of Paper II) that could detect several analytes at the same time [128] and the feasibility of preparing both sensor components, the ISE and the RE in a solid state is of primary importance for the mass production of low-cost, miniature sensor systems [95,98,108,129], since also mass-production and low cost are linked together [8,23] as are low-cost and disposability of sensors [122].

Disposable sensors are desirable from many perspectives. They can be very practical analytical tools in an on-site testing [89]. In order to be able to prepare disposable sensors, the price must be kept low by for example using commercially existing, in-expensive materials [97,130]. To have truly disposable sensors, both the ISE and the RE should be disposable, as is the case of all-plastic potentiometric cells reported by Michalska's group, where PEDOT-PSS (Baytron P) functions both as an ion-to-electron transducer and an electrical contact, when a conventional, PVC-based ion-selective and reference membrane are applied on them [131-133]. Disposable pH sensors have been presented by Musa et al.[134], using though a Ag/AgCl quasi RE, and Noh et al. [98], whose RE was based on a pH buffered polymer junction.

Unfortunately, at the moment, the piece-to-piece reproducibility of solid-contact ISEs with plastic ionophore-based membranes is not ideal and does not allow a replacement of one electrode with another without calibration [4,135]. Much attention has been paid to the reproducibility of sensors from the same manufacturing batch and to the batch-to-batch reproducibility [75], which seems promising with mass-fabrication techniques available and adaptable for solid-contact ISEs [24,129].

3.1 The Stability and the reproducibility of the standard potential E° in potentiometric sensors

The reproducibility and stability of the standard potential E° for the ISEs is a challenge [8]. Reproducible slope values for solid-state ISEs are often reported in the literature, while no mention of the reproducibility of the standard potential in time, or between electrodes are given. This would be very important information for the reader to be able to evaluate the stability of the presented configuration, and especially the quality of the used solid-solid contact, as pointed out by Lindner and Gyurcsányi [74].

One cause of the potential drifts of the solid-state ISEs is the accumulation of a thin aqueous layer behind the ISM [136,137]. It can be found at the interface of the electric contact in the case of a coated wire electrode (CWE) arrangement or at the ISM | solid-contact interface, if such exists, due to the tendency of the polymeric membranes to sorb water [4]. The water layer functions as a miniature internal filling solution, and as the membrane is permeable to ions, the composition of the water layer is influenced by that of the sample [136,138]. One big branch of ISE research is dedicated to the understanding of the mechanism of this water layer formation and its influence on the potential stability, and to the attempts to avoid accumulation of water by for example using as hydrophobic solid-contact and membrane materials as possible [136,139-141].

This instability of the E° can be compensated with frequent calibrations [23] as is done with automated clinical analyzers [18], but the E° must be stable and reproducible at least in between two calibrations [127]. The importance of a reproducible standard potential is becoming more and more evident, as the current research trends are heading for disposable sensors, point-of-care diagnostics and remote environmental sensing [14,23,122,142], all of which would benefit greatly from a calibration-free configuration or at least from very simple calibrations [26,129].

Generally, a lack of a comprehensive standard protocol for the evaluation of the new ISEs and the solid contacts has been found to be problematic in the evaluation of results from different research groups [68,74,143,144]. There are though some generally adopted tests like the water layer test discussed already earlier [136] and the current-reversal chronopotentiometry test for the evaluation of the stability of the solid-contact [45,145]. Considering the importance of a reproducible and stable E° in current application fields, description of the E°-characteristics must become a standard procedure when introducing new solid-contact materials for ISEs, as is already done in many cases [146,147]. It is nevertheless more common to report the reproducibility of the calibration slope and drif of the electrode potential in some particular solution.

There have been several strategies to improve the stability and the reproducibility of the standard potential of potentiometric sensors. Vázquez et al. [147] showed that it is possible to increase the stability of the E° by cross-linking the ion-to-electron transducing conducting polymer layer with multivalent cations. Best results were obtained when ruthenium redox couple ($Ru(NH_3)_6^{2+/3+}$) was used with complexing agent. The E° values of four identical electrodes had standard deviation of ± 7mV after one day of conditioning, and ±9 mV after 14 days of conditioning. Their mean potential was though 15 mV lower because of the long term drift. Gyurcsányi et al. [51] compared the potential stability and the reproducibility of K^+-selective microfabricated ISEs when Cl^- -containing hydrogel or a conducting polymer polypyrrole (PPy) doped with a redox pair hexacyanoferrate(II)/(III) (FeCN) was used as inner contact. After 9 hours of conditioning, the standard deviation of the potential of seven hydrogel-contact electrodes was 24 mV, while that of the PPy/FeCN solid contact electrodes was 19.8 mV

Zou et al. [135] showed that doping the PVC-based ion selective membrane with Co(II)/Co(III) redox couple could improve the reproducibility of the E°. The standard deviation of the E° of three gold electrodes (coated wire arrangement) could be improved from ± 63.5 mV to ±1.7 mV when an optimized ratio of the redox pair was added to the membrane. The standard deviation was further decreased down to ±1.1 mV when a redox-active self-assembled monolayer was used as solid contact under the redox pair containing ISM [135].

Lindfors et al. [141] have studied effect of a hydrophobic conducting polymer poly(3-octylthiophene) (POT) as a solid contact in Ca^{2+} selective electrodes. They report that the standard deviation of the reproducibility of the E° for CWE-type Ca-ISE can be improved from ±27.6 mV to ±6.7 mV when POT is introduced solid contact and three replica electrodes were studied in both cases.

The choice of a suitable cleaning step before the deposition of a conducting polymer solid contact has been found important [148]. The post polymerization treatments of the conducting polymer film with cyclic voltammetry [149] or with a constant potential [150] were found to improve the reproducibility of the E°. Reproducibility of the E° of the conducting polymer-based solid-state ISEs is the topic of Papers IV and V.

4. Solid contact materials in ion selective electrodes

In well-functioning ISEs, a reversible mechanism for the changing of the ionic conductivity of the membrane to the electronic conductivity of the circuit is required. In the absence of the traditional internal reference electrode and the filling solution with its well-defined redox system, a material capable of similar ion-to-electron transduction is needed in solid-state constructions to avoid a blocked interface between the membrane and the metal. This transducer layer is referred to as the solid contact, and it should provide a stable potential between the ISM and the metal [4,71].

Many different types of electroactive materials showing mixed electronic and ionic conductivities have been studied as solid contact materials [8]. Generally, a good solid contact material should show either a large redox capacitance [45] or a large double layer capacitance in order to be able to stabilize the potentiometric response of a solid-state ISE [8,51,74].

The introduction of the conducting polymers as solid contacts of the ISEs in the early 90's [79] put them in the focus of the ISE research community, and significant improvements in analytical performance of this type of ISEs have been seen over the years [8,151]. Conducting polymers are considered as one of the most promising transducer materials for ISEs despite some drawbacks.

4.1 Conducting polymers

4.1.1 Introduction

Conducting polymers (CP) were discovered in the mid-70's by Alan Heeger, Alan MacDiarmid and Hideki Shirakawa [152,153] who received together the Nobel prize in chemistry year 2000 "for the discovery and development of conductive polymers" [154]. The finding of the conducting polymers was so exciting because they possessed a unique combination of properties not found in any other known materials; electrical and optical properties of metals or semiconductors and advantageous mechanical properties and processing advantages of polymers [155]. The applications and the research branches for the conducting polymers are numerous, ranging from batteries, sensors and different types of membranes to light emitting diodes (LEDs) and photovoltaic devices (i.e. solar cells) to biological and biomedical directions [156].

Traditionally, polymers and plastics have been categorized as insulators [157]. When all the valence electrons of the carbon backbone are covalently bound, there are no mobile electrons available to participate in the charge transduction [157]. The electronic configuration of the conducting polymers is nevertheless fundamentally different from the common polymers [155]. Their backbone consists of alternating single and double bonds that are formed between sp^2p_z – hybridized carbon atoms, and are thus also called "conjugated polymers" [157].

While the three sp^2 orbitals form covalent σ-bonds to the neighboring carbons or other atoms, the p_z –orbital forms a π-bond that is delocalized over several atoms [157].

In their pristine, un-doped state, all conducting polymers are semiconductors or insulators. In analogy with processes of the semiconductor physics, the oxidation of the CP is called p-doping. In p-doping, an electron is removed from the π-electron system leaving a positive hole behind. This hole functions as a positive charge carrier. Correspondingly, the reduction of a pristine CP is called n-doping, where an electron is added to the delocalized π-orbital [157]. The positive p-doping (oxidation) is much more common than the reductive, negative n-doping [158]. P-doping is the valid process for the CP poly(3,4-ethylenedioxythiophene) (PEDOT) used in this work.

In order to maintain the charge neutrality in the system, counter-ions are introduced to the polymer structure during the doping process [130,157,159]. The conductivity of the CP can increase even with 10-12 orders of magnitude, from insulating to metallic, when transformed from an un-charged (un-doped) to a charged (doped), conducting form [130,155,158].

Conducting polymers can be divided into redox-polymers and electronically conducting polymers. In the redox polymers the electroactive sites are localized in the polymer structure and charge propagates by an electron exchange reaction (electron hopping). In the electronically conducting polymers, electrons are delocalized through the conjugated system [158]. It is the latter group that is relevant for this work.

The electrical conductivity results from the existence of the charge carriers and from the ability of those charge carriers to move [157]. In the conducting polymers the delocalization of the electrons along the conjugated backbone provides a path for mobile charges, that is, for intrachain conductivity [155]. The conductivity between two polymer chains, that is interchain conductivity, is considered to occur via an electron hopping and tunneling that are the conduction mechanisms of redox polymers with localized electroactive sites [158]. From these two, the charge propagation between the chains rather than within the chains is more likely to be the rate limiting process that determines the conductivity of the material [160]. The intrachain conduction with delocalized electrons can be described with a band model that is used for metals and semiconductors [158].

Understanding and clarifying the charge propagation in conducting polymers is one of the most important topics of the field [158,160]. There are several models describing the mechanism of the charge transport, of which the one extreme is the delocalized band model, where charges and unpaired electrons are delocalized over a large number of monomer units, and the chemical model, where the charge is localized in the polymer chain or delocalized over some monomer units [158]. According to the latter model better suited for the organic polymers from a chemist's point of view rather than a physicist's [160], storage of a charge on the polymer chain leads to a structural relaxation that in its turn localizes the charge, though this relaxation extends over several atoms in the chain [155]. These partly delocalized defects that are generally considered to be the charge carriers existing in conducting polymers are classified as solitons, polarons and bipolarons [158]. A soliton is a neutral or a charged defect state, a domain boundary between two degenerate ground states in polymers like polyacetylene [155,158,160]. Most of the conjugated polymers have non-degenerate ground states, which mean that they can exist in two different structures, whose energy levels are not equal to each other and the other form is thus energetically more favorable [157]. Polarons occur in polymers with non-degenerate ground states. They have a neutral and a charged soliton in the same chain and are actually radical ions associated with a local geometrical distortion. Bipolarons are defined as a pair of like charges, di-ions, associated with strong local lattice distortion occurring usually at the higher doping states [158,160].

Doped, conjugated polymers are good conductors for two reasons: firstly, the doping introduces charge carriers into the π-electron system, and since every repeat unit is a potential redox site, the CP can be doped to a relatively high density of charge carriers. Secondly, the π-bonding results in the delocalization of π-electrons along the polymer chains and between chains, and the broad π-electron bandwidth in the energy gap can result in relatively high charge carrier mobilities [130,157].

The shared interest of chemists and physicists towards conducting polymers as materials creates interdisciplinary possibilities, but also a risk of misunderstandings arising from different terminological backgrounds [160,161]. In addition to concept of doping used by physicists, even the term charging of conducting polymer can be used to describe an insertion of positive or negative charges on the polymer backbone by oxidation or reduction, while discharging is used to describe the opposite process [158-160].

4.1.2 Synthesis of conducting polymers

Synthesis i.e. polymerization of conducting polymers can be done either chemically or electrochemically. The first method is favored, when wanting to produce large quantities, and the latter when good control of the film formation and thus good quality of the film is of importance [158]. In electropolymerization, the synthesis of the conducting polymer and its doping/charging occurs simultaneously, as the monomer in the solution is oxidized with help of an applied potential or a current, and the charge of the forming film is balanced with counter-ions from the solution [158,160]. An experimental set-up for electropolymerization is shown schematically in Figure 6. The polymerization solution containing the dissolved monomer is often de-aerated by purging it with nitrogen or argon gas in order to prevent oxygen from interfering in the reaction.

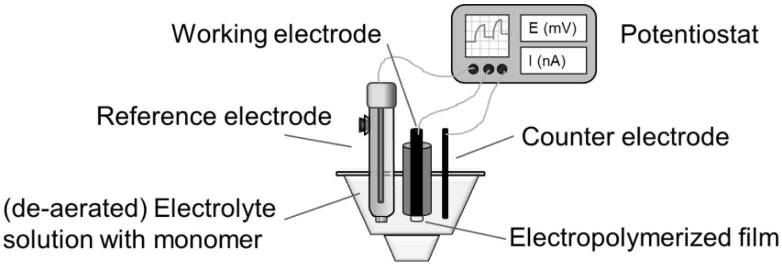


Figure 6. *A schematic picture of an electropolymerization set-up*

There is no general, comprehensive scheme for the electropolymerization process of conducting polymers, but it has been widely accepted that the first step is the formation of radicals from monomers [158,159] usually by oxidizing them. This tends to require a slightly higher potential than the reversible redox reaction of the CP film. The oxidation of the monomer is most often irreversible [158], as can also be the oxidation of the formed film if too high potentials are applied. Then the film is overoxidized and may loose its electroactivity [162]. Experimental conditions for the electropolymerization, like the applied polymerization potential or the scan rate in the case of a polymerization with cyclic voltammetry, or the current density in the case of a galvanostatic polymerization, have a strong influence on the proceeding reaction step [158]. Radical cations can form dimers, which can react further with other radicals in a stepwise chain growth, or then radicals may react with monomers in the neutral state. There can be parallel dimerization reactions occurring simultaneously, and the concentration of cation radicals close to the electrode surface should be kept relatively high [158].

The film morphology is strongly dependent on the composition of the polymerization solution and especially on the type of the counter ion incorporated into the film to balance the charge [158]. After the electrosynthesis of the CP-film, its oxidation state can be easily changed electrochemically, accompanied by movement of counter-ions in or out of the film [157,158].

4.1.3 Conducting polymers in potentiometric sensors

The unique combination of electrical, electrochemical and optical properties of conducting polymers enables conversion of chemical information into a measurable electrical or optical signal. CPs can therefore be used as transducers in chemical sensors, as indicated in Figure 1 [1,6]. Conducting polymer films can show a potentiometric sensitivity to cations or anions, even selectivity in some cases, depending on the composition of the film [163-167]. Selectivity can be conveniently attained by covering the CP-film with an ion-selective membrane (ISM), in which case the role of the CP film is to function as ion-to-electron transducer (solid contact) between the ISM and the electrical contact, not as sensing layer [79].

Conducting polymers can form an ohmic contact to materials with a high work function, like many metals and carbon materials, thus ensuring a good electrical conduction across this interface [8]. Deposition of the CP layer onto an electrode surface can be done for example by drop-casting in the case of CPs that can be dissolved or dispersed in some liquid. Many of the conducting polymers are though poorly soluble in common organic solvents, which hampers their processing [8,155,168]. An electropolymerized CP film stays on the electrode due to adhesion and van der Waals forces, and no chemical bonds exist between an electronically conducting surface and the CP film [158].

The properties of the CP film can be adjusted for example by functionalization of the backbone or by immobilization of functional doping ions into the structure [6,169]. The use of conducting polymers as sensing layers in potentiometric sensors has been widely studied [1]. CPs can have a mixed response towards anions, cations, pH and redox species, which complicates their use as reliable sensing layers [1]. Covering the CP film with an ion-selective membrane reduces this problem to some extent, combining simultaneously the high selectivity of the ionophore-based membrane and redox-properties of the CP layer, ensuring a good ion-to-electron transduction. The CP works as a solid contact as discussed above [1] and shown in Figure 5d. Even mixing of a conducting polymer directly into the membrane cocktail, from which "single-piece" ISEs could be obtained, has been studied [170].

The solution to protect the CP with an ISM is not water proof, so to speak, as water, dissolved oxygen and some ions may diffuse through the membrane. This means that the film undergoes to some extent similar side reaction processes than if it was in a direct contact with the sample solution [1,8,60]. It is evident though that the ISM slows down these side reactions considerably compared to a CP-film that is directly exposed to the sample solution [8].

The potential of the CP-film influences the overall potential of the measured SC-ISE system, as it is the sum of all phase-boundary potentials, as indicated in Figure 3. Ideally, if the potential of the CP film is stable, it can be incorporated in the E°-term of Equation 13. The potential of the conducting polymer is however an interplay between the redox state and ion activities in the film, determined by the composition of the solution it is in contact with [163,164].

The redox state (doping level) and the consequent ionic content of a CP film can be controlled electrochemically [157], which is utilized in the Paper IV, or by adjusting the chemical potential through soaking [171], or both. During the electrochemical oxidation or reduction of CPs, the overall electroneutrality of the CP phase is maintained by ion exchange processes between the polymer film and the contacting solution. Ions, solvent molecules and other neutral molecules can enter and leave the film during chargig/discharging (oxidation/reduction) porcesses depending on their physical properties like size and charge and on specific interactions with the polymer itself [158]. If the counter ion introduced to the film during the synthesis is a small, mobile doping anion (B^-), it can move freely in and out of the film during the oxidation/reduction of the polymer backbone, as shown in Equation 22. In the case of a bulky immobile anion (A^-), small mobile cations (C^+) will move in and out of the film balancing the charge of the original doping ion when the polymer backbone is oxidixed or reduced, shown in Equation 23 [157,166]. Subscripts film and solution in Equations 22 and 23 denote the respective phases.

$$(CP^{+}B^{-})_{film} + e^{-} \leftrightharpoons (CP^{0})_{film} + B^{-}_{solution} \qquad (22)$$

$$(CP^{+}A^{-})_{film} + C^{+}_{solution} + e^{-} \leftrightharpoons (CP^{0}A^{-}C^{+})_{film} \qquad (23)$$

Which of these processes occurs upon the reduction/oxidation (or doping/undoping) of the CP, depends on the type of ions in the polymer matrix [157]. Many CPs (and their derivatives) have been studied as solid contacts, like polypyrrole (PPy), poly(3-octylthiophene) (POT), polyanilines (PANI) and the (3,4-ethylenedioxythiophene) (PEDOT) [1,8]. The CP chosen for this work is PEDOT.

4.1.4 Poly(3,4-ethylenedioxythiophene) PEDOT

Poly(3,4-ethylenedioxythiophene), or PEDOT, was developed in the mid-1980's in Germany at research laboratiories of Bayer AG [172,173]. The molecular structure of PEDOT is shown in Figure 7.

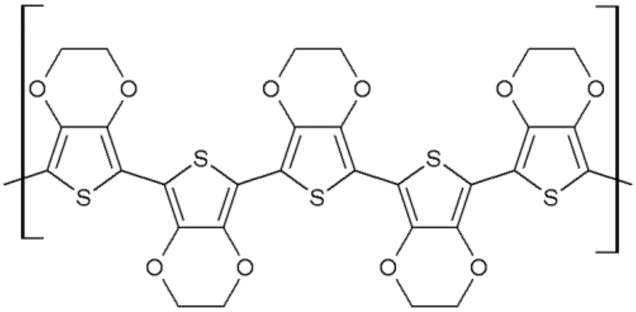


Figure 7. *The molecular structure of Poly(3,4-ethylenedioxythiophene), PEDOT*

PEDOT shows a very good electrochemical stability and durability during potential cycling, varying humidity, light and temperature, compared to many other conducting polymers [168,174,175]. It can be prepared by a chemical polymerization from the EDOT monomer using oxidizing agents, or by an electrochemical polymerization, which results in good yields and in high conductivity [174]. PEDOT can be made processable by using a polyelectrolyte like poly(styrene sulfonic acid) (PSS) as a dopant, thus forming a stable, highly conductive (10 S/cm) dispersion in water, known as the commercial product Baytron P [172,176]. This dispersion can be spin-cast, dip-coated or even printed on various substrates resulting in thin films of optical quality, an essential feature for plastic electronic applications like light emitting diodes (LED) or thin film transistors [157].

Because of the stability, processability and attractive characteristics attributed to Baytron P, it has been studied and used as a ion-to-electron transducer in ISEs [131,132,147]. The electropolymerization of PEDOT for sensor applications is also common and well-studied approach [45,138,149,177,178].

The symmetry of cyclic voltammograms recorded for PEDOT films indicates a high reversibility of the doping process, a good feature for an ion-to-electron transducer. It is also insensitive to dissolved oxygen and less influenced by CO_2 in contact with measurement solution compared to polypyrrole [138].

In this work, a PEDOT film that is to function as an ion-to-electron transducer of ISEs is prepared by electropolymerization in presence of chloride to form PEDOT(Cl) films (papres I and II) or in presence of PSS^- to form PEDOT(PSS) films (Papers IV and V).

4.2 Nanostructured carbon materials as solid contacts

A remarkable improvement in the stability of ISEs was the introduction of self-assembled lipophilic monolayers (SAM) between an ISM and a metal electrode, since they were shown to prevent the troublesome water-layer formation worsening the analytical performance of coated-wire type solid-state electrodes [136]. Even better analytical characteristics (Nernstian response) for the ISE were obtained when the SAM solid-contact was also redox-active, i.e. it was able to carry out the ion-to-electron transduction [139]. This, together with impressive results obtained by DeMarco’s group with a synchrotron radiation X-ray photoelectron spectroscopy, confirming a surface-confined ion-to-electron transduction process for a conducting polymer solid-contact in the case of a bulky ion in the membrane [67], suggests that a charge transfer reaction taking place at the surface of a solid contact is adequate. Thus also the high double layer capacitance associated with large surface area materials as solid contacts is justified.

One approach to improve the performance of ISEs has indeed been the use of different nanostructured materials [74,90,128] from various carbon structures to metal nanoparticles like platinum [146] and gold [50] as solid contacts. Niu's group has recently reported of solid-state-ISEs utilizing monolayer-protected clusters as solid contacts providing remarkable reproducibility and signal stability also for E° [48].

In recent years, different novel carbon materials have been a subject to an intensive research. Many of them, like single walled carbon nanotubes [69], multi walled carbon nanotubes [47,179], carbon black [180], graphene [181] and three-dimensionally ordered macroporous carbon [68,182] have been used as solid-contact materials in ISEs with good results. Also composites of conducting polymers and carbon, like PEDOT doped with carbon nanotubes, have been studied as solid contacts [46].

These materials were tested as solid contacts in ISEs in hope to overcome some problems related to the use of conducting polymers, like the light and redox sensitivity [128,150]. The stabilizing effect they have on the potential of an ISE is related to the high double layer capacitance that they possess due to a very large surface area of the interface between the nanomaterial and the ion-selective membrane [68,70,128].

In Paper III flexible carbon cloth was both characterized as a potential solid-contact material and used as a combined electrical conductor and an ion-to electron transducer to prepare an all-solid-state ISE and RE. Due to their high specific surface area, carbon cloths have been used in different capacitor-constructions, fuel cells and as adsorbents in different environmental applications to remove air and water pollutants [183].

5. Characterization techniques

The main technique used in this work was open circuit potentiometry. Potentiometric measurements in the Paper I were done with a homemade multichannel millivolt-meter connected to a PC, and in all the other papers with a Lawson EMF16 Interface potentiometer (Lawson Labs, Inc.) with high input impedance of 10^{15} Ω . An extra reference electrode was connected to one of the measurement channels whenever possible in order to confirm proper functioning of the main reference electrode. Chronoamperometry and chronopotentiometry used in Papers IV and V and electrochemical impedance spectroscopy used in Paper III are briefly discussed.

5.1 Chronoamperometry and chronopotentiometry

Observing how an electrical property of the cell changes with time as a response to some applied signal is a very good method to study the working electrode [13]. In chronoamperometry, current is measured as a function of time, when different potentials or no potential is applied to the system. It is a useful technique for evaluation of diffusion coefficients, rates of electrode processes, adsorption parameters and rates of coupled chemical reactions [15]. Concerning conducting polymers, chronoamperometry can be used to study the charge transport, the diffusion, phase formation, the phase transition and the relaxation [158]. In chronopotentiometry, the potential is measured as a function of time, while a controlled constant current or a series of different current steps are applied [13,15].

Chronoamperometric and chronopotentiometric measurements are usually done in a three-electrode cell with a counter electrode (CE), a working electrode (WE) and a reference electrode (RE) using a potentiostat, as shown in Figure 6 [35]. The potential of the WE is controlled vs. the RE, while the current flows between the CE and the WE [15,34]. The potentiostat itself is a feedback circuit based on an operational amplifier circuit that reduces the current between the WE and the RE to zero thus reducing the unwanted IR-drop close to zero [13,34]. The IR drop exists always when a current I is forced through a solution, according to

$$\Delta E = \Delta V - IR_{solution} \tag{24}$$

where ΔE is the measured potential and $R_{solution}$ is the solution resistance. The IR-drop varies with the solution resistance and the current passing, and can thus distort measurement results [34]. In the zero-current potentiometry this is not a problem, since only a negligible current is allowed to flow through the system, but if higher currents are involved, a three-electrode cell and a potentiostat capable of processing the IR drop should be used [34].

All electropolymerizations of this work were done in a galvanostatic mode using an Autolab General Purpose Electrochemical System (AUT20.FRA2-Autolab and AUT30.FRA2-Autolab, Eco Chemie, B.V., the Netherlands). The chronopotentiometric and chronoamperometric measurements in the Paper IV were performed with an IVIUMSTAT (Ivium Technologies, The Netherlands).

5.2 Electrochemical impedance spectroscopy (EIS)

Impedance can be understood as a generalized resistance that takes into account phase differences [184]. It is an excellent tool for characterizing electrochemical systems, whose behavior is determined by a number of strongly coupled processes that proceed at different rates [185]. In electrochemical impedance spectroscopy (EIS), a small, sinusoidally varying perturbation (excitation) potential is applied to an electrochemical cell, and the resulting time-dependent current is recorded with a time-lag (a phase shift or a phase angle φ) between the applied potential and the resulting current [80,186]. The time dependency of the applied potential and the measured current is shown in Equations 25 and 26, where ω is the angular frequency 2πf.

$$e = \Delta E \ \sin(\omega t) \tag{25}$$

$$i = \Delta I \ \sin(\omega t + \ \phi) \tag{26}$$

The potential is typically set to oscillate around the equilibrium potential [34] or some other potential of interest [40] with a very small amplitude, around 5-10 mV. Due to the sinusoidal oscillation of the excitation signal, the net perturbation is zero and the system can be studied in steady state conditions [40,186]. Keeping the amplitude of the excitation potential small allows linear treatment of the current-potential relationship with a simplified Buttler-Volmer equation [34,187].

With an analogy to the Ohms law, the impedance Z of a system is given by Equation 27 where e and i are the frequency dependent voltage and current respectively [10]. Z is often presented in a complex notation where the real (Z′) and the imaginary (Z′′) components represent the resistive and the reactive part of the impedance as given in Equation 28 where $j = \sqrt{-1}$ [41,186,187]

$$Z = \frac{e}{i} \tag{27}$$

$$Z = Z' - jZ'' \tag{28}$$

One of the most typical ways to represent impedance data is in the Argand diagram (Nyquist plot) where Z′′ is on the y-axis and Z′ on the x-axis, both as a function of the frequency [34] . In a vector form their modulus ǀZǀ shows also the phase angle φ, as seen in Figure 8a.

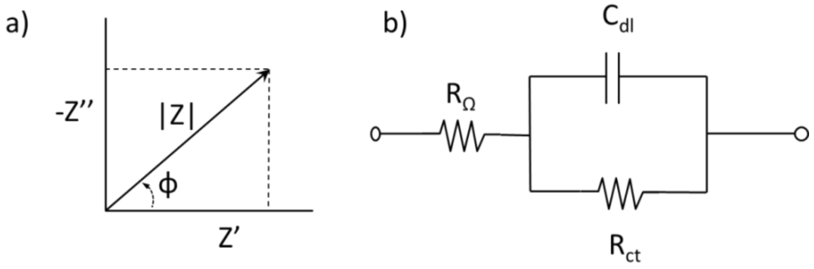


Figure 8. *a) A complex and a vector representation of the impedance and b) the Randles equivalent circuit where* R_s *represents the solution resistance,* R_{ct} *the charge transfer resistance,* C_{dl} *the double layer capacitance and* Z_w *the Warburg impedance*

Another possible graphical representation of the impedance data is the so called Bode-plot, where log ǀZǀ and the phase angle φ are plotted versus the logarithm of the frequency. The Bode-plot representation allows a better inspection of the frequency-dependency of the systems impedance than the complex plane graph, where frequencies are not visible unless separately indicated for each or selected data points. Thus the Bode-plot is better suited for systems with several time constants [80].

The frequency range of an impedance measurement should be chosen wide enough in order to obtain information on processes occurring in and dominating the studied system on different time scales [34,187]. A typical range is 100 kHz – 10 mHz.

The impedance measurement can be done either in a two-electrode symmetrical cell with two identical electrodes or in a conventional three-electrode cell with a working electrode, a counter electrode and a reference electrode [188]. Use of the two-electrode measurement configuration can be especially useful when incorporation of a reference electrode to the system would be difficult due to the cell geometry, or due to a lack of a suitable reference for the electrolyte used, as was the case in impedance measurements for Paper III.

The analysis of an impedance spectra of an electrochemical system often requires a construction of an equivalent circuit that mimics the behavior of the electrode reaction. Equivalent circuits consist of passive elements, resistors and capacitors, all of which have a physical meaning in the electrochemical cell like the solution resistance or the double layer capacitance at the electrode | solution interface [10,34,40,186,187]. Modelling and understanding the dynamic behavior of electrochemical systems with equivalent circuits is both a powerful as well as a vulnerable point of impedance spectroscopy. Very useful information can be obtained with a good model, but one should always bear in mind that several different equivalent circuits may give the same impedance spectra, and that same circuit may fit several models. In other words, there is a danger of oversimplification and misinterpretation [10,34,184]. EIS is a powerful characterization tool, but it should be used in combination with other techniques or on known systems to make reliable conclusions [187].

The impedance of an electrochemical cell is often modelled with the so called Randles equivalent circuit that is shown in Figure 8 b. The total current through the surface induced by the oscillating excitation potential is the sum of the faradaic processes and the double layer charging. The latter one gives rise to a nearly ideal double layer capacitance C_{dl}. The Faradaic processes are represented by the charge transfer resistance R_{ct} and the Warburg impedance Z_w that can be understood as a resistance to the mass transfer. All charge must pass the solution resistance, which is given by R_S [40].

Passive elements used in equivalent circuits are ideal resistors and ideal capacitors, which is not the case in real electrochemical systems, even though the real impedance Z′ behaves largely like a resistor and the imaginary impedance Z′′ as a capacitor [186]. To make the model fit better to a real spectrum, a constant phase element may be added to the equivalent circuit to compensate for the non-idealities such as a non-uniform surface [184]. The uncertainty of the conclusions made from EIS-spectra increase naturally with an increasing complexity of the system, when the number of the fitting parameters expands [34].

Electrochemical impedance spectroscopy has been found to be a good tool for studying electroactive polymers (that is, redox polymers with immobilized redox centers and conducting polymers with delocalized charges) [158,189-191]. The small perturbation of the exciting signal allows for the system to be investigated under nearly steady-state conditions, which is a clear benefit as larger perturbations would shift parts of the polymer film to excited states causing inhomogeneity of the film [40,191]. An EIS experiment that covers a large range of frequencies, and thus time scales, can give information on many different processes occurring in an electrochemical cell, such as charge transfer kinetics at the surface of the electrode and charge transport and diffusion in the solution phase [34,158,192].

EIS has been used to study the charge transfer kinetics at the ISM | solution interface [193] and also the charge transfer, the ion diffusion and the capacitance of a conducting polymer | solution interface [194-197]. Also doping of conducting polymers, ion diffusion inside the film, capacitive properties and separation of the faradaic and capacitive processes of conducting polymer films can be studied with EIS [191,192]. Even other types of solid-contacts like carbon-based materials [70,198] and monolayer-protected clusters [48] have been characterized with EIS.

The impedance measurements for this work were done with Autolab General Purpose Electrochemical System (AUT30.FRA2-AUTOLAB, Eco Chemie, B.V., the Netherlands).

6. Results and discussion

6.1 Solid-contact reference electrodes based on lipohilic salts

When this thesis was started, the lack of a solid-state reference electrode was striking. As both the half-cells (the ion-selective and the reference) should be in a solid state in order to have an all-solid-state sensor, aiming for a solid state reference electrode was a natural starting point. Inspired by some earlier experimental observations in our laboratory, and in agreement with the early theoretical discussion by Vincze and Horvai concerning partitioning of lipophilic ions between the membrane and the sample phase [111], a very systematic study of different ammonium and borate salt containing reference membrane candidates was performed.

6.1.1 Preparation of studied reference membrane candidates

Two types of membranes were prepared. Three different "single salt" membranens contained commercially available tetrabutylammonium-tetrabutylborate (TBA-TBB), tetrabutylammonium-tetraphenylborate (TBA-TPhB) or tetraheptylammonium-tetraphenylborate (T(hept)A-TPhB). To obtain more information on the effect of the alkyl chain length (from ethyl to octyl and dodecyl), various tetra(alkyl)ammonium chlorides were also mixed with potassium-tetra(phenyl)borate forming "double salt" membranes on the assumption that when dissolved in the membrane phase, KCl and tetra(alkyl)ammonium-tetra(phenyl)borate would dope the membrane. During an earlier work [199], the optimum weight percentage of TBA-TBB in the reference membrane was found to be 12.5% (w/w) and thus the corresponding molar amount of other salts was used for the respective membranes studied. The remaining membrane constituents were 1/3 poly(vinyl chloride) (PVC) and 2/3 o-NPOE as a plasticizer.

During this work the standard procedure adopted was to always include an extra reference electrode as a working electrode in all potentiometric measurements, in order to guarantee the validity of the results and the proper functioning of the main reference. This was done because the reference electrode is known to be one of the most common sources of errors in potentiometric measurements. All the prepared electrodes were measured according to the same measurement protocols (given in Table 1), aiming to evaluate the performance of the prepared reference membrane candidates in different situations. The focus was on their insensitivity towards changes in the electrolyte concentration and composition, as well as on their response time.

Table 1 *The potentiometric measurement protocols for evaluating the studied reference membrane candidates*

Protocol	order of solution	solution	time
I (long KCl)	1	0.1 M KCl	10 min
	2	0.01 M KCl	10 min
	3	0.1 M KCl	10 min
	4	0.01 M KCl	10 min
	5	0.1 M KCl	10 min
II (salts)	1	0.1 M KCl	10 min
	2	0.0001 M KCl	10 min
	3	0.0001 M NaCl	10 min
	4	0.1 M NaCl	10 min
	5	0.1 M KCl	10 min
III (fast-response)	odd	0.01 M KCl	30 s
	even	0.1 M KCl	30 s
IV (overnight)	1	0.01 M KCl	18 hours

Two types of disk electrode substrates were used, a Ag/AgCl and a glassy carbon (GC), onto which a 10 mC film of PEDOT doped with Cl^- had been electropolymerized galvanostatically using a constant 0.02 mA/cm^2 current. Two replicate electrodes were prepared by drop-casting 100 μl of the respective membrane cocktail on the Ag/AgCl and GC/PEDOT(Cl) substrates.

6.1.2 Comparison of the different membranes

When comparing the three "single salt" membranes, it was obvious that the membrane containing TBA-TBB showed the best insensitivity towards the changes in the electrolyte, but its response was rather slow. The other two "single salt" membranes showed a faster response, but were more sensitive to the changes in the concentration. The dissociation of TBA-TBB into two similar sized and equally mobile equitransferent ions of moderate lipophilicity was hypothesized to be the origin of its desired insensitivity. The molecular structure of TBA-TBB is shown in Figure 9. An unbalanced partitioning of a cation and an anion of a lipophilic salt (or an ionic liquid) between the membrane phase and the sample phase would lead to ion-exchange, and thus to an ionic sensitivity of the membrane [96]. The purity of the commercially available TBA-TBB salt was only 98% (Fluka), during the time of the research conducted for Paper I. Later, the manufacturer decreased it to 97%, so that the effect of impurities on the response cannot be completely excluded.

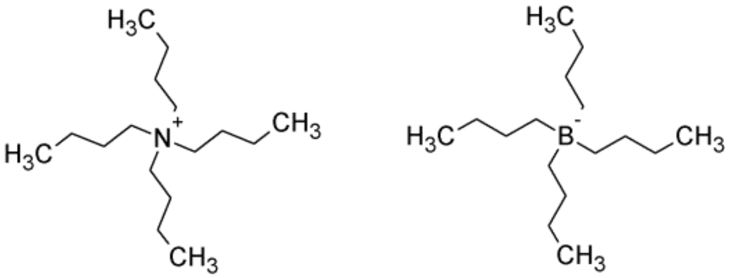


Figure 9. *The molecular structure of the tetrabutylammonium-tetrabutylborate salt*

In the "double salt" approach, KCl was also present in the membrane (as K^+ and Cl^- ions). Some confluence can be found with the approach used by Michalska's group, where in addition to introducing both cationic and anionic sites to the membrane, KCl was also added to the membrane alongside AgCl containing traces of metallic silver [66,116,131]. For two "single salts" (TBA-TPhB) and (T(hept)A-TPhB), corresponding "double salts", i.e. tetra(butyl or heptyl)ammonium-Cl^- and K-tetra(phenyl) were available, and thus the "single" and "double salt" approaches could be compared for these membranes.

In both cases, the response time and the insensitivity towards the concentration changes improved when the "double salt" approach was used. The difference was more pronounced when comparing (T(hept)A-TPhB) and (T(hept)A-Cl^- + K^+-TPhB) with a larger lipophilic cation, for which the "double salt" membrane showed a clearly better reproducibility of the potential.

When comparing all the membranes containing a double salt, (tetra(alkyl)ammonium chlorides mixed with potassium-tetra(phenyl)borate), no clear trend on the effect of the alkyl chain length on the reference membrane performance was found. The T(propyl)ACl+TPhB membrane showed though the best potential reproducibility, a good response time and the smallest sensitivity towards changes in the concentration.

A potential drawback of the “double salt” approach is the relatively high leakage of the highly water soluble KCl from the small KCl “reservoir” of the membrane.

6.1.3 *TBA-TBB reference membrane*

After the critical evaluation of all studied membranes, the TBA-TBB membrane was chosen as the solid-state reference for this work. The representative potential response of this membrane on two different substrates is shown in Figure 10.

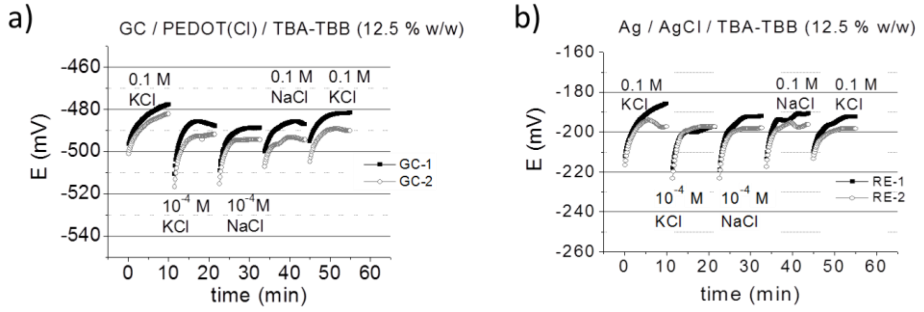


Figure 10. *The potential behavior of the PVC reference membrane containing TBA-TBB salt on a) a GC/PEDOT(Cl) or b) a Ag/AgCl substrate in a test where the electrolyte was varied from 10^{-1} M KCl to 10^{-4} M KCl, 10^{-4} M NaCl, 10^{-1} M NaCl and 10^{-1} M KCl, 10 min each. Measurements are done after an 18 h soaking in 10^{-2} M KCl. [b) is modified Figure 2 in Paper I]*

Immersion of the electrodes into a new solution is always followed by a typical potential drift from lower potentials stabilizing after a few minutes of contact with the solution.

The mechanism of the potential formation is considered to depend on the partitioning of TBA-TBB between the membrane and the sample phase, as shown in Equations 29 and 30 [4]

$$a_{TBA(memb)}a_{TBB(memb)} = k_{TBA-TBB}a_{TBA(aq)}a_{TBB(aq)} \tag{29}$$

$$\frac{C_{TBB(memb)}}{a_{TBB(aq)}} = \frac{k_{TBA-TBB}a_{TBA(aq)}}{C_{TBA(memb)}} \tag{30}$$

and the measured potential is the phase boundary potential given in Equation 31 [2,111] where k_i is the partition coefficient determined by the phase transfer free energy, a_i the activity of the ion i in the aqueous sample phase and $[i^z]_{memb}$ the concentration of the ion i in the membrane phase. All these parameters are determined only by TBA^+ and TBB^- ions. As the membrane is the only source of the TBA^+ and TBB^- ions, the potential of the prepared reference electrode is independent from the composition of the sample.

$$E_{PB} = E° + \frac{RT}{zF}\ln\left(\frac{k_i a_{i(aq)}}{[i^z]_{memb}}\right) \tag{31}$$

The charge transfer at the CP | membrane interface for the GC/PEDOT-Cl/membrane electrodes is expected to be well defined. The small doping ion Cl^- is able to move between the membrane and the CP phase, and as shown recently by De Marco [67], even large lipophilic ions from membrane (TBA^+ or TBB^- in this case) can move into the CP phase. In the case of the "double salts", Cl^- is readily present in the membrane phase, and thus there is a well-defined charge transfer also with the Ag/AgCl substrate. Assumption of the participation of the Cl^- ion to the charge transfer is supported also by the improved response of the "single salt" electrodes after an overnight conditioning in 0.01 M KCl (protocol IV), during which some KCl can move into the membrane phase.

6.2 All-solid-state potentiometric sensors with a TBA-TBB reference membrane

From the data presented here, and on the basis of the discussion in literature, it seems that reference electrodes based on lipophilic salts will not be able to reach the superior reliability level of Ag/AgCl/Cl^- -reference system. In addition to the requirement for equal lipophilicities and diffusion coefficients in the membrane phase for the anion and the cation of the salt pair, the lipophilicity of the analyte ion pairs in the sample phase should be balanced in order to avoid response [85, 94, 111]. This contradicts the requirement for the potential independency of the reference electrode from the sample. Reference membranes based on lipophilic ions can nevertheless be good quasi reference electrodes in chosen applications. It is evidently very advantageous to be able to prepare both electrodes in a potentiometric sensor, the ion-selective and the reference electrode, using the same technology and in a solid state [73,90], and this is where the strength of the TBA-TBB reference membrane can be found.

The reference membrane developed in Paper I has been used to complete the all-solid state potentiometric sensors in Papers II and III. It was one component of the multi-electrode platform where gold/PEDOT(Cl) was used as the substrate (Paper II) and drop-cast directly on a carbon cloth substrate (Paper III).

The TBA-TBB membrane has also been applied on screen-printed carbon electrodes, onto which PEDOT(Cl) was first electropolymerized [129], proving that this membrane works well on many different substrates. In recent, yet unpublished results, the TBA-TBB –reference membrane was successfully used in combination with paper-based ISEs, where filter paper impregnated with carbon nanotube ink was covered with an ion selective membrane [52] or a reference membrane.

6.2.1 All-solid-state multielectrode

The multielectrode platform used in Paper II was developed by collaborators at the AGH-University of Science and Technology in Krakow. It contained 7 gold disks of 1 mm diameter integrated into a polyacrylate-based body and soldered to individual wires for connection to a potentiometer. The construction of the pen-like multielectrode probe is schematically shown in Figure 11. PEDOT(Cl) was electropolymerized onto surfaces of all 7 gold disks to function as an ion-to-electron transducer. The Au/PEDOT(Cl) spot in the middle of the array was then covered with a reference membrane based on TBA-TBB described above, and the 6 remaining electrodes were covered with 3 x conventional Pb^{2+} -selective and 3x conventional pH selective membranes, allowing simultaneous measurement of the pH and the Pb^{2+} activity in triplicate. The water layer test [136] performed with the multielectrode reveals, as expected, that PEDOT(Cl) film works as a water layer suppressant.

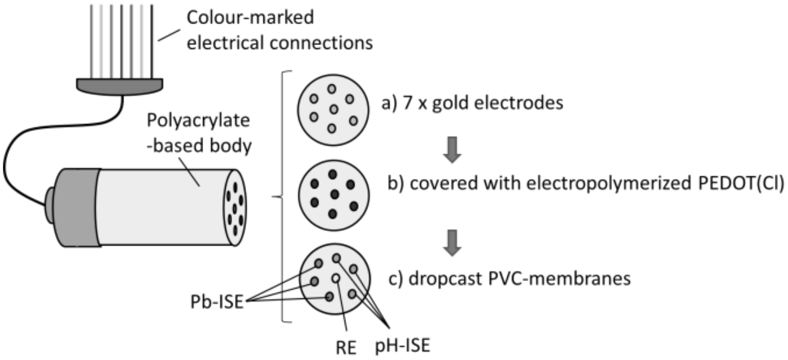


Figure 11. *A schematic presentation of the multielectrode platform and the stepwise preparation of ISEs and the RE on it*

The calibration of the solid-contact pH electrodes versus the solid-contact RE using the platform shown in Figure 11 gave a Nernstian response in the pH range 2-9, and the respective calibration of Pb^{2+} electrodes versus the SC-RE resulted in a detection limit of 10^{-8} M (2ppb) in the solutions with background pH of 3.3. A continuous use of the electrode over a three days period did not change detection limits or slopes of calibration curves significantly.

The all-solid-state multielectrode was used successfully for pH measurements in real environmental samples such as river water, rain water and tap water. Measured values were compared to those obtained with a conventional commercial pH-meter, and the deviation was in all cases smaller than 0.52%. The multielectrode probe with a RE, pH and Pb^{2+} selective electrodes was also used to speciate lead in a water sample, as lead in aqueous solutions is known to complex with a variety of anions in a pH –dependent manner, influencing the potentiometric reponse of the Pb-ISE [200,201]. From the experiments in Paper II it can be concluded that integration of solid-contact ISEs and REs in this kind of a potentiometric sensor array is possible, and can be used for environmental analysis. Recently a potentiometric multielectode probe with a similar enginering has been shown to work with even other types of sensing layers and with very small sample volumes down to 10-30µl [202].

The work with all-solid-state sensors was continued with screen printed carbon substrates, with an aim to produce low-cost disposable potentiometric sensors for the environmental detection of lead [129]. Also in this case the TBA-TBB reference membrane was used in combination with an electropolymerized PEDOT(Cl) layer as an ion-to-electron transducer. All-solid-state potentiometric lead-sensors prepared on screen printed carbon electrodes were able to detect nanomolar levels of lead in environmental samples, which is very promising considering early-state alarm applications for the environmental monitoring.

6.2.2 All-solid-state sensor based on carbon cloth

In Paper III, the TBA-TBB reference membrane and a conventional K$^+$-selective membrane were drop-cast on a piece of carbon cloth (cc) rolled inside a hollow PVC cylinder, as schematically shown in Figure 12.

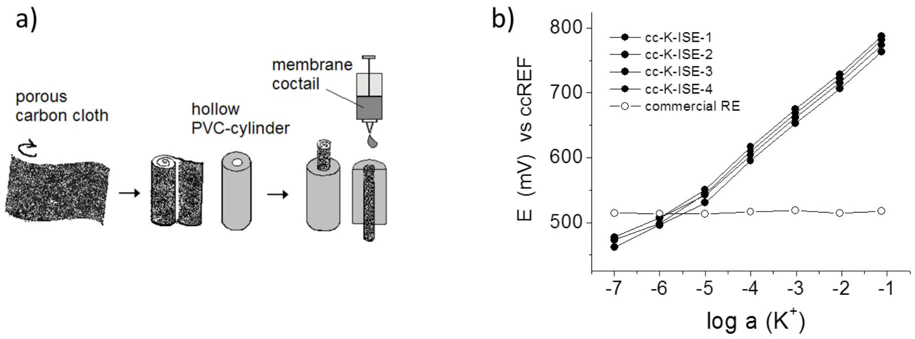


Figure 12. *a) A schematic presentation of the preparation of a solid-state RE and a solid-state ISE on carbon cloth and b) a calibration of four identical ccK$^+$-ISEs vs a ccRE. A conventional Ag/AgCl/3MKCl/1MLiAc RE is added to the calibration to validate the proper functioning of the solid-state RE. [Figure 1 and modified Figure 7 in Paper III]*

These solid-state potassium selective electrodes and solid-state reference electrodes prepared on carbon cloth will be referred to as ccK$^+$-ISE and ccRE respectively. Again, a solid-state RE and a solid-state ISE are prepared in the same way. The carbon cloth functioned simultaneously as an electronic conductor, to which the crocodile clips from the multichannel potentiometer wires could be attached, and as an ion-to-electron transducer, whose operation was based on the large surface area and thus large double-layer capacitance of the carbon cloth [68,70,128].

A calibration of the ccK$^+$-ISEs vs ccRE resulted in Nernstian slopes in the activity range from 10^{-1} to 10^{-5} M KCl in the absence of a supporting electrolyte, as seen in Figure 12 b). A commercial, conventional Ag/AgCl/3MKCl/1MLiAc reference electrode was added to one of the measurement channels to validate the results.

There is some offset between the calibration curves of four ccK$^+$-ISEs resulting from deviating standard potentials, which is a typical problem for solid-state ISEs as discussed in section 3.1. In this case, the deviation is probably partly due to the manual production with differing geometries for hand-rolled carbon cloth, and slightly varying amounts of drop-cast membrane cocktail that was let to be absorbed to the rolled carbon cloth without any further control. An optimization and a careful control of the preparation is expected to improve the reproducibility of standard potentials.

The potentials of the ccRE and ccK$^+$-ISEs prepared in this way were followed over a long period of time versus a conventional Ag/AgCl/3M KCl/1M LiAc RE in order to obtain information on their long term stability. Potentials of the cc-based electrodes were followed in 0.1 M KCl for 25 days, during which electrodes were continuously immersed in 0.1 M KCl, and results are shown in Figure 13a.

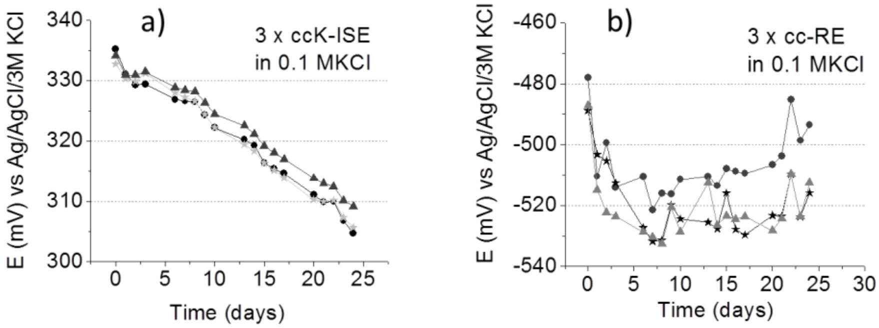


Figure 13. *The potential stability of three a) ccK$^+$-ISEs and b) cc-REs measured against a conventional Ag/AgCl/3MKCl reference electrode during a continuous conditioning in 0.1 M KCl. The freshly prepared cc-electrodes were conditioned for 1h in the 0.1 M KCl before the measurement was started (day 0)* [Figure 8 and 9 in Paper III]

The ccK$^+$-ISEs showed a slow potential drift of approximately 1mV/day (42μV/h) which is satisfactory drift rate and shows that an ion-to-electron transduction based on a large double layer capacitance can result in relatively stable potentials. Also, the 3 ccK$^+$-ISEs shown Figure 13a behave very similarly with each other which is also promising. A critical inspection of measurements raises also the question on the stability of the conventional RE against which the ccK$^+$-ISEs were measured over such a long period of time. The internal Ag/AgCl element is sensitive to changes of the Cl$^-$ concentration in the internal filling compartment that may occur due to evaporation of the filling solution, even if the electrode is well-maintained. Potential drifts of ccK$^+$-ISEs is decreased to 0.36 mV/day (15μV/h) after conditioning in 0.1 M KCl for over one month (42 days).

The stability and the potential reproducibility of ccREs in 0.1 M KCl over the 25 days period (fig. 13 b) was found to show larger variations in potentials in a random manner instead of a clear drift pattern of ccISEs. Their potentials were nevertheless maintained within a certain range, and could be considered satisfactory taking into account that the membrane used is not an ideal reference but more like a pseudo-reference element that nonetheless enables all-solid-state measurements with various substrates. It was found promising that despite the described lack in long term stability, the ssRE could very well complete the potentiometric sensor, as during single calibration, for example on the day 25, the ccK$^+$-ISE measured vs ccRE gave a good near-Nernstian slope.

The performance of the TBA-TBB-based reference electrodes could possibly be improved further in the future by for example optimizing the ion-to-electron transduction at the back side of the membrane

6.3 Impedance study of the ion-to-electron transduction process for carbon cloth and PEDOT as a solid-contacts

The ion-to-electron transduction process of a solid-contact layer between the ISM and the electronic conductor can be either a faradaic redox process, as in the case of conducting polymers, or a capacitive process based on the charging of the double layer for large surface area carbon materials as discussed earlier. In Paper III these processes are studied for three solid contacts: carbon cloth, PEDOT doped with a bulky anion poly(sodium 4-styrenesulfonate) (PEDOT(PSS)) and PEDOT doped with a small mobile anion PEDOT(Cl), with help of electrochemical impedance spectroscopy (EIS) in an electrolyte medium with similarity to an ion selective membrane.

Two identical GC-disk electrodes coated with the studied solid-contact material were inserted in a glass tube with a small opening in the middle as shown in Figure 14.

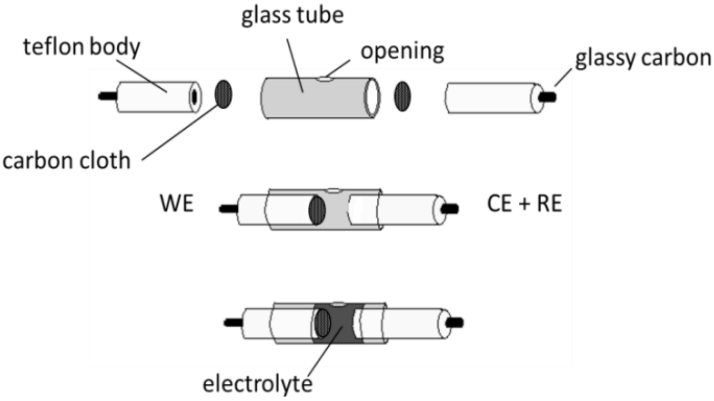


Figure 14. A schematic picture of the two-electrode set-up for EIS measurements in Paper III [modified Figure 2 in Paper III]

PEDOT films were electropolymerized on the GC surface, and circular pieces of carbon cloth was adhered to electrodes with carbon glue. The electrolyte solution could be inserted via a small opening in the glass tube functioning as the cell, so that the electrolyte was sandwiched between the two identical electrodes.

Four different electrolyte solutions were chosen for the study. One was an aqueous 0.1 M KCl solution, i.e. a small mobile cation and a small mobile anion, and three others were ISM-resembling matrices where a salt was dissolved in a plasticizer: 0.1 M tridodecylmethylammonium chloride (TDMACl) in o-nitrophenyl octyl ether (o-NPOE) (i.e. a small mobile anion and a bulky cation), 0.1 M potassium tetrakis(4-chlorophenyl)borate (KTpClPB) in o-NPOE (i.e. a small mobile cation and a bulky anion) and 0.1 M tetrabutylammonium-tetrabutylborate TBA-TBB in oNPOE, containing the moderately lipophilic large salt used in the reference membrane described earlier.

The impedance measurements were done in a frequency range from 10 kHz to 0.01 Hz using a 10 mV perturbation amplitude. The distance between the identical electrodes was 5.5 mm, and one of them was connected as the working electrode while the other was a combined counter and reference electrode. Measurements are presented in the form of Bode-plots in Paper III, where the logarithm of the impedance modulus |Z|, and the phase angle φ are plotted versus the logarithm of the frequency. Examples of the EIS-results are shown Figures 15 and 16 a and b, where the electrolytes are $KCl_{(aq)}$ and $TDMACl_{(o\text{-}NPOE)}$ respectively.

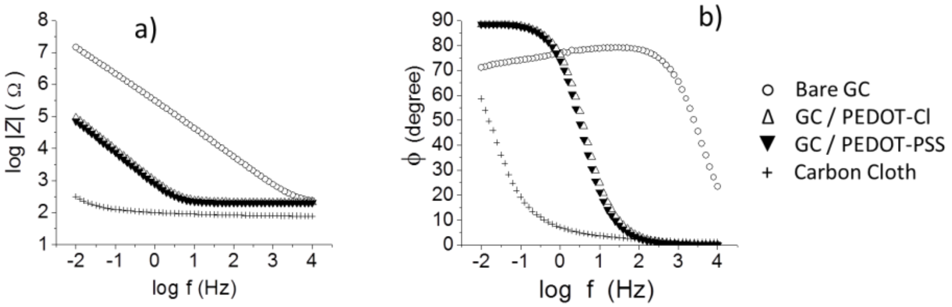


Figure 15. *Bode plots, i.e. the impedance modulus a) and phase b) for all studied electrodes in a* KCl_{aq} *electrolyte [Figure 3 in Paper III]*

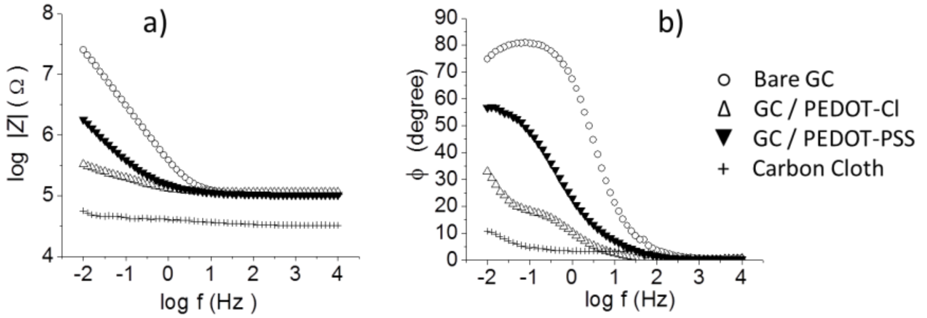


Figure 16. *The impedance a) modulus and b) phase for all studied electrodes in a* $TDMACl_{(o\text{-}NPOE)}$ *electrolyte [Figure 4 in Paper III]*

The Bode-plot representation allows qualitative comparison of the electrodes with different solid contacts attached on the glassy carbon (GC) surface. Also a bare GC was included in the study for comparison. Regarding the ion-to-electron transduction process, the low-frequency end of the measurement is of a special interest [45]. In all electrolytes the trend is clear, the impedance is highest for the bare GC and lowest for the carbon cloth, while the impedance of PEDOT-films is in between these two, and is influenced by the doping ion and the electrolyte solution.

The impedance response of PEDOT films is influenced by the mobility of the doping anion in the film together with the presence and the mobility of suitable charge balancing ions in the electrolyte phase. The impedance of the anion sensitive PEDOT(Cl) is lower than the impedance of the cation sensitive PEDOT(PSS) [163,164] in an electrolyte containing a small mobile anion, $TDMA^+Cl^-_{(o\text{-}NPOE)}$. Cl^- incorporated to the film as a doping anion is mobile, and can leave and enter the PEDOT film as a result of its oxidation and reduction, so that the charge balance is maintained. Correspondingly, the impedance of PEDOT(PSS) is lower than that of PEDOT(Cl) in solution with a small mobile cation $K^+TpClPB^-_{(o\text{-}NPOE)}$, as K^+ may enter and leave the film and balance the charge of the immobile PSS^--doping ion when PEDOT is reduced or oxidized. In $KCl_{(aq)}$, impedances of the PEDOT(PSS) and the PEDOT(Cl) films are similar, as both a mobile anion and a mobile cation are present in equal concentrations. In all cases, the low-frequency impedance of the PEDOT film is significantly lower than that of the bare GC, confirming that PEDOT is able to mediate the ion-to-electron transduction more efficiently than bare GC both in organic and aqueous solutions [45].

Carbon cloth showed smaller impedance at low frequencies compared to PEDOT(PSS), PEDOT(Cl) and bare glassy carbon in all electrolytes used, suggesting that it is the most efficient ion-to-electron transducer from these. It should though be taken into consideration that the piece of carbon cloth attached to an electrode surface covered the whole disk (the GC and the insulating body, diameter 8 mm) and had a thickness of ca. 0.55 mm, while the PEDOT film was electrodeposited only on the conducting GC surface (diameter 3 mm) and had a thickness of approximately 1 μm [194]. The active surface area of the carbon cloth was evaluated to be 2.6×10^5 times larger than the geometric area, and thus the low impedance observed in the measurements may be related to the large area available for the double layer charging.

Based on the EIS results discussed in this section, and on the application of carbon cloth in all-solid-state potentiometric sensing in the previous section, it can be concluded that alongside with other novel carbon materials with high surface area, carbon cloth is one of the promising solid-contact alternatives. Carbon cloth -based ISEs were recently proven to work in a drug release study, where they showed a long lifetime for over 6 months and an analytical performance comparable to the more traditional UV-method [203]. An advantage of carbon cloth is that it can function both as an ion-to-electron transducer and an electronic conductor at the same time, and that it is very bendable and flexible. A challenge with carbon cloth is the carbon dust shedding from it during handling, but it is not expected to be a problem too difficult to solve.

6.4 Electrochemical control of the standard potential E° of a solid state ISE with a CP as a solid contact

As stated earlier, the stability of the standard potential for solid-state ISEs is one of the key problems standing in the way of modern applications for disposable home-diagnostics and reliable, remote environmental monitoring with potentiometric sensors. In Papers IV and V this problem is addressed for conducting polymer–based solid-state ISEs. Even if the analytical response of an ISE is formed at the ISM|sample solution interface, the redox-state and the ionic content of the CP-film under the ISM contributes to the overall measured signal of the ISE as a component of the standard potential (E°). The redox state of the conducting polymer solid contact can be controlled electrochemically by current pulses or by applying a constant potential.

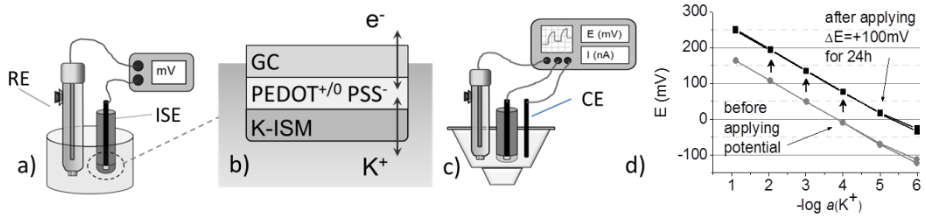


Figure 17. *A schematic picture of a) a potentiometric open-circuit measurement b) an ion-to-electron transduction c) a measurement set-up to apply a potential or a current to an ISE and d) a shift of the calibration curve by oxidation of the conducting polymer layer working as the ion-to-electron transducer*

PEDOT(PSS) films covered with valinomycin-based K^+-selective membranes were chosen as a model system for this part of the work. Thus, Equation 23 can be written

$$(PEDOT^{+}PSS^{-})_{film} + K^{+}_{membrane} + e^{-} \leftrightharpoons (PEDOT^{0}PSS^{-}K^{+})_{film} \qquad (32)$$

If no external control is applied, equilibrium between the CP-film and the solution at a specific ratio of oxidized and neutral PEDOT is established according to Equation 32 [145], determining the standard potential of the solid-contact ISE.

6.4.1 Control of the E° by applying current or potential

According to the hypothesis in this work, the equilibrium of Equation 32 can be shifted with help of an applied current or an applied potential. A negative current or a potential more negative than the equilibrium potential would reduce the PEDOT film and the reaction in Equation 32 would proceed from left to right, while a positive current or a potential more positive than the equilibrium potential would oxidize the PEDOT film and the reaction in Equation 32 would proceed from right to left.

The influence of the quality and characteristics of the ISM covering the PEDOT(PSS) film on the redox-reaction in Equation 32 was evaluated by studying three different membranes: two valinomycin based K^+-selective membranes with and without the lipohilic additive tetradodecylammonium tetrakis(4-chlorophenyl)borate (ETH-500) that lowers the membrane resistance, and a cation sensitive membrane containing potassium tetrakis(4-chlorophenyl)borate (KTpClB). Membrane compositions are given in Table 2

***Table 2** The composition of membranes used in the study*

components	**SC-ISE(i)**	**SC-ISE(ii)**	**SC-ISE(iii)**
valinomycin	1%	1%	-
KTFPB	0.5%	0.5%	-
ETH-500	-	1%	-
KTpClPB	-	-	1%
DOS	65.5%	65%	-
o-NPOE	-	-	66%
PVC	33.3%	32.5%	33%

All electrodes were calibrated in KCl solutions with concentrations ranging from 10^{-1} M to 10^{-8} M and back to 10^{-1} M vs. a Ag/AgCl/3M KCl/1M LiAc before any treatment, to be sure that they were working as expected.

The electrochemical treatment to shift the reaction in Equation 32 was done with help of a potentiostat in a conventional 3-electrode cell with a glassy carbon rod as the counter electrode, a conventional Ag/AgCl/3M KCl electrode as the reference and a SC-ISE under study as the working electrode. The electrolyte was 0.1 M KCl without any de-aeration. The potential of the SC-ISE was shifted by applying a potential deviating from its OCP for 10-74 hours, while measuring the current simultaneously, or by applying ±1nA current pulses while measuring the potential. These experiments were done in a Faraday cage in order to avoid disturbances, and it was found that both methods supported the hypothesis and could be used to adjust the E° of SC-ISEs with conducting polymer transducer.

In an experiment were the E° was shifted by applying a potential deviating ±100mV from the OCP for 24h, while measuring the current flowing due to the oxidation/reduction, it was observed that the resistance of the membrane had an influence on how close to the applied value the potential could be shifted. This can be seen in Figure 18, where the OCP of each electrode is first measured for 10 hours in 0.1 M KCl (10th hour shown in Figure 18a), after which an approximately 100mV more negative potential (indicated with stars) is applied for 24 hours followed immediately by a measurement of the OCP in the same 0.1 M KCl electrolyte.

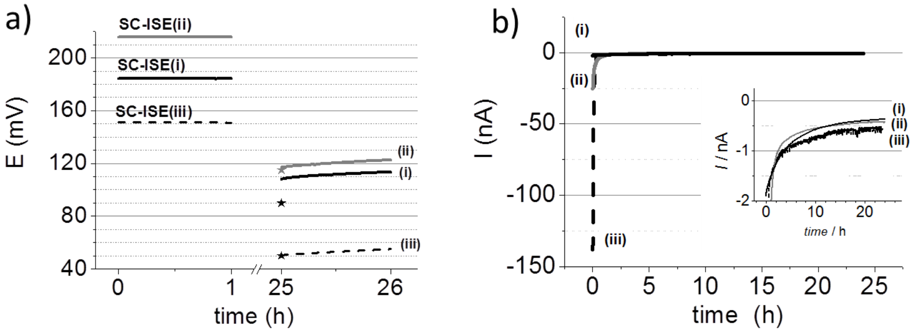
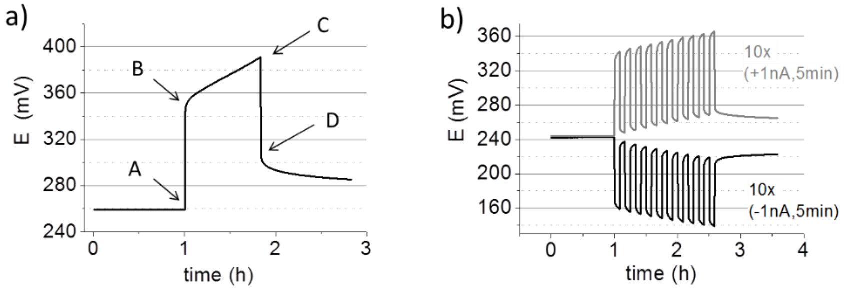


Figure 18. *a) Open-circuit potentials of all SC-ISE types studied in this work before and after applying a ΔE= -100 mV for 24 h (the applied potential indicated with a star) and b) currents measured while applying the potential [modified from Figure 1 in Paper VI]*

The membrane resistance of the SC-ISE(i) was highest of the studied electrodes, in the range of tens of MΩ, followed by the SC-ISE(ii) with a few MΩ resistance, and finally the SC-ISE(iii) had the lowest membrane resistance of a few hundred kΩ. It is evident that the potential of conducting polymer SC-ISEs can be shifted in this way, and also that the more resistive the membrane, the further away from the applied potential the OCP remains after a 24h treatment. The potential of all studied electrodes could nevertheless be shifted to the close proximity of the applied potential. The OCP of SC-ISEs was recorded for 10 h after the potential treatment (1st hour is shown in Figure 18a), and it could be seen that it slowly drifted back towards the original potential. The drift was nevertheless rather slow, and a calibration curve with a good near-Nernstian (~ 59 mV/dec) slope could be obtained with the adjusted E°.

Adjustment of the E° with help of current pulses was also possible, as can be seen in Figure 19, where the principle of the current-induced E° -adjustment (Fig. 19 a) as well as the reversibility of the method (Fig 19b) is shown with the SC-ISE(i). A positive current shifts the potential up and a negative current shifts the potential down.



Figure

Figure 19. *a) The principle of adjusting the E° with current pulses: the current is turned on at "A" and off at "C", and the OCP is recorded before and after the pulses, and a typical potential response of the SC-ISE(i) when applying a +1 nA current in one 50-min pulse and b) in ten 5-min pulses with 5 min relaxation at open circuit in between each pulse using +1nA and -1nA currents [Figure 3a and 5a Paper IV]*

6.4.2 An instrument-free method to control the E° of a SC-ISE by short-circuiting it with a conventional RE

The drive to prepare calibration-free ISEs is mainly derived from a desire to simplify the measurements. Thus, it would be a great advantage if the stabilization of the standard potential could be done without any sophisticated instrumentation. In Paper V, a possibility to adjust the E° of SC-ISEs according to the principle described above, by inducing a potential difference by short-circuiting it with a conventional large-capacitance reference electrode, is studied, as shown schematically in Figure 20.

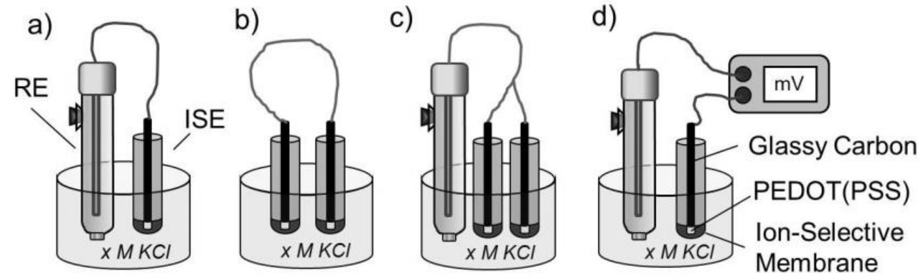


Figure 20. *A schematic picture of short-circuiting a solid-contact ISE with a) a conventional large-capacitance reference electrode b) with another similar solid-contact ISE and c) a conventional RE and another SC-ISE. An open-circuit potential measurement performed before and after the short-circuiting treatment as well as electrode components is shown in d)[Figure. 1 in Paper V]*

In such a case, the reaction in Equation 32 proceeds until the potential difference between the reference electrode and the SC-ISE is close to zero (i.e. until there is no potential difference between the short-circuited electrodes) depending on the membrane resistance. This corresponds to situation where where the E° was shifted by applying 0mV with an instrument. Conventional reference electrodes are designed to tolerate passages of small currents, and thus the short-circuiting does not influence the potential of the reference electrode.

While doing a literature search for Papers IV and V, it was found that short-circuiting of two Ag/AgCl reference electrodes, to render them symmetric, was used already in 1992 by Rumpf et al. [127]. This gave further optimism for hoping success to the proposed concept, and indeed, the short-circuiting method for controlling the E° of SC-ISEs has been found to be very promising. The electrode-to-electrode reproducibility was satisfactory, while the reproducibility of a calibration curve of a specific electrode, when short-circuited for adequate time in similar conditions, showed an excellent reproducibility of the calibration curve, as demonstrated in Figure 21 b.

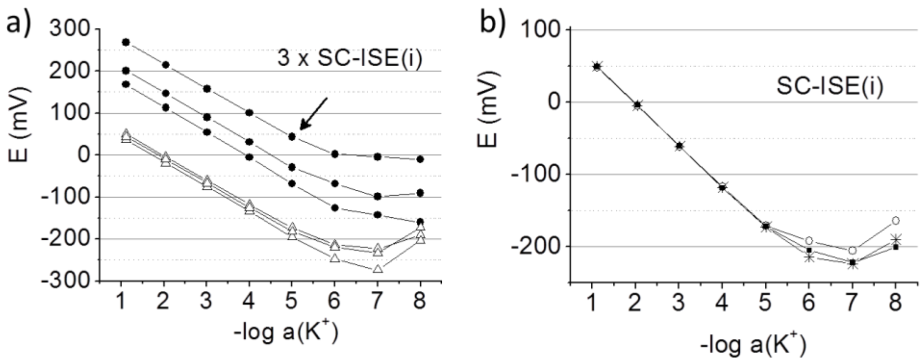


Figure 21. *a) Calibration slopes of 3 similar SC-ISE(i)s before any treatment and after short-circuiting them together with each other and a conventional Ag/AgCl/3MKCl RE in 0.1 M KCl for 3 days and b) calibration curves of the SC-ISE(i) marked in a) with an arrow after short-circuiting it in 0.1 M KCl with a conventional Ag/AgCl/3M KCl reference for 3 days (*) and an additional overnight after it (○), disconnected and let to relax for 3 days and short-circuiting again in 0.1 M KCl for 3 days (■) [Figure 2 in Paper V]*

The control of the E° with the short-circuiting process is an on-off type of a situation, which means that one should wait for equilibrium in order to obtain reproducibility, even if it might take several days. The equilibration process slows down towards the end as the driving force decreases. Yet, as the short-circuiting does not need any extra instrument, this set-back can be circumvented in possible practical applications, as the electrodes could be e for example stored short-circuted for all times except during the measurement.

The means to influence the position of the potential that the equilibration reaction is striving for is to control the short-circuiting solution, as the electrode potential is determined at the interface of the ISM and that solution. This is demonstrated in Figure 22, where a cation sensitive SC-ISE(iii) was short-circuited with a conventional RE in KCl-solutions of different concentrations.

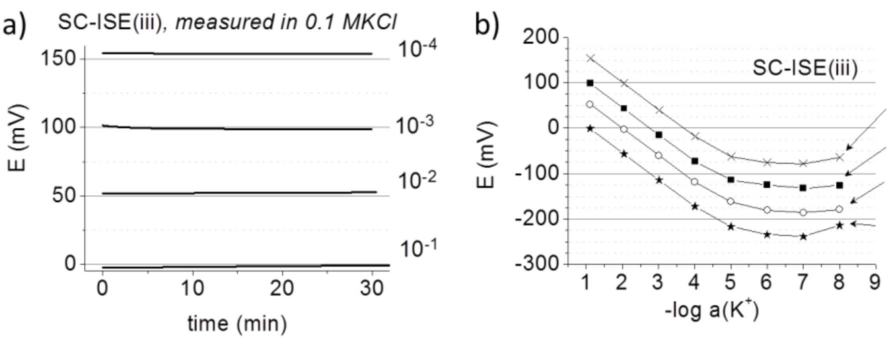


Figure 22. *a) Open-circuit potentials of a cation sensitive SC-ISE(iii) measured in 0.1 M KCl vs. Ag/AgCl/3M KCl/1M LiAc after short-circuiting with a conventional Ag/AgCl/3M KCl RE in* 10^{-1} *M KCl for 3 days, with a Ag/AgCl/3M KCl/0.1 MKCl in* 10^{-2} *M KCl for 3 days and in* 10^{-3} *M KCl for overnight and with a Ag/AgCl/3M KCl/1M LiAc in* 10^{-4} *M KCl overnight and b) calibration curves measured after short-circuitings [Fig 5 in Paper V]*

After the treatment, the OCP of the electrode was zero, or close to zero, in a solution with the same concentration as the short-circuiting electrolyte, as can be seen in Figure 22b showing the calibration of the SC-ISE(iii) after the treatment. Inserts 10^{-1} – 10^{-4} indicate the concentration of the short-circuiting electrolyte. This increases the flexibility of the short-circuiting method for the E° adjustment considerably, as the shift to the equilibrium potential does not need to be so dramatic.

7. Conclusions and closing remarks

The research related to the potentiometric sensors is a very rewarding field of science because it encloses work with novel materials such as conducting polymers and different three dimensional carbon structures, and shows a potential for very practical and important applications within healthcare and environmental issues, both truly important in today's world. Many of the very crucial problems restraining the exciting applications of potentiometric sensing in remote environmental locations and in vanguard diagnostics have been either solved or are under an intensive research within the ISE community at the moment and it seems that they will be solved within a reasonable time.

The research presented in this PhD thesis was tackling some of the main issues related to the field, i.e. the lack of a solid-state reference electrode (the Holy Grail) and the inadequate stability and reproducibility of the standard potential of solid contact ISEs. An effort was also made to improve the understanding of the processes occurring between the ion-to-electron transducer and the ion-selective membrane. Each of these topics was brought a step or two forward, even if implementation to practical analysis in portable potentiometric sensing still remains for the future.

The solid-state reference membrane developed in the beginning of the project has proven to be a good partner to solid state ISEs in many cases discussed above, though its limitations, the slowness of the response and the slight irreproducibility of the potential must be kept in mind. Nevertheless, the studied membrane did perform satisfactorily in the environmental measurements reported in Paper II and with the new solid contact material, carbon cloth, described in Paper III. In these sensor assemblies, it is a great advantage that the reference electrode can be prepared from similar materials and by similar techniques as the ISE, or the ISEs in the case of the multielectrode platform in Paper II. The TBA-TBB-reference membrane has in addition proven to be able to work on very exciting substrates like paper-based electrodes.

The electrochemical impedance measurements in Paper III confirmed the already well-known fact that GC/PEDOT improves the ion-to-electro transduction process compared to bare GC, but also that carbon cloth performed this task even more effectively, bearing in mind that the thickness of the carbon cloth in the study was much larger than that of the PEDOT film. Because of its mechanical flexibility, carbon cloth could be an especially promising new solid-contact material among the other high-surface-area carbon materials. In addition, Papers I-III settle themselves on the continuum of the ISE research from traditional electrodes containing the filling solution towards all-solid-state sensors.

Papers IV and V addressed the stability and the reproducibility of the sensor response, which are of utmost importance in all applications exploiting potentiometric sensing. The finding of Paper IV that the standard potential of the ISEs with a conducting polymer as a solid contact, can be adjusted by reducing or oxidizing the conducting polymer solid-contact in a predictable manner by applying a current or a potential is very promising.

The instrument-free method to control the E° and thus the position of the calibration slope presented in Paper V contains very exciting possibilities concerning simplified calibration procedures in remote locations and manufacturing calibration-free disposable sensors. Short-circuiting as the driving force for the oxidation/reduction of the CP solid-contact is inexpensive, simple and robust. The piece-to-piece reproducibility of the E° between different sensors requires still some improvement, but the day-to-day reproducibility of a chosen sensor was excellent. Most of the experiments in Papers IV and V were still done using a conventional macroscopic, liquid-containing reference electrode. In the future, the possibilitiy to use the short-circuiting method in an all-solid-state configuration will be studied.

8. References

[1] J. Bobacka, A. Ivaska, A. Lewenstam, *Electroanalysis.* **2003,** 15, 366-374.

[2] P. Buhlmann, L.D. Chen, *Supramol. Chem. : Mol. Nanomater.* **2012,** 5, 2539-2579.

[3] C.H. Hamaan, A. Hamnett, W. Vielstich, *Electrochemistry*, WILEY-VCH Cerlag GmbH & Co. KGaA, Weinheim **2007.**

[4] K.N. Mikhelson, *Ion-Selective Electrodes*, Springer-Verlag, Berlin Heidelberg **2013.**

[5] Tierney, J. Michael,*Handbook of Chemical and Biological Sensors,* (Eds. J.S. Schlultz, R.F. Taylor), IOP Publishing Ltd, Bristol, UK **1996.**

[6] J. Bobacka, *Encycl. Sens.* **2006,** 2, 279-294.

[7] E. Bakker, K. Chumbimuni-Torres, *J. Braz. Chem. Soc.* **2008,** 19, 621-629.

[8] J. Bobacka, A. Ivaska, A. Lewenstam, *Chem Rev.* **2008,** 108, 329-351.

[9] E. Bakker, E. Pretsch, *Anal Chem.* **2002,** 74, 420A-426A.

[10] J. Janata, *Principles of Chemical Sensors*, Plenum Press, New York **1989.**

[11] E. Lindner, B.D. Pendley, *Anal. Chim. Acta.* **2013,** 762, 1-13.

[12] J. Wang,*Handbook of Chemical and Biological Sensors,* (Eds. J.S. Schlultz, R.F. Taylor), IOP Publishing Ltd, Bristol, UK **1996**.

[13] K.B. Oldham, J.C. Myland, *Fundamentals of Electrochemical Science,* Academic Press, Inc., San Diego, California **1994.**

[14] E. Bakker, V. Bhakthavatsalam, K.L. Gemene, *Talanta.* **2008,** 75, 629-635.

[15] D.T. Sawyer, A. Sobkowiak, J.L.J. Roberts, *Electrochemistry for Chemists*, John Wiley & Sons, Inc., New York **1995.**

[16] E. Bakker, P. Bühlmann, E. Pretsch, *Electroanalysis.* **1999,** 11, 915-933.

[17] A. Lewenstam, *Electroanalysis.* **2014,** 26, 1171-1181.

[18] A. Lewenstam,*Comprehensive Analytical Chemistry vol. 49,* (Eds. S. Alegeret, A. Merkoci), Elsevier, Amsterdam, The Netherlands and Oxford, UK **2007**, pp. 5-5-24.

[19] N.J. Ronkainen, H.B. Halsall, W.R. Heineman, *Chem. Soc. Rev.* **2010,** 39, 1747-1763.

[20] Y. Wang, H. Xu, J. Zhang, G. Li, *Sensors.* **2008,** 8, 2043-2081.

[21] P. Bergveld, *Biomed. Microdevices.* **2000,** 2, 185-195.

[22] Diagnostics for All, 2014, 09/17, http://www.dfa.org/

[23] D. Diamond, *Anal. Chem.* **2004,** 76, 278A-286A.

[24] A. Radu, S. Anastasova, C. Fay, D. Diamond, J. Bobacka, A. Lewenstam, *Sensors, 2010 IEEE.* **2010,** 1487-1490.

[25] P.C. Hauser, D.W.L. Chiang, G.A. Wright, *Anal. Chim. Acta.* **1995,** 302, 241-248.

[26] A. Radu, S. Anastasova-Ivanova, B. Paczosa-Bator, M. Danielewski, J. Bobacka, A. Lewenstam, D. Diamond, *Anal. Methods.* **2010,** 2, 1490-1498.

[27] A. Lewenstam, M. Maj-Zurawska, A. Hulanicki, *Electroanalysis* **1991,** 3, 727-734.

[28] E. Bakker, P. Buehlmann, E. Pretsch, *Chem. Rev.* **1997,** 97, 3083-3132.

[29] A. Lewenstam, *Scand. J. Clin. Lab. Invest. , Suppl.* **1994,** 54, 11-19.

[30] T. Sokalski, A. Ceresa, T. Zwickl, E. Pretsch, *J. Am. Chem. Soc.* **1997,** 119, 11347-11348.

[31] A. Radu, S. Peper, E. Bakker, D. Diamond, *Electroanalysis.* **2007,** 19, 144-154.

[32] inW.P. Carey, B.R. Kowalski,*Handbook of Chemical and Biological Sensors,* (Eds. J.S. Schlultz, R.F. Taylor), IOP Publishing Ltd, Bristol, UK **1996**.

[33] X. Ge, Y. Kostov, L. Tolosa, G. Rao, *Anal. Chim. Acta.* **2012,** 734, 79-87.

[34] D. Pletcher, *A First Course in Electrode Processes*, Royal Society of Chemistry, Cambridge, UK **2009.**

[35] G. Inzelt,*Handbook of Reference Electrodes,* (Eds. G. Inzelt, A. Lewenstam, F. Scholz), Springer-Verlag, Berlin, Heidelberg **2013.**

[36] D.J.G. Ives, G.J. Janz, *Reference Electrodes, Theory and Practice,* Academic Press Inc., New York and London **1961.**

[37] J. Koryta, J. Dvorak, L. Kavan, *Principles of Electrochemisry*, John Wiley and Sons Ltd., Chichester, England **1993.**

[38] E.A. Guggenheim, *J. Phys. Chem.* **1930,** 34, 1540-1543.

[39] E.A. Guggenheim, *J. Phys. Chem.* **1929,** 33, 842-849.

[40] A.J. Bard, L.R. Faulkner, *Electrochemical Methods, Fundamentals and Applications*, John Wiley & Sons, Inc. **2001.**

[41] V.S. Bagotsky, *Fundamentals of Electrochemistry*, John Wiely & Sons, Inc., Hoboken, New Jerssey **2006.**

[42] T. Sokalski, M. Maj-Zurawska, A. Hulanicki, A. Lewenstam, *Electroanalysis.* **1999,** 11, 632-636.

[43] R.P. Buck, E. Lindner, *Pure Appl. Chem.* **1994,** 66, 2527-2536.

[44] E. Bakker, *Anal. Chem.* **1997,** 69, 1061-1069.

[45] J. Bobacka, *Anal. Chem.* **1999,** 71, 4932-4937.

[46] Z. Mousavi, J. Bobacka, A. Lewenstam, A. Ivaska, *J. Electroanal. Chem.* **2009,** 633, 246-252.

[47] Z. Mousavi, A. Teter, A. Lewenstam, M. Maj-Zurawska, A. Ivaska, J. Bobacka, *Electroanalysis.* **2011,** 23, 1352-1358.

[48] M. Zhou, S. Gan, B. Cai, F. Li, W. Ma, D. Han, L. Niu, *Anal Chem.* **2012,** 84, 3480-3483.

[49] C. Lai, M.A. Fierke, A. Stein, P. Buhlmann, *Anal Chem.* **2007,** 79, 4621-4626.

[50] E. Jaworska, M. Wojcik, A. Kisiel, J. Mieczkowski, A. Michalska, *Talanta.* **2011,** 85, 1986-1989.

[51] R.E. Gyurcsányi, N. Rangisetty, S. Clifton, B.D. Pendley, E. Lindner, *Talanta.* **2004,** 63, 89-99.

[52] M. Novell, M. Parrilla, G.A. Crespo, F.X. Rius, F.J. Andrade, *Anal Chem.* **2012,** 84, 4695-4702.

[53] P. Lingenfelter, I. Bedlechowicz-Sliwakowska, T. Sokalski, M. Maj-Zurawska, A. Lewenstam, *Anal Chem.* **2006,** 78, 6783-6791.

[54] A. Hulanicki, A. Lewenstam, *Anal. Chem.* **1981,** 53, 1401-1405.

[55] A. Hulanicki, T. Sokalski, A. Lewenstam, *Mikrochim. Acta.* **1988,** 3, 119-129.

[56] E. Bakker, *Journal of The Electrochemical Society.* **1996,** 143, L83-L85.

[57] T. Sokalski, W. Kucza, M. Danielewski, A. Lewenstam, *Anal. Chem.* **2009,** 81, 5016-5022.

[58] E. Pergel, R.E. Gyurcsanyi, K. Toth, E. Lindner, *Anal. Chem.* **2001,** 73, 4249-4253.

[59] E. Lindner, R.E. Gyurcsanyi, R.P. Buck, *Electroanalysis.* **1999,** 11, 695-702.

[60] A. Michalska, J. Dumanska, K. Maksymiuk, *Anal. Chem.* **2003,** 75, 4964-4974.

[61] A. Konopka, T. Sokalski, A. Michalska, A. Lewenstam, M. Maj-Zurawska, *Anal. Chem.* **2004,** 76, 6410-6418.

[62] J. Sutter, A. Radu, S. Peper, E. Bakker, E. Pretsch, *Anal. Chim. Acta.* **2004,** 523, 53-59.

[63] J. Sutter, E. Lindner, R.E. Gyurcsanyi, E. Pretsch, *Anal. Bioanal. Chem.* **2004,** 380, 7-14.

[64] L.Y. Heng, E.A.H. Hall, *Anal. Chim. Acta.* **1996,** 324, 47-56.

[65] N.M. Ivanova, M.B. Levin, K.N. Mikhelson, *Russ. Chem. Bull.* **2012,** 61, 926-936.

[66] A. Kisiel, A. Michalska, K. Maksymiuk, E.A.H. Hall, *Electroanalysis.* **2008,** 20, 318-323.

[67] J. Veder, M.R. De, K. Patel, P. Si, E. Grygolowicz-Pawlak, M. James, M.T. Alam, M. Sohail, J. Lee, E. Pretsch, E. Bakker, *Anal Chem.* **2013,** 85, 10495-10502.

[68] M.A. Fierke, C. Lai, P. Buhlmann, A. Stein, *Anal. Chem.* **2010,** 82, 680-688.

[69] G.A. Crespo, S. Macho, F.X. Rius, *Anal. Chem.* **2008,** 80, 1316-1322.

[70] G.A. Crespo, S. Macho, J. Bobacka, F.X. Rius, *Anal. Chem.* **2009,** 81, 676-681.

[71] B.P. Nikolskii, E.A. Materova, *Ion-Selective Electrode Reviews.* **1985,** 7, 3-39.

[72] U. Guth, F. Gerlach, M. Decker, W. Oelssner, W. Vonau, *J. Solid State Electrochem.* **2009,** 13, 27-39.

[73] A. Lewenstam,*Handbook of Reference Electrodes,* (Eds. G. Inzelt, A. Lewenstam, F. Scholz), Springer-Verlag, Berlin, Heidelberg **2013**

[74] E. Lindner, R.E. Gyurcsanyi, *J. Solid State Electrochem.* **2009,** 13, 51-68.

[75] K. McElhoney, G.D. O'Neil, N.A. Chaniotakis, S.P. Kounaves, *Electroanalysis.* **2012,** 24, 2071-2078.

[76] R.W. Cattrall, H. Freiser, *Anal. Chem.* **1971,** 43, 1905-1906.

[77] R.P. Buck, *Anal. Chem.* **1976,** 48, 23R-39R.

[78] T.A. Fjeldly, K. Nagy, *J. Electrochem. Soc.* **1980,** 127, 1299-1303.

[79] A. Cadogan, Z. Gao, A. Lewenstam, A. Ivaska, D. Diamond, *Anal. Chem.* **1992,** 64, 2496-2501.

[80] D. Pletcher, R. Greef, R. Peat, L.M. Peter, J. Robinson, *Instrumental Methods in Electrochemistry*, Horwookd Publishing Limited, West Sussex, England **1985.**

[81] A.D. McNaught, A. Wilkinson, **2014-02-24,** 2014. IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Blackwell Scientific Publications, Oxford (1997). http://goldbook.iupac.org/S05917.html

[82] P. Spizer, S. Wunderli, K. Maksymiuk, A. Michalska, A. Kisiel, Z. Galus, et al.,*Handbook of Reference Electrodes,* (Eds. G. Inzelt, A. Lewenstam, F. Scholz), Springer-Verlag, Berlin, Heidelberg **2013**.

[83] G. Inzelt,*Handbook of Reference Electrodes,* (Eds. G. Inzelt, A. Lewenstam, F. Scholz), Springer-Verlag, Berlin, Heidelberg **2013**

[84] S. Ito, H. Hachiya, K. Baba, Y. Asano, H. Wada, *Talanta.* **1995,** 42, 1685-1690.

[85] G.D. O'Neil, R. Buiculescu, S.P. Kounaves, N.A. Chaniotakis, *Anal. Chem.* **2011,** 83, 5749-5753.

[86] D. Diamond, E. McEnroe, M. McCarrick, A. Lewenstam, *Electroanalysis.* **1994,** 6, 962-971.

[87] R. Mamińska, A. Dybko, W. Wróblewski, *Sensors Actuators B: Chem.* **2006,** 115, 552-557.

[88] Ł Tymecki, E. Zwierkowska, R. Koncki, *Anal. Chim. Acta.* **2004,** 526, 3-11.

[89] A. Mroz, *Analyst* **1998,** 123, 1373-1376.

[90] A. Michalska, *Electroanalysis.* **2012,** 24, 1253-1265.

[91] A. Kisiel, K. Kijewska, M. Mazur, K. Maksymiuk, A. Michalska, *Electroanalysis.* **2012,** 24, 165-172.

[92] T. Blaz, J. Migdalski, A. Lewenstam, *Analyst* **2005,** 130, 637-643.

[93] R.P. Buck, E. Lindner, *Anal. Chem.* **2001,** 73, 88A-97A.

[94] E. Bakker, *Electroanalysis.* **1999,** 11, 788-792.

[95] J. Ha, S.M. Martin, Y. Jeon, I.J. Yoon, R.B. Brown, H. Nam, G.S. Cha, *Anal. Chim. Acta.* **2005,** 549, 59-66.

[96] D. Cicmil, S. Anastasova, A. Kavanagh, D. Diamond, U. Mattinen, J. Bobacka, A. Lewenstam, A. Radu, *Electroanalysis.* **2011,** 23, 1881-1890.

[97] W. Liao, T. Chou, *Anal. Chem.* **2006,** 78, 4219-4223.

[98] J. Noh, S. Park, H.C. Kim, T.D. Chung, *Bull. Korean Chem. Soc.* **2010,** 31, 3128-3132.

[99] A. Michalska, A. Kisiel, K. Maksymiuk,*Handbook of Reference Electrodes,* (Eds. G. Inzelt, A. Lewenstam, F. Scholz), Springer-Verlag, Berlin, Heidelberg **2013**

[100] H. Suzuki, T. Hirakawa, S. Sasaki, I. Karube, *Sens. Actuators, B.* **1998,** 46, 146-154.

[101] H. Suzuki, A. Hiratsuka, S. Sasaki, I. Karube, *Sens. Actuators, B.* **1998,** 46, 104-113.

[102] H. Suzuki, H. Shiroishi, S. Sasaki, I. Karube, *Anal. Chem.* **1999,** 71, 5069-5075.

[103] S.D. Collins, *Sensors Actuators B: Chem.* **1993,** 10, 169-178.

[104] N. Kwon, K. Lee, M. Won, Y. Shim, *Analyst* **2007,** 132, 906-912.

[105] A. Simonis, H. Luth, J. Wang, M.J. Schoning, *Sens. Actuators, B.* **2004,** 103, 429-435.

[106] P.J. Kinlen, J.E. Heider, D.E. Hubbard, *Sens. Actuators, B.* **1994,** 22, 13-25.

[107] M.A. Nolan, S.H. Tan, S.P. Kounaves, *Anal. Chem.* **1997,** 69, 1244-1247.

[108] D. Desmond, B. Lane, J. Alderman, J.D. Glennon, D. Diamond, D.W.M. Arrigan, *Sens. Actuators, B.* **1997,** 44, 389-396.

[109] Z. Mousavi, K. Granholm, T. Sokalski, A. Lewenstam, *Analyst,* **2013,** 138, 5216-5220.

[110] K. Granholm, Z. Mousavi, T. Sokalski, A. Lewenstam, *J. Solid State Electrochem.* **2014 ,** 18, 607-612.

[111] A. Vincze, G. Horvai, *Proc. Electrochem. Soc.* **1997,** 97-19, 550-555.

[112] T. Zhang, C. Lai, M.A. Fierke, A. Stein, P. Buhlmann, *Anal. Chem.* **2012,** 84, 7771-7778.

[113] T. Kakiuchi, T. Yoshimatsu, N. Nishi, *Anal. Chem.* **2007,** 79, 7187-7191.

[114] Y. Mi, S. Mathison, E. Bakker, *Electrochem. Solid-State Lett.* **1999,** 2, 198-200.

[115] K.M. Mangold, S. Schäfer, K. Jüttner, *Synth. Met.* **2001,** 119, 345-346.

[116] A. Kisiel, H. Marcisz, A. Michalska, K. Maksymiuk, *Analyst.* **2005,** 130, 1655-1662.

[117] H.J. Lee, U.S. Hong, D.K. Lee, J.H. Shin, H. Nam, G.S. Cha, *Anal. Chem.* **1998,** 70, 3377-3383.

[118] K. Nagy, K. Eine, K. Syverud, O. Aune, *J. Electrochem. Soc.* **1997,** 144, L1-L2.

[119] S.P. Kounaves, M.H. Hecht, J. Kapit, K. Gospodinova, L. DeFlores, R.C. Quinn, W.V. Boynton, B.C. Clark, D.C. Catling, P. Hredzak, D.W. Ming, Q. Moore, J. Shusterman, S. Stroble, S.J. West, S.M.M. Young, *Journal of Geophysical Research: Planets.* **2010,** 115, - E00E10.

[120] S.P. Kounaves, M.H. Hecht, S.J. West, J. Morookian, S.M.M. Young, R. Quinn, P. Grunthaner, X. Wen, M. Weilert, C.A. Cable, A. Fisher, K. Gospodinova, J. Kapit, S. Stroble, P. Hsu, B.C. Clark, D.W. Ming, P.H. Smith, *Journal of Geophysical Research: Planets.* **2009,** 114,E00A19.

[121] E. Pretsch, *TrAC* **2007,** 26, 46-51.

[122] C. Zuliani, D. Diamond, *Electrochim. Acta.* **2012,** 84, 29-34.

[123] M. Valcárcel, S. Cárdenas, *TrAC* **2005,** 24, 67-74.

[124] M.M. Marei, T.J. Roussel, R.S. Keynton, R.P. Baldwin, *Anal. Chim. Acta.* **2013,** 803, 47-55.

[125] H. Kahlert, T. Steinhardt, J. Behnert, F. Scholz, *Electroanalysis.* **2004,** 16, 2058-2064.

[126] M.D. DeGrandpre, M.M. Baehr, T.R. Hammar, *Anal. Chem.* **1999,** 71, 1152-1159.

[127] G. Rumpf, U. Spichiger-Keller, H. Buehler, W. Simon, *Anal. Sci.* **1992,** 8, 553-559.

[128] A. Duzgun, G.A. Zelada-Guillen, G.A. Crespo, S. Macho, J. Riu, F.X. Rius, *Anal. Bioanal. Chem.* **2011,** 399, 171-181.

[129] S. Anastasova, A. Radu, G. Matzeu, C. Zuliani, U. Mattinen, J. Bobacka, D. Diamond, *Electrochim. Acta.* **2012,** 73, 93-97.

[130] A.G. MacDiarmid, *Synth. Met.* **2001,** 125, 11-22.

[131] A. Kisiel, A. Michalska, K. Maksymiuk, *Bioelectrochemistry.* **2007,** 71, 75-80.

[132] A. Michalska, M. Ocypa, K. Maksymiuk, *Electroanalysis.* **2005,** 17, 327-333.

[133] A. Michalska, K. Maksymiuk, *Anal. Chim. Acta.* **2004,** 523, 97-105.

[134] A.E. Musa, F.J. del Campo, N. Abramova, M.A. Alonso-Lomillo, O. Domínguez-Renedo, M.J. Arcos-Martínez, M. Brivio, D. Snakenborg, O. Geschke, J.P. Kutter, *Electroanalysis* **2011,** 23,115-121.

[135] X.U. Zou, J.H. Cheong, B.J. Taitt, P. Buhlmann, *Anal. Chem.* **2013,** 85, 9350-9355.

[136] M. Fibbioli, W.E. Morf, M. Badertscher, N.F. De Rooij, E. Pretsch, *Electroanalysis.* **2000,** 12, 1286-1292.

[137] M.R. De, J. Veder, G. Clarke, A. Nelson, K. Prince, E. Pretsch, E. Bakker, *Phys Chem Chem Phys.* **2008,** 10, 73-76.

[138] M. Vazquez, J. Bobacka, A. Ivaska, A. Lewenstam, *Sens. Actuators, B.* **2002,** 82, 7-13.

[139] M. Fibbioli, O. Enger, F. Diederich, E. Pretsch, K. Bandyopadhyay, S. Liu, L. Echegoyen, P. Buhlmann, *Chem. Commun.* **2000,** 339-340.

[140] T. Lindfors, F. Sundfors, L. Hoefler, R.E. Gyurcsanyi, *Electroanalysis.* **2011,** 23, 2156-2163.

[141] T. Lindfors, L. Hofler, G. Jagerszki, R.E. Gyurcsanyi, *Anal Chem.* **2011,** 83, 4902-4908.

[142] J. Phair, L. Newton, C. McCormac, M.F. Cardosi, R. Leslie, J. Davis, *Analyst,* **2011,** 136, 4692-4695.

[143] A.V. Mueller, H.F. Hemond, *Anal. Chim. Acta.* **2011,** 690, 71-78.

[144] A. Michalska, M. Wojciechowski, E. Bulska, K. Maksymiuk, *Talanta.* **2010,** 82, 151-157.

[145] J. Bobacka, A. Lewenstam, A. Ivaska, *J Electroanal Chem.* **2001,** 509, 27-30.

[146] B. Paczosa-Bator, L. Cabaj, R. Piech, K. Skupien, *Analyst,* **2012,** 137, 5272-5277.

[147] M. Vazquez, P. Danielsson, J. Bobacka, A. Lewenstam, A. Ivaska, *Sens. Actuators, B.* **2004,** 97, 182-189.

[148] T.V. Shishkanova, G. Broncova, M. Krondak, D. Sykora, V. Kral, *J. Mater. Sci.* **2011,** 46, 7594-7602.

[149] Z. Mousavi, J. Bobacka, A. Lewenstam, A. Ivaska, *J. Electroanal. Chem.* **2006,** 593, 219-226.

[150] T. Lindfors, *J. Solid State Electrochem.* **2009,** 13, 77-89.

[151] J. Bobacka, *Electroanalysis.* **2006,** 18, 7-18.

[152] C.K. Chiang, C.R. Fincher Jr., Y.W. Park, A.J. Heeger, H. Shirakawa, E.J. Louis, S.C. Gau, A.G. MacDiarmid, *Physical Review Letters.* **1977,** 39, 1098-1098-1101.

[153] H. Shirakawa, E.J. Louis, A.G. MacDiarmid, C.K. Chiang, A.J. Heeger, *J. Chem. Soc. , Chem. Commun.* **1977,** 578-580.

[154] "The Nobel Prize in Chemistry 2000". *Nobelprize.org.* Nobel Media AB 2013. Web. 18 May 2014, http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2000/

[155] A.J. Heeger, *Synth. Met.* **2001,** 125, 23-42.

[156] G.G. Wallace, G.M. Spinks, L.A.P. Kane-Maguire, P.R. Teasdale, *Conductive Electroactive Polymers: Intelligent Polymer Systems,* Taylor & Francis Group, Boca Raton **2009.**

[157] A.J. Heeger, S.N. Sariciftci, E.B. Namdas, *Semiconducting and Metallic Polymers,* Oxford University Press, Oxford, New York **2010.**

[158] G. Inzelt, *Conducting Polymers, A New Era in Electrochemistry,* Springer-Verlag, Berlin Heidelberg **2008.**

[159] J. Heinze, B.A. Frontana-Uribe, S. Ludwigs, *Chem. Rev.* **2010,** 110, 4724-4771.

[160] J.L. Bredas, G.B. Street, *Acc. Chem. Res.* **1985,** 18, 309-315.

[161] N. Hall, *Chem. Commun.* **2003,** 1-4.

[162] B. Krische, M. Zagorska, *Synth. Met.* **1989,** 28, 257-262.

[163] A. Lewenstam, J. Bobacka, A. Ivaska, *J. Electroanal. Chem.* **1994,** 368, 23-31.

[164] J. Bobacka, Z. Gao, A. Ivaska, A. Lewenstam, *J. Electroanal. Chem.* **1994,** 368, 33-41.

[165] A. Cadogan, A. Lewenstam, A. Ivaska, *Talanta.* **1992,** 39, 617-620.

[166] J. Migdalski, T. Blaz, A. Lewenstam, *Anal. Chim. Acta.* **1996,** 322, 141-149.

[167] A. Michalska, A. Ivaska, A. Lewenstam, *Anal. Chem.* **1997,** 69, 4060-4064.

[168] F. Jonas, L. Schrader, *Synth. Met.* **1991,** 41, 831-836.

[169] U. Lange, N.V. Roznyatovskaya, V.M. Mirsky, *Anal. Chim. Acta.* **2008,** 614, 1-26.

[170] J. Bobacka, T. Lindfors, M. McCarrick, A. Ivaska, A. Lewenstam, *Anal. Chem.* **1995,** 67, 3819-3823.

[171] J. Migdalski, T. Błaż, A. Lewenstam, *Electrochim. Acta.* **2014,** 133, 316-324.

[172] L. Groenendaal, F. Jonas, D. Freitag, H. Pielartzik, J.R. Reynolds, *Adv Mater.* **2000,** 12, 481-494.

[173] F. Jonas, G. Heywang, W. Schmidtberg, J. Heinze, M. Dietrich, *Eur. Pat. Appl.* **1989,** 1989-106236; 1988-3813589, 15.

[174] G. Heywang, F. Jonas, *Adv Mater.* **1992,** 4, 116-118.

[175] M. Dietrich, J. Heinze, G. Heywang, F. Jonas, *J Electroanal Chem.* **1994,** 369, 87-92.

[176] F. Jonas, W. Krafft, *Eur. Pat. Appl.* **1991,** 1990-124841; 1990-4003720, 17.

[177] A. Michalska, A. Galuszkiewicz, M. Ogonowska, M. Ocypa, K. Maksymiuk, *J. Solid State Electrochem.* **2004,** 8, 381-389.

[178] Z. Mousavi, J. Bobacka, A. Ivaska, *Electroanalysis.* **2005,** 17, 1609-1615.

[179] G.A. Crespo, D. Gugsa, S. Macho, F.X. Rius, *Analytical & Bioanalytical Chemistry.* **2009,** 395, 2371-2376.

[180] B. Paczosa-Bator, *Talanta.* **2012,** 93, 424-427.

[181] F. Li, J. Ye, M. Zhou, S. Gan, Q. Zhang, D. Han, L. Niu, *Analyst.* **2012,** 137, 618-623.

[182] C. Lai, M.A. Fierke, A. Stein, P. Buehlmann, *Anal. Chem.* **2007,** 79, 4621-4626.

[183] A.L. Cukierman, *ISRN Chem. Eng.* **2013,** 261523, 31.

[184] J.R. Macdonald, W.B. Johnson,*Impedance Spectroscopy, Theory, Experiment and Applications,* (Eds. E. Barsoukov, J.R. Macdonald), John Wiley & Sons, Inc., Hoboken, New Jersey **2005**.

[185] E. Barsoukov, J.R. Macdonald, John Wiley & Sons, Inc., Hoboken, New Jersey **2005**.

[186] P.M.S. Monk, *Fundamentals of Eletroanalytical Chemistry*, John Wiley & Sons Ltd., West Sussex, England **2001.**

[187] M.E. Orazem, B. Tribollet, *Electrochemical Impedance Spectroscopy,* John Wiley & Sons, Inc., Hoboken, New Jersey **2008.**

[188] N. Bonanos, J.C.H. Steele, E.P. Butler,*Impedance Spectroscopy, Theory, Experiment and Applications,* (Eds. E. Barsoukov, J.R. Macdonald), John Wiley & Sons, Inc., Hoboken, New Jersey **2005**.

[189] M. Ates, *Progress in Organic Coatings.* **2011,** 71, 1-10.

[190] G. Láng, G. Inzelt, *Electrochim. Acta.* **1999,** 44, 2037-2051.

[191] M.M. Musiani, *Electrochim. Acta.* **1990,** 35, 1665-1670.

[192] N.S. Sundaresan,*Electrical and Optical Polymer Systems: Fundamentals, Methods and Applications,* (Eds. D.L. Wise, G.E. Wnek, D.J. Trantolo, T.M. Cooper, J.D. Gresser), Marcel Dekker Inc., New York **1998**

[193] K.N. Mikhelson, *Chem. Anal. (Warsaw, Pol.).* **2006,** 51, 853-867.

[194] J. Bobacka, A. Lewenstam, A. Ivaska, *J. Electroanal Chem.* **2000,** 489, 17-27.

[195] J. Bobacka, M. Grzeszczuk, A. Ivaska, *Electrochim. Acta.* **1992,** 37, 1759-1765.

[196] J. Bobacka, M. Grzeszczuk, A. Ivaska, *J. Electroanal. Chem.* **1997,** 427, 63-69.

[197] F. Sundfors, J. Bobacka, A. Ivaska, A. Lewenstam, *Electrochim. Acta.* **2002,** 47, 2245-2251.

[198] U. Mattinen, S. Rabiej, A. Lewenstam, J. Bobacka, *Electrochim. Acta.* **2011,** 56, 10683-10687.

[199] U. Mattinen, MSc thesis, Åbo Akademi University **2008,**

[200] A. Ceresa, E. Bakker, B. Hattendorf, D. Guenther, E. Pretsch, *Anal. Chem.* **2001,** 73, 343-351.

[201] M. Guziński, G. Lisak, J. Kupis, A. Jasiński, M. Bocheńska, *Anal. Chim. Acta.* **2013,** 791, 1-12.

[202] T. Blaz, B. Bas, J. Kupis, J. Migdalski, A. Lewenstam, *Electrochem. Commun.* **2013,** 34, 181-184.

[203] H. Vakili, N. Genina, H. Ehlers, J. Bobacka, N. Sandler, *Pharmaceutics.* **2012,** 4, 366-376

Åbo Akademi
University

ISBN 978-952-12-3189-6
Painosalama Oy – Åbo, Finland 2015