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Investigation of Mg²⁺-selective electrodes for measurements in biological fluids

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Investigation of Mg$^{2+}$-selective electrodes for measurements in biological fluids

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1. Zusammenfassung


Herkömmliche Methoden zur Bestimmung von Selektivitätskoeffizienten basieren auf der semiempirischen Nicolsky-Eisenman-Gleichung. Dieser Formalismus kann zu irreführenden Resultaten führen, wenn die Ladung von Mession und Störion verschieden sind. Um aussagekräftigere Selektivitätskoeffizienten zu erhalten, wurde eine neue Definition eines konsistenten Selektivitätskoeffizienten $K_{ij}^{cono}$ vorgeschlagen, der auf einer Modifikation der Nicolsky-Eisenman-Beziehung beruht. Als Folge davon wurden die für klinische Messungen erforderlichen Selektivitätskoeffizienten angepasst.

Das Selektivitätsmuster von ionenselektiven Elektroden ist hauptsächlich durch den Ionophor und andere Membrankomponenten bestimmt. In der vorliegenden Arbeit wurde nachgewiesen, dass die Zusammensetzung des Innenelektrolyten der Elektrode ebenfalls
1. Zusammenfassung

die Selektivität und Detektionsgrenze in einem gewissen Ausmass beeinflussen kann. Es zeigte sich, dass sich hohe Mg\(^{2+}\)-Konzentrationen in der Innenlösung günstig auf die Selektivität der Elektrode auswirkten. Störionen (z.B. Ca\(^{2+}\)) in der Innenlösung hatten demgegenüber einen gegenteiligen Einfluss auf das Selektivitätsverhalten.

Bei Messungen in Blutproben leiden Mg\(^{2+}\)-selektive Elektroden oft unter Interferenzen, die auf Silikone zurückgeführt werden. Es wird vermutet, dass sich diese lipophilen Verbindungen von Blut-Teströhrchen ("Vacutainer") lösen und an der Oberfläche der Elektrodenmembranen adsorbieren. Es konnte gezeigt werden, dass diese Interferenzen mit einer geeigneten Membranmatrix reduziert oder völlig eliminiert werden können. Elektrodenmembranen aus weichgemachtem PVC, die einen geeigneten Anteil an Polyurethan und Silikonöl enthielten, schnitten in dieser Hinsicht am besten ab und zeigten dabei eine zufriedenstellende Selektivität und Sensitivität.
2. Summary

The aim of this work was to develop magnesium-selective electrodes for the direct measurements of free magnesium ion active molality ($a_{Mg^{2+}}$) in whole blood and blood serum. The $Mg^{2+}$ is considered to be clinically and physiologically more relevant than the total concentration of magnesium.

Tripodal malonyl diamide derivatives and dipodal malonyl diamide derivatives of diazacrown ethers have been shown experimentally to be effective synthetic host compounds for $Mg^{2+}$. They exhibited high preference for $Mg^{2+}$ over $Ca^{2+}$. In this work, these newly synthesised $Mg^{2+}$-selective ionophores were systematically investigated in respect to selectivity patterns and lipophilicities. The addition of lipophilic borate salts (anionic sites) can significantly reduce the bulk membrane resistance and the charge transfer resistance at the membrane / solution interface. This was supported by impedance studies of $Mg^{2+}$-selective membranes. The stoichiometries of ion-ionophore complexes were estimated by optimizing the molar ratio of borate/ionophore in the PVC membrane with respect to the selectivity pattern. These results will help in the future development of more selective $Mg^{2+}$-selective ionophores.

The conventional methods to determine selectivity coefficients are based on the semi-empirical Nicolsky-Eisenman equation. This formalism can lead to misleading results in the case of different charges of the primary and interfering ions. In order to obtain a more reasonable selectivity coefficient, a new definition of the selectivity coefficient $K_{i,j}^{\text{const}}$ was proposed on the basis of a modification of the Nicolsky-Eisenman equation. The required selectivity coefficients of ISE in blood analysis were recalculated on the basis of this new approach.

Although the selectivity pattern of ISE is mainly established by the intrinsic property of the ionophore and other membrane components, the inner filling solution proved to affect the selectivity and detecting limit to some extent. A high concentration of the primary ion ($Mg^{2+}$) in the inner solution proved to be beneficial for the selectivity of
2. Summary

Mg$^{2+}$-ISEs. The existence of interfering ions (e.g. Ca$^{2+}$) in the inner solution decreases the ISE’s preference of Mg$^{2+}$ over interfering ions in sample solution.

In determining the ionized magnesium molality in blood samples, Mg$^{2+}$-selective electrodes often suffer from the so-called “silicone-interference” which causes an unstable emf response. The lipophilic silicone substances dissolved from the blood collecting tube (Vacutainer) were assumed to be adsorbed onto the electrode membrane surface. It was shown in this work that the addition of suitable quantities of polyurethane and silicone oil to the PVC membrane matrix could reduce or even exclude the “silicone interference” and such electrodes still showed a satisfactory selectivity and sensitivity.
3. Introduction

A chemical sensor is a device that transforms chemical information into an analytically useful signal. This can range from the concentration of a specific sample component to total composition analysis. Ion-selective electrodes (ISEs) are electrochemical sensors that allow the potentiometric determination of the activity of an analyte ion in the presence of other interfering ions. ISEs today form one of the most important groups of chemical sensors [1,2]. The intensive research for novel electrode materials and new constructions has subsequently given way to more detailed studies on ion selectivity and electrode mechanisms as well as the practical applications of ISEs. A broad range of electrodes, specific for various anions and cations is available, and are routinely used in diagnostic instruments, in clinical analyzers, in process control, and in environmental analyses [3-6].

The history of ion-selective electrodes goes back to the early 1930’s with the application of the pH glass electrode. In 1932, Arnold Beckman developed the modern glass pH electrode [7], which was the first commercial chemical sensor. In 1937 Kolthoff and Sanders [8] published a paper which made use of solid state electrodes, such as the silver halide and fluoride-selective electrodes. In the late 1950s, Eisenman and coworkers carried out a systematic study on various types of glass which led to the development of electrodes that were selective for certain monovalent ions such as Na⁺, K⁺, Ag⁺, Tl⁺, Li⁺ and Cs⁺ [9]. The basic concept of chemical sensors owes much to the investigation of Moore and Pressman in the 1960’s on the effect of naturally occurring neutral antibiotics on biological membrane systems. In the meantime, Ross and Simon had applied for a patent covering the K⁺-selective electrode, and a new class of chemical sensors based on neutral carriers had been introduced [10]. G.J. Moody, R.B. Oke and J.D.R. Thomas introduced plasticized PVC as the matrix of the electrode membrane to prevent the membrane components from being hydrated while allowing the active components to be sufficiently mobile [11]. Since the end of the 1960’s, ISEs have proved to be important in analytical chemistry and many of the electrodes have been successfully commercialized.
Along with the intensive investigation and increasing development of science and technology, various chemical sensors have been developed for different applications. It has been estimated that by 1990 more than 7000 papers on ISEs had been published [7]. ISEs today form one of the most important groups of chemical sensors. On the other hand, bulk membrane optodes (optical selective sensors) have been known for only about 10 years. Optodes are optical sensors which consist of the selective ligand for recognition for the analyte and an indicator dye for the optical transduction. A large number of such optodes have been developed in a short time since ionophores originally developed for ISEs can often be used without further modification. Moreover, the two types of sensors rely on very similar chemical processes. The commercial market for the two types of sensors is forecast to grow rapidly over the next few years, especially in the field of biomedicine in which the main application is the measurement of blood parameters.

Magnesium which contributes 1.94% to the mass of the earth is an extremely important divalent cation in the intracellular space controlling some 300 enzymatic reactions, involving e.g. ATPases, creatine kinase, alkaline phosphatase and enolase. Total magnesium deficiency can lead to neuromuscular, psychiatric and cardiovascular disorders [12]. It has also been associated with sleep disturbances, as well as anxious and depressive disorders [13].

The total magnesium content of the human body is in the range of 20 to 80 g for an adult corresponding to approximately 1 to 2 mol. Of the total magnesium concentration, 53% is present in the bones, 27% in muscles, 19.2% in soft tissues with only a small percentage in blood (0.8%), of which 0.3% corresponds to plasma and 0.5% to erythrocytes [14]. Human plasma magnesium can be divided into three fractions: 1. protein-bound magnesium, mainly bound to albumin (76%) and to a lesser extent to globulins (24%), 2. ionized magnesium (Mg$^{2+}$), which is the biologically active fraction in the extracellular fluid, and 3. magnesium complexed with anions such as phosphate, bicarbonate, and citrate [15,16]. The ionized magnesium concentration in the healthy adult serum pool was evaluated to be 0.57 mmol/L (0.185 mmol/L activity) with a degree of complexation of 24% (AAS, total
concentration in the same serum pool: 0.75 mmol/L) [17]. Analytical techniques with high accuracy and precision to measure extracellular Mg\(^{2+}\) are urgently needed for physiological applications [16,18-21]. Over the years, many different methods have been developed for determining total and ionized magnesium concentration in serum/plasma. In 1947, the first colorimetric method for measuring Mg\(^{2+}\) in biological fluids was described [22] and studies are still being published about this technique [23]. Other methods involve enzymology [24,25], fluorometry [26,27], flame-emission spectrometry [28] or atomic absorption spectrometry (AAS). The latter method has been proposed as the candidate reference method for the determination of magnesium in human serum/plasma [29,30]. Alternatively, in situ analysis can be carried out by X-ray fluorescence [31]. Ion-selective electrodes (ISEs) have been used in measuring the activity of the ionized Mg\(^{2+}\). \(^{31}\)P and \(^{25}\)Mg-NMR spectroscopy is a reliable method for the determination of Mg\(^{2+}\) activity or concentration [32].

The direct determination of the free Mg\(^{2+}\) activity or concentration has become possible with the Mg\(^{2+}\)-selective electrodes [33]. Since the first application of the K\(^{+}\)-selective polymeric membrane electrodes based on valinomycin in a commercial analyzer (STAT-ION) in 1972, neutral ligands for most of the biologically relevant ions have been developed. Mg\(^{2+}\)-selective electrodes have been studied intensively in order to discriminate between Mg\(^{2+}\) and Ca\(^{2+}\) in aqueous samples. Owing to the low intracellular Ca\(^{2+}\) concentration, satisfactory intracellular Mg\(^{2+}\) determinations were possible with microelectrodes of low selectivity against Ca\(^{2+}\) since the early 1980s [34]. For the direct measurement of extracellular Mg\(^{2+}\), successful Mg\(^{2+}\)-selective electrodes had been reported only since 1990 [17,35] based on the syntheses of ionophores, ETH 5282 and ETH 7025. The Mg\(^{2+}\) selectivity coefficient over calcium ion \(\log K^\text{ref}_{\text{Mg, Ca}}\) reached -0.8 (ETH 5282) and -1.0 (ETH 7025), respectively. Up to now, about 50 relevant magnesium ionophores for Mg\(^{2+}\)-selective electrodes have been synthesized [1,36-38] and various new electrodes have been developed by Prof. W. Simon and Prof. U. Spichiger at the ETH, Zürich. In recent years, many new Mg\(^{2+}\) ionophores have been synthesized and characterized in our laboratory and other laboratories [1,36-38]. The response mechanism of polymeric selective membrane
and the bio-compatibility for physiological analysis were investigated \[39-42\]. However, reliable Mg\(^{2+}\)-selective electrodes have only been used routinely in clinical chemistry for a few years \[43,44\]. The clinical analyzers measuring Mg\(^{2+}\) were commercialized by AVL, Kone and Nova \[43,45,46\].

In this work, electrodes using the newly synthesized Mg\(^{2+}\)-selective ionophores were studied systematically. The functions of lipophilic borate salts in the electrode membranes are discussed, based on the experimental results. Two different types of ionophores (tripodal malonic acid diamide derivatives and dipodal malonic acid amide derivatives with diazacrown ether) were compared in PVC membranes regarding selectivity, lipophilicity and stoichiometry. Selectivity coefficients based on the conventional Nicolsky-Eisenman equation have been much discussed recently, especially the discrepancies when the primary ion and interfering ion have different charges \[47\]. In order to express the selectivity of ISEs, a modification of the conventional Nicolsky-Eisenman equation was made. A charge independent selectivity coefficient was derived from the Nicolsky-Eisenman equation. Using this selectivity coefficient, it was possible to calculate the required selectivity coefficients for reliable clinical measurements. To exclude the so-called “silicone interference” in measuring blood samples with PVC membrane-based Mg\(^{2+}\)-selective electrodes, membrane matrices were tested with numerous polymers and a matrix of PVC and polyurethane showed satisfactory results.
4. Theory of ion-selective electrodes

4.1 Classification of ion-selective electrodes

Ion-selective electrodes (ISEs) can be classified into different groups according to the properties of their membranes.

4.1.1 Glass membrane electrodes

Glass pH electrodes have been widely used since the early 1930s to quantify the concentration of H⁺ in solution [7]. Glass membrane electrodes are usually formed from silicate or alumino-silicate or multi-component glass. These electrodes operate on the principle that when a glass membrane is immersed into an aqueous solution, an ion-exchange mechanism with the fixed SiO⁻ group in the glass membrane boundary region is initiated. The glass is made of a solid silicate matrix within which alkali metal ions are mobile. When this glass membrane is brought into contact with an aqueous solution, its surface becomes hydrated to a depth of about 100 nm and the alkali metal cations (principally Na⁺) from the glass matrix can be exchanged for other ions in the solution, preferably H⁺. Thus, an electrostatic potential across the membrane is created which is a linear function of the pH of the solution. The glass pH electrodes realized the idea of specific sensing and they have been the most frequently and successfully used electrochemical sensors.

\[
Na^+_{(glass)} + H^+_{(aq)} \leftrightarrow H^+_{(glass)} + Na^+_{(aq)}
\]

Doping the glass membrane with different proportions of aluminum oxide and other metal oxides can produce selective glass membrane electrodes for other cations such as lithium, sodium, potassium, silver, or ammonium [48-50].
4. Theory of ion-selective electrodes

4.1.2 Solid-state membrane electrodes

Solid-state membrane electrodes are used primarily as sensors for those kinds of ions forming either insoluble salts or insoluble stable complexes with the ionic sites of the membrane material [51]. Various crystalline materials can be used as membrane materials, including single crystals, cast or sintered materials, pressed polycrystalline pellets, as well as heterogeneous combinations of precipitates held in hydrophobic polymer binders. One of the first usable electrodes based on crystalline material was realized in 1961 by Pungor and Hollos-Rokosi [52] using silver iodide precipitate, embedded in an inert matrix as an iodide-sensitive membrane. The potentiometric response of the solid-state membrane to the target ion is governed by the boundary potential at the membrane-solution interface where the sensing ion is precipitated by the reaction with the membrane component. The selectivity of the electrodes for the target ion against the interfering ions can be achieved by different solubility products $K_{sp(M+)}$ of their precipitate on the membrane surface. For example, when the silver halide membrane serves as a sensor for halide ions, the selectivity sequence is $S^> > I^- > Br^- > SCN^- > Cl^-$. By making use of the same principle, electrodes based on LaF$_3$ single crystal show a high preference for fluoride over other halide ions but are subject to some interference by hydroxyl ions.

The number of solid-state materials is limited. Among those used or considered for selective electrode applications are [51]:

- rare earth- and alkaline earth metal fluorides;
- halides of silver, lead, mercury and thallium;
- sulfides and other chalcogenides of silver, copper, lead, mercury, cadmium and zinc;
- silver thiocyanate, cyanide, azide, chromate and phosphate;
- mixtures of different halides or chalcogenides with silver sulfide.
4. Theory of ion-selective electrodes

4.1.3 Liquid membrane electrodes

In conventional liquid membrane electrodes, the carriers or ligands are immobilized in an inert matrix such as diaphragm or polymer. According to the different membrane response mechanisms, there are two different groups of liquid membrane electrodes namely (a) liquid ion-exchanger membrane electrode; (b) neutral carrier and charged carrier based liquid membrane electrodes. In (b), only the neutral carrier based liquid membrane electrodes are discussed.

a). Liquid ion-exchanger membrane electrodes consist of an organic water-immiscible liquid phase incorporating mobile ionic additives, such as hydrophobic acids, bases, and salts. The electrically charged ion-exchanger in liquid-membrane generally shows permselectivity for oppositely charged ions without complexation in polar membrane solvents. The ion-exchange is usually expressed as:

$$ I - E_{(memb)} + J_{(aq)} \rightleftharpoons J - E_{(memb)} + I_{(aq)} $$

where $E_{(memb)}$ is the ion exchanger in the membrane phase and $I$, $J$ are the two ions involved in the process. The selectivity between different counter-ions of the same charge is governed by the extraction behaviour of the solvation membrane medium. The partition of compounds in the two phases of different polarities can be denoted by the lipophilicity of the compound. The lipophilicity of ions is represented by the Hofmeister Series for organic, apolar phases and is driven by the hydration enthalpy of ions [53]:

$$ \text{ClO}_4^- > \text{SCN}^- > \Gamma^- > \text{NO}_3^- > \text{Br}^- > \text{Cl}^- > \text{HCO}_3^- > \text{AcO}^- > \text{SO}_4^{2-} \sim \text{HPO}_4^{2-} $$
$$ \text{R}^+ > \text{Cs}^+ > \text{Rb}^+ > \text{K}^+ > \text{NH}_4^+ > \text{Na}^+ > \text{Li}^+ \sim \text{Ba}^{2+} > \text{Sr}^{2+} > \text{Ca}^{2+} > \text{Mg}^{2+} $$

b). The class of neutral carrier-based liquid membrane electrodes was first introduced with the valinomycin-based K$^+$-selective electrodes by W. Simon [54]. The electrode membrane usually contains the electrically neutral, ion-specific complexing ligand (or ion-carrier, ionophore) which is dissolved in the lipophilic organic solvent in the membrane matrix. Polymeric membrane based ISEs are the most versatile chemical
4. Theory of ion-selective electrodes

sensors and have been reported for over 60 different analytes [55]. The selective complexation occurs between the ligand and target ion at the membrane-solution interface where a boundary potential is generated. As the ligand can specifically complex the target ion in the presence of interfering ions, the selectivity of membrane electrodes for target ions against interfering ions relies mainly on the stability of the respective ion/ionophore complexes. This means that such type of electrodes can be selective to the target ion, which does not match the “Hofmeister Series”. For example, the liquid membrane electrode based on the neutral ionophore ETH 5506 exhibits a 100 fold higher sensitivity for Mg$^{2+}$ over Ca$^{2+}$ ($\log K_{Mg, Ca}^{pre} = 1.9 \pm 0.06$) [56].

4.1.4 Asymmetric electrodes

In the context of chemical sensors for various fields, ISEs with special characteristics have been developed. Many gas-sensitive electrodes and enzyme electrodes have been developed with the potentiometric detection unit based on the conventional electrodes discussed above. Microelectrodes have been designed to measure ion activities inside living cells [57,58] or in small samples. They can be constructed by immobilizing the ion-exchanger and ionophore in the tips of micropipettes. To eliminate the internal filling solution, the solvent polymeric membrane may be brought into direct contact with a metal wire or other conducting materials, semi-conductors / insulators. By coating thin wires with the solvent polymeric material, miniaturization of potentiometric sensors becomes possible. Ion-selective field effect transistors (ISFETs) [59] are the hybrids of ion-selective electrodes and metal-oxide field effect transistors (MOSFETs). In the ISFET, the metal gate of a MOSFET is in direct contact with either a solid or liquid ion-selective membrane.

Table 4.1. Characteristics of ion-selective electrodes

<table>
<thead>
<tr>
<th>Sensitive layer</th>
<th>Inner electrode</th>
<th>Nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>Electrolyte, AgCl/Ag</td>
<td>Symmetric electrodes</td>
</tr>
<tr>
<td>Polymeric membrane</td>
<td>Electrolyte, AgCl/Ag</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solid contact</td>
<td>Asymmetric electrode</td>
</tr>
<tr>
<td>Solid state, crystals</td>
<td>Solid contact</td>
<td></td>
</tr>
</tbody>
</table>
4. Theory of ion-selective electrodes

4.2 Ionic strength and activity coefficients

Ion-selective electrodes measure the activities (active molalities or molal activities) of ions in the sample solution which effectively contribute to the potential response of the electrode membrane. The ion activity (active molality) is obtained from the concentration by taking into account the activity coefficient \( \gamma \).

\[
a_i = \gamma_i \cdot m_i \quad (4-1)
\]

or

\[
a_i = \gamma_i \cdot c_i \quad (4-2)
\]

where factor \( \gamma_i \neq 1 \) is the activity coefficient for a single ion; \( m_i \) is the mass concentration (molality) of single ion in solution (mol/kg solvent) in Eq. 4-1. Usually in the case of a dilute solution, \( m_i \) is equal to the volume concentration \( c_i \) (mol/L solvent) in Eq. 4-2. A simplified model was proposed by Debye and Hückel using a solution with a strong electrolyte where the ions are not hydrated but only involved in electrostatic interactions \[60,61\]. For alkali and alkaline earth salts, the Debye and Hückel approach gives a fair approximation of the mean activity coefficient \( \gamma^* \) for a single ion and its counter-ion.

\[
\log \gamma^* = \frac{z^+ z^- A \sqrt{I}}{1 + B a \sqrt{I}} \quad (4-3)
\]

where:

- \( A, B \): Debye-Hückel constants (for water at 25°C: \( A = -0.509 [(\text{mol} \, \text{l}^{-1})^{0.5}] \), 
  \( B = 0.328 [(\text{mol} \, \text{l}^{-1})^{0.5} \, \text{Å}^{-1}] \);
- \( z^+, z^- \): charge numbers of cation and anion of relevant electrolyte;
- \( I \): ionic strength of solution;
- \( a, C \): constants for fitting the theoretical relationship to the measured \( \gamma^* \).

The ionic strength \( I \) of the electrolyte solution is related to the electrostatic interaction between ions which corresponds to their ionic charges and ionic radius.
4. Theory of ion-selective electrodes

\[ I = \frac{1}{2} \sum z_i^2 m_i \]  \hspace{1cm} (4-4)

\( m_i \): mass concentration (molality) of single ion in solution (mol/kg solvent);
\( z_i \): charge of any electrolyte species in the sample solution.

According to the approximation proposed by Debye and Hückel \([60]\), the activity coefficient of a single ion, which is immeasurable, is given by:

\[ \log \gamma_+ = \frac{|z_+/z_-|}{|z_-/z_+|} \log \gamma_{\pm} \]  \hspace{1cm} (4-5)

\[ \log \gamma_- = \frac{|z_-/z_+|}{|z_+/z_-|} \log \gamma_{\pm} \]  \hspace{1cm} (4-6)

Figure 4.1 illustrates the relationship between \( \gamma_\pm \), \( \gamma_+ \) and \( \gamma_- \), using MgCl\(_2\) solution as an example.

![Graph](image)

Figure 4.1. The mean and single-ion activity coefficients of MgCl\(_2\) as functions of the logarithm of the ionic strength. The curves were plotted using Eq. 4-3, 4-5 and 4-6. The upper curve gives the single anion activity coefficient function for Cl\(^-\), \( \gamma_- \), and the bottom curve shows the single cation activity coefficient function for Mg\(^{2+}\), \( \gamma_+ \). Circles are measured \( \gamma_\pm \).

In the Debye-Hückel approximation, the ions are not hydrated and are only involved in electrostatic interactions between ions. Many other approximations based on
In the Debye-Hückel approximation, the ions are not hydrated and are only involved in electrostatic interactions between ions. Many other approximations based on Debye-Hückel model have been made taking the hydration sphere of water molecules into account \cite{62} as well as in mixed electrolyte solutions \cite{63,64}. Investigations have also been made for biological fluids \cite{64,65}, in which the interactions between ions in mixed electrolyte solutions as well as the interactions between neutral compounds and ions have been taken into account. To date, the Pitzer approximation seems to be ideally suited for mixed electrolytes in biological fluids. For monovalent ions, both approximations, Debye-Hückel and Pitzer, show good agreement. In contrast, there is considerable difference for divalent ions \cite{62}. In this thesis, the activity coefficients of all aqueous solutions were calculated with a computer program based on the Pitzer approximation since most solutions used here are mixed electrolyte solutions.
4. Theory of ion-selective electrodes

4.3 The principles of ion-selective electrodes

The conventional layout of a potentiometric cell is given in Figure 4.2. One half-cell consists of an ion-selective membrane, an inner filling solution and an internal reference electrode. The other half-cell consists of a reference electrode dipping into a reference electrolyte. The two half-cells are contacted by an intermediate salt bridge electrolyte which is normally housed in the reference electrode body. The total potential difference between the two electrodes (electromotive force, emf) is measured by a voltmeter. In this work, the cation is considered as the analyte for illustrating the principle of ion-selective electrodes. Anion-selective electrodes rely on the same principle.

![Figure 4.2. Schematic diagram of an ISE-based potentiometric cell](image)

The configuration of a typical membrane electrode cell may then be represented as follows:

$$E_i$$  $$E_2$$  $$E_3$$  $$E_1$$  $$E_4$$  $$E_5$$  $E_{\text{ref}}$

| Hg/Hg$_2$Cl$_2$ | KCl (satd.) | salt bridge | sample solution | membrane | internal solution | AgCl/Ag |

reference electrode  ion-selective electrode (ISE)
4. Theory of ion-selective electrodes

The total potential difference measured between the two terminals of the cell is composed of several local potential differences in the potentiometric cell.

\[ E = (E_1 + E_2 + E_3 + E_4 + E_5) + E_J + E_M = E_0 + E_J + E_M \]  

\[ E_0 = E_1 + E_2 + E_3 + E_4 + E_5 \]

where

- \( E \) electromotive force (emf);
- \( E_0 \) reference potential, including the potential contributions from \( E_1 \) to \( E_5 \);
- \( E_J \) liquid junction potential;
- \( E_M \) membrane potential.

For a given potentiometric cell and a fixed temperature, \( E_0 \) is constant and not affected by the sample composition. The liquid junction potential \( E_J \) arises at the junction between the sample solution and the salt bridge solution, which may vary according to the activities of both the bridge electrolyte and the sample electrolyte. Methods to reduce \( E_J \) will be discussed in section 4.3.1.

The potential difference across the ion-selective membrane \( E_M \) depends on the activity (less precisely on the concentration) of the ion on both sides (see Eq. 4-17). \( E_M \) consists of three parts: the internal membrane contribution \( E_D \) and two membrane-liquid interfacial contributions, \( E_B^+ \) and \( E_B^- \).

\[ E_M = E_D + E_B^+ + E_B^- \]  

\( E_D \) is the diffusion potential that comes from charge diffusion within the bulk membrane. \( E_B^+ \) and \( E_B^- \) are the phase boundary potentials that originate at the surfaces of sample solution-membrane and membrane-inner contacting reference solution.

If the activity of the inner filling solution is maintained constant, the membrane potential \( E_M \) provides information about the analyte activity in the sample solution.
4.3.1 Liquid junction potential

At the junction between the bridge electrolyte of the reference electrode and the sample solution (diaphragm, free flowing capillary etc.), the ions in both solutions diffuse into each other along their concentration gradients. The difference in mobility of the ions causes a charge separation and an electric field is formed which hinders further charge separation. After some time, a steady state is reached in which the ions no longer separate and all ions move at the same rate along the chemical potential gradient. This charge separation across the liquid junction generates a potential difference, the liquid junction potential $E_J$ (Figure 4.3).

![Figure 4.3. Charge separation and the liquid junction potential. $a_1$ and $a_2$ are the activities of electrolyte at the borders of the diffusion layer, $a_1 > a_2$.](image)

In the above assembly (see Figure 4.2), $E_3$ and $E_J$ are examples of this type of potential. On the assumption that the inner electrolyte concentration of the reference electrode and the solution temperature do not change, $E_3$ remains constant. The potential contribution of $E_J$ may vary with the composition and the temperature of the sample solution. A change of the liquid junction potential has however, the same effect, on the measured potential as a change in the concentration of the primary ion in the sample on the ion-selective electrode. Thus, estimations of $E_J$ are of practical interest either to eliminate it or to numerically correct it using the Henderson equation.
4. Theory of ion-selective electrodes

[66]. This equation is derived from the Nernst-Planck equation under the assumptions of zero current and linear activity profiles for all ions within the membrane:

\[
E_j = \frac{\sum z_m u_m a_m - \sum z_x u_x a_x}{\sum z_m^2 u_m a_m + \sum z_x^2 u_x a_x} \cdot \frac{RT}{F} \ln \frac{\sum z_m^2 u_m a_m (0) + \sum z_x^2 u_x a_x (0)}{\sum z_m^2 u_m a_m (d) + \sum z_x^2 u_x a_x (d)}
\]  

(4-10)

where,
- \(z_m, z_x\) charge numbers of the cation and anion;
- \(u_m, u_x\) mobilities of the cation and anion (\(\text{cm}^2 \text{s}^{-1} \text{J}^{-1} \text{mol}\));
- \(a_m, a_x\) active molalities of the cation and anion (\(\text{mol kg}^{-1}\));
- \(\Delta a_m, \Delta a_x\) difference of active molalities of the cation and anion between the sample solution and the bridge solution (\(\text{mol kg}^{-1}\));
- (0), (d) (0) represents the sample solution; (d) represents the bridge solution;
- \(R\) gas constant (8.314 \(\text{J K}^{-1} \text{mol}^{-1}\));
- \(T\) absolute temperature (K);
- \(F\) Faraday constant (96487 C mol\(^{-1}\)).

Figure 4.4. Liquid junction potential \(E_j\) as a function of the single-ion activity of the sample cation (\(\text{Na}^+, \text{K}^+, \text{Ca}^{2+}\) and \(\text{Mg}^{2+}\), chloride salts) for 1M and 3M KCl bridge electrolytes. Calculations are based on Eq. 4-10 using single ion activities.
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Figure 4.5. Liquid junction potential as a function of the single-ion activity of the sample cation (Mg$^{2+}$, Ca$^{2+}$ within the physiological range) for 1M KCl bridge electrolyte. Activity coefficients were calculated according to Pitzer and $E_J$ according to Eq. 4-10.

(a) $c_{Mg^{2+}}$ (0.3 mM-0.6mM) in the following physiological background solution:
   
   $c_{Ca^{2+}}=1.3$ mM; $c_{K^+}=4.0$ mM; $c_{Na^+}=140.0$ mM; chloride salts.

(b) $c_{Ca^{2+}}$ (1.0 mM-1.3 mM) in the following physiological background solution:
   
   $c_{Mg^{2+}}=0.6$ mM; $c_{K^+}=4.0$ mM; $c_{Na^+}=140.0$ mM; chloride salts.

In order to exclude or minimize the contribution of the liquid junction potential to the measurements, two factors were taken into account for salt bridge electrolyte. (1) A molar concentrations of the bridge electrolyte ($\alpha_m(\bar{d})$, $\alpha_m(\bar{d}) \gg \alpha_m(0)$, $\alpha_m(0)$) minimizes the variation of $E_J$. Therefore, varying electrolyte activity in a sample solution cannot significantly change the value of $E_J$. (2) Cations and anions in the salt bridge solution should have the same absolute charge number and similar absolute mobilities (equitransferent solution), e.g. KCl ($\mu_{K^+} = 8.10 \times 10^8$ cm$^2$ s$^{-1}$ J$^{-1}$ mol, $\mu_{Cl^-} = 8.11 \times 10^8$ cm$^2$ s$^{-1}$ J$^{-1}$ mol) in order to minimize $E_J$ ($\sum z_m \mu_m a_m(0) = \sum |z_x| \mu_x a_x(0)$).

The double-bridge system is generally used as the reference electrode (see Figure 4.2) in which a saturated KCl solution is used as the inner bridge electrolyte solution and 3M KCl as the outer one (Figure 4.4). In clinical application, the outer bridge solution is usually 1M KCl (Figure 4.5). When the target ions in a sample solution is one of
4. Theory of ion-selective electrodes

the bridge electrolyte ions (K⁺ or Cl⁻) or when certain sample ions react with either or of both, e.g. Ag⁺, Pb²⁺ etc., KCl can be replaced by LiOAc or NH₄NO₃ solutions as the outer bridge solution. This is because in the latter two solutions, the cation has the similar mobility values to that of its counterion [51].

4.3.2 Membrane potential

The membrane potential $E_M$ consists of two boundary potentials, $E_B$, and a diffusion potential $E_D$. The boundary potential (Donnan potential) is produced by the ion exchange equilibria at the phase boundaries between the membrane and both the sample solution and the inner electrolyte, respectively. $E_D$ arises from the diffusion of the ions within the membrane itself. In Figure 4.6, two boundary potentials, $E_B$ and $E_B^*$, are located at the interfaces x=0 and x=d, respectively.
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Figure 4.6. Schematic representation of the electrode assembly

- $a_i(^i)$, $a_j(^i)$: activities of primary and interfering ions in the bulk of sample solution;
- $a_i(^\prime \prime)$, $a_j(^\prime \prime)$: activities of primary and interfering ions in the bulk of inner electrolyte;
- $a_i(0)$, $a_j(0)$: activities of I and J in the membrane boundary (0);
- $a_i(d)$, $a_j(d)$: activities of I and J in the membrane boundary (d);
- $c_{i,\text{tot}}$: total ligand concentration within the membrane;
- $c_R$: concentration of the anionic sites $R^-$ in the membrane;
- $\phi$: local electrostatic potential.
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$E_M$ is the difference of the electronic potentials $\phi'$ and $\phi''$ between both electrolyte solutions adjacent to the membrane.

$$E_M = \phi'' - \phi' = [\phi(0) - \phi''] - [\phi(d) - \phi''] + [\phi(d) - \phi(0)]$$

The symbol $\phi$ in Eq. 4-11 denotes the local electrostatic potentials at the boundaries of the membrane surfaces, (0) and (d), and the solutions, ('$\phi''$) and ('$\phi'$), respectively.

The following three assumptions have been made for the above model:

1. A chemical equilibrium exists between the aqueous sample solution and the membrane phase;
2. The activity coefficients of all ions and ion-ligand complexes of the same charge are identical and the activity of neutral molecules (e.g., the ligand) is equal to their concentration;
3. The concentrations of ligand and anionic site are constant throughout the membrane.

4.3.3 Diffusion potential (membrane internal)

When a membrane separates two solutions of different species or different active molalities, a diffusion process takes place within the membrane phase driven by the activity gradient. Different ionic mobilities of cations and anions cause a charge separation and establish a potential difference. The potential difference itself will simultaneously influence the diffusion process to keep the membrane phase in a zero current state. Ions of higher mobility are slowed down by the electric field while ions of lower mobility are speeded up. When the steady state for the diffusion is reached, the potential difference within the diffusion layer is called the diffusion potential $E_D$ (Figure 4.7). The diffusion potential is concisely described as [51]:

$$E_D$$
4. Theory of ion-selective electrodes

\[ E_D = -\frac{RT}{F} \int_{a_i}^{a_0} \frac{t_i}{z_i} d \ln a_i \text{ (memb)} \]  

(4-12)

where \( a_0 \) and \( a_d \) denote the active molalities of ion I at the two borders of the membrane phase \( x=0 \) and \( x=d \); \( z_i \) the charge number of ion I; \( t_i \) the electrical transference number of ion I. When steady state conditions are reached within the membrane (membrane conditioning), the diffusion potential \( E_D \) is assumed to be constant. \( E_D \) is an important part of the \( E_M \) and the concept can also be applied to the liquid junction potential \( E_J \).

![Diagram of membrane diffusion potential](image)

**Figure 4.7.** Membrane diffusion potential, \( E_D \). \( a_0 \) and \( a_d \) are the activities of electrolyte in the inner solution and the sample solution. The \( E_D \) is the diffusion potential across the membrane.
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4.3.4 Boundary potential (Donnan potential)

The phase boundary potential difference arises mainly from the non-uniform distribution of electrically charged species between two phases. In a general sense, the electrical boundary potential plays a dominant role in the charge-transfer reactions at the interfaces. When an ion-selective membrane contacts an aqueous solution, the ions in the solution interact with the ligand within the membrane phase (see Figure 4.8), which generates the charge separation or interfacial electric field. When the electric field reaches the charge-balance, the potential difference across the two phases denotes the phase boundary potential or Donnan potential, $E_B$.

Based on the above assumptions for the model in Figure 4.6, the boundary potentials $E_{B'}$ and $E_{B''}$ for all ions $I$ of the same charge $z_i$ can be described as [67]:

$$E_{B'} = \frac{RT}{z_i F} \sum \frac{k_i a_i'}{\ln \gamma_i c_{i,0}}$$  

$$E_{B''} = \frac{RT}{z_i F} \sum \frac{k_i a_i''}{\ln \gamma_i c_{i,d}}$$  

with

$$k_i = \exp \left\{ \frac{\mu_i^{0} (aq) - \mu_i^{0} (memb)}{RT} \right\}$$  

where

$a_i'$ activity of ion $I$ in the aqueous sample solution;

$a_i''$ activity of $I$ in the inner electrolyte solution;

$c_{i,0}$ concentration of $I$ in the membrane layer contacting the sample solution;

$c_{i,d}$ concentration of $I$ in the membrane layer contacting the inner solution;

$\mu_i^{0} (aq)$ chemical standard potential of $I$ in the aqueous phase;

$\mu_i^{0} (memb)$ chemical standard potential of $I$ in the membrane phase;

$k_i$ partition coefficient of $I$ between the aqueous solution and the membrane phase.
In conclusion, when \( I \) is the only target ion in sample solution, the membrane potential \( E_M \) can be described according to Eq. 4-12, 4-13 and 4-14:

\[
E_M = \frac{RT}{z_i F} \left\{ \ln \left( \sum_i k_i a_i \right) - \ln \left( \sum_i k_i c_{i,0} \right) \right\} - \frac{RT}{z_i F} \ln a_i - \ln \left( \sum_i c_{i,d} \right) \ln a_i (\text{memb.})
\]  

(4-16)

As the neutral ligand-based membrane contains a defined number of anionic sites that control the total concentration of the cationic species in the membrane, \( c_{i,0} \) and \( c_{i,d} \) are independent of any activity change in the aqueous solution. Assuming that \( a_i (\text{memb}) \) in the membrane phase is constant over time, Eq. 4-16 can be simplified to:

\[
E_M = \text{constant} + \frac{RT}{z_i F} \ln a_i
\]  

(4-17)

### 4.3.5 Nernst and Nicolsky-Eisenman equation

When the ion-selective electrode responds selectively to the target ion in the sample solution, a potential difference is generated across the two sides of the electrode membrane, which is ideally, a linear function of the logarithm of the analyte activity (primary ion \( I \)). By including the constant inner electrolyte \( a_i^\infty \) into the “constant” term, \( E_M \) can be described by using the Nernst equation [68]:

\[
E_{\text{ISE}} = \text{constant} + \frac{RT}{z_i F} \ln a_i = E_i^0 + s_i \log a_i
\]  

(4-18)

\[
s_i = 2.303RT / z_i F = 59.16 \text{mV} / z_i (25^\circ C)
\]  

(4-19)

\( E_{\text{ISE}} \): potential difference between inner filling solution and sample solution;

\( E_i^0 \): constant potential difference including the boundary potential difference between the internal filling solution and the membrane.
The intercept $E_i^0$ of the linear response function is a temperature-dependent constant and the slope $s_i$ is identical to the Nernstian factor.

However, the validity of this linear relationship is limited. It depends on the particular target ion, the composition of the sample solution and the experimental conditions. Since the ionophores rarely show ideal selectivity for $I$, the electrodes respond not only to $I$ but also to $J$ (interfering ion). In order to describe the performance of ISEs in the presence of interfering ions, Nicolsky and Eisenman introduced a semi-empirical extension for the response of a potentiometric cell to a mixed solution containing both ions, $I$ and $J$ [69,70]:

$$E_i = E_i^0 + s_i \log \left[ a_i(IJ) \sum_{ij} K_{i,j}^{pot} (a_j(IJ))^{z_i/z_j} \right]$$

(4-20)

It is useful to subdivide the intercept $E_i^0$:

$$E_i^0 = E_i^0 + E_R + E_j$$

(4-21)

$E_i$: electromotive force of the cell assembly;

$E_i^0$: cf. Eq. 4-18;

$E_R$: constant potential difference consisting of $E_1, E_2, E_4$ and $E_5$ (see section 4.3.1). $E_R$ is independent of change in sample composition;

$E_j$: liquid junction potential difference generated between reference electrolyte and sample solution (see Figure 4.2);

$a_i(IJ)$: active molality of $I$ in the mixed solution;

$a_i(IJ)$: active molality of $J$ in the mixed solution;

$z_i, z_j$: charges of $I$ and $J$;

$K_{i,j}^{pot}$: potentiometric selectivity coefficient of primary ion $I^{z_i}$ against interfering ion $J^{z_j}$; An ideal selective electrode would show all $K_{i,j}^{pot} = 0$.

The Nicolsky-Eisenman equation reduces to the Nernstian equation in either of the two cases:
4. Theory of ion-selective electrodes

1. Electrode has a perfect selectivity; all $K_{i,j}^{\text{pot}} = 0$;

2. Sample solution contains no species other than $I$, which makes all $a_j$ equal to zero.

According to Eq. 4-18 and Eq. 4-20, 1 mV change of response potential ($\Delta E_i = 1$ mV) results in $z_i \times 0.4\%$ change in the activity of the primary ion $I^{z_i}$ ($\Delta a_i \approx z_i \times 0.4\% \times a_i$). When measuring divalent cations, e.g. $\text{Mg}^{2+}$, 1 mV emf change corresponds to about 8% activity variation of the $\text{Mg}^{2+}$ concentration in the sample solution.

The Nicolsky-Eisenman equation adequately describes the electrode response to the mixed solution containing the primary and interfering ions of the same charge. The selectivity coefficient $K_{i,j}^{\text{pot}}$ is the weighing factor to characterize the selectivity of electrodes for $I$ against $J$. However, if $I$ and $J$ exhibit different charges, the theoretical electrode function obtained with the above formalism is incorrect [47,71,72]. Therefore, $K_{i,j}^{\text{pot}}$, calculated from the Nicolsky-Eisenman equation can be misleading when describing discrimination for primary ions against interfering ions. Nonetheless, the reporting of selectivity coefficient on the basis of the traditional Nicolsky-Eisenman equation is still recommended, given that Nernstian response slopes are observed for ions that are highly discriminated against [73]. In recent years, there has been a lot of discussion about how the electrode response function should be described and the potentiometric selectivity should be reported [47,72,74]. These problems will be discussed in Section 4.5.
4.4 Selectivity of neutral ligand based electrode membranes

Selectivity denotes the capability of an electrode to recognize the primary ion in presence of interfering species, which is specified as the selectivity coefficient $K_{ij}^{\text{pot}}$ (see Eq. 4-20).

For a neutral ligand based cation-selective membrane (anion-selective membranes are treated similarly), the complexation of the primary ion $I^{z_i}$ and the neutral ligand $L$ at the sample-membrane interface ($IL_{n}^{z_i}$, $n$: stoichiometry of the complex) aids the distribution of the primary ion (complexed form) in the membrane phase. When the interfering ion $J^{z_j}$ is present in the sample solution, there is competition between both primary and interfering ions ($JL_{m}^{z_j}$, $m$: stoichiometry of the complex) for the ligand. This can be expressed by the following equation:

$$K_U
$$
$$IL_{n}^{z_i} \text{(memb)} + \frac{Z_i}{Z_j}J^{z_j} \text{(aq)} \rightleftharpoons I^{z_i} \text{(aq)} + \frac{Z_i}{Z_j}JL_{m}^{z_j} + (n - m)L \text{(memb)} \quad (4-22)$$

where

$K_U$: ion-exchange constant

(aq): aqueous phase;

(memb): membrane phase.

The above equilibrium can be split into the following two equilibria:

$$nL \text{(memb)} + I^{z_i} \text{(aq)} \rightleftharpoons IL_{n}^{z_i} \text{(memb)}$$

$$mL \text{(memb)} + J^{z_j} \text{(aq)} \rightleftharpoons JL_{m}^{z_j} \text{(memb)}$$

$$k_i = \frac{[I^{z_i}] \text{(memb)}}{[I^{z_i}] \text{(aq)}} \quad (4-23)$$
4. Theory of ion-selective electrodes

\[ k_j = \frac{[I^{z_j}_{\text{memb}}]}{[J^{z_j}_{\text{aq}}]} \]  \hspace{2cm} (4-24)

- \( k_i, k_j \): partition coefficients of I and J in aqueous phase and membrane phase;
- \([I^{z_i}_{\text{aq}}] \): total concentration of I in aqueous phase;
- \([J^{z_j}_{\text{aq}}] \): total concentration of J in aqueous phase;
- \([I^{z_i}_{\text{memb}}] \): total concentration of I in membrane phase;
- \([J^{z_j}_{\text{memb}}] \): total concentration of J in membrane phase.

The ion-exchange constant \( K_U \) of the above equilibrium Eq. 4-22 can be expressed as:

\[ K_U = \frac{(k_j)^{z_j/z_j}}{k_i} \left( \frac{[I^{z_i}_{\text{aq}}]}{[J^{z_j}_{\text{aq}}]} \right)^{z_i/z_j} \]

\[ = \left( \frac{\alpha_i \gamma_i}{\gamma_j \alpha_j} \right) \left( \frac{[J^{z_j}_{\text{memb}}]}{[I^{z_i}_{\text{memb}}]} \right)^{z_i/z_j} \]

\[ = \left( \frac{\gamma_i^{z_i/z_j}}{\gamma_j^{z_j/z_j}} \right) \left( \frac{[I^{z_i}_{\text{memb}}]}{[J^{z_j}_{\text{memb}}]} \right)^{z_i/z_j} \]  \hspace{2cm} (4-25)

When the Nicolsky-Eisenman equation is applied, the selectivity coefficient of the ISE, \( K_{i,j}^{\text{pot}} \), is described by Eq. 4-26 when the electrode shows the same potential value in a pure solution of cation I and in a pure solution of J.

\[ K_{i,j}^{\text{pot}} = \frac{a_i}{a_j^{z_i/z_j}} \]  \hspace{2cm} (4-26)

From Eq. 4-25 and Eq. 4-26, the selectivity coefficient is obtained as:

\[ K_{i,j}^{\text{pot}} = K_U \left( \frac{\gamma_i^{z_i/z_j}}{\gamma_j^{z_j/z_j}} \right) \left( \frac{[I^{z_i}_{\text{memb}}]}{[J^{z_j}_{\text{memb}}]} \right)^{z_i/z_j} \]  \hspace{2cm} (4-27)

The selectivity of a neutral ligand based electrode membrane depends on:
- the concentration of the lipophilic anionic sites in the membrane;
- the extraction property of the plasticizer;
- the intrinsic selectivity behaviour of the ligand, i.e. stability constant of the complex.
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4.4.1 Effect of lipophilic ionic sites in the membrane

The addition of a lipophilic ionic salt (e.g. tetraphenylborate salt for cationic ISEs) to the neutral ligand based ion-selective membrane can establish permselectivity of the membranes [75,76]. The interference by lipid-soluble anions in the sample can also be reduced [77]. The concentration of anionic membrane components affects the ion selectivity, primarily, by controlling the concentration of exchangeable cations and, in addition, by influencing the concentration of free ionophores available for the complexation of cations.

The existence of lipophilic anionic sites in the membrane phase, in most cases, improves the extraction of cation and cation-ligand complexes into the membrane phase. The activation barrier for the interfacial exchange of cation and the cation-ligand complex is lowered as well and hence the response time is shortened. The amount of anionic sites affects the selectivity of the electrode primarily by controlling the concentration of both the exchangeable cations and the free ionophore available for complexation at the membrane/solution interface [78].

![Figure 4.8. Model of the neutral ligand (L)-based membrane containing anionic sites (R'). A is the counterion in the sample solution; Il, is the complex of I and L with the stoichiometry of n; JLm is the complex of J and L with the stoichiometry of m.](image)

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The ratio of the anionic site/ligand in the membrane composition is optimized with respect to the best selectivity of the primary ion over the interfering ions \([78]\). The stoichiometry of the ion-ligand complex can be calculated from the theoretical treatment of the optimum ratio of anionic site/ligand. Within the bulk membrane, the stoichiometric relationship between the cation and the ligand can only be described on a statistically basis.

**The two limiting cases for the addition of counterions in the membrane**

The emf-response of a neutral-ligand based membrane electrode to samples containing \(I^i\) is described by the following approximation:

$$E = E_0 + \frac{RT}{z_i F} \ln \frac{k_i a_{i'}^i}{[I^i]^z}$$  \(4-28\)

The second term of Eq. 4-28 corresponds to the potential difference at the interface between the sample solution and the ion-selective membrane, and \(E_0\) includes all other potential contributions. The quantity \(a_{i'}^i\) is the activity of the primary ion in the sample solution, \([I^i]\) is the concentration of the same species (uncomplexed primary ion) at the membrane/solution interface and \(\gamma_i\) is the activity coefficient of \(I^i\) in the lipophilic membrane solvent (plasticizer). For a given concentration of ligand and plasticizer, \(\gamma_i\) is constant in the membrane phase. \(k_i\) is the partition coefficient of \(I^i\) between the aqueous phase and membrane phase.

At the membrane/solution interface, the neutral ligand \(L\) forms complexes with \(I^i\) and \(J^{z_j}\) of the forms \(II^{z_i}_n\) and \(JL^{z_j}_m\). The stability constants \(\beta_{n,n}\) and \(\beta_{m,n}\), \(n, m:\) stoichiometries of \(II^{z_i}_n\) and \(JL^{z_j}_m\) are defined as follows.

$$\beta_{n,n} = \frac{\gamma_{n,n} [II^{z_i}_n]}{\gamma_i[I^{z_i}] \gamma_L[L]^n}$$  \(4-29\)

$$\beta_{m,n} = \frac{\gamma_{n,n} [JL^{z_j}_m]}{\gamma_j[J^{z_j}] \gamma_L[L]^m}$$  \(4-30\)
where species in brackets denote concentrations. The activity coefficient of the neutral ligand \( L (\gamma_L) \) is assumed to be constant in all cases. For a given concentration of the ligand and complex in the membrane, the ratio of the two ionic activity coefficients \( \left( \frac{\gamma_{II_n}}{\gamma_L} \right) \) is constant. The concentration of \( I^{+} \) at the membrane-solution interface is determined by two factors:

- the concentration of anionic sites in the membrane which limits the amount of cationic species;
- the concentration of the uncomplexed carrier which depends on the cation-ligand complexation equilibria at the membrane/solution interface, and which controls the concentration of free cations.

In the **limiting case a**, there is an excess of carrier at the membrane-sample boundary (concentration \([L]\), in Eq. 4-32) which results in a nearly complete complexation of the primary ions (Eq. 4-31). Hence, the amount of free cations is small and according to Eq. 4-28, the corresponding emf (Eq. 4-33) is relatively large:

\[
\left[ L_i^{n} \right] = \beta_{II_n} [L]^{n} \frac{\gamma_{II_n}}{\gamma_L} \approx \left[ R^{-} \right] / z_i
\]  

(4-31)

\[
[L] = [L]_{tot} - n [II_n] \approx [L]_{tot} - \left[ R^{-} \right] / z_i
\]  

(4-32)

where \([L]_{tot}\) is the total carrier concentration in the membrane phase.

\[
E_i = E_0 + \frac{RT}{z_i F} \ln \left( \frac{[L]_{tot} - [R^{-}]^{1/n} (n/\omega_i)^{n}}{[R^{-}] / z_i} \right) \frac{\gamma_{II_n}}{\gamma_L}
\]  

(4-33)

In the **limiting case b**, the carrier concentration is too low for an adequate complexation of cations (Eq. 4-34). Accordingly, the concentration of the free cations is larger:
4. Theory of ion-selective electrodes

\[ [I] \approx [R^-] \left( \frac{1}{z_i} \right) - [L]_{\text{tot}} \quad (4-34) \]

\[ E_i = E_0 \left( \frac{RT}{z_i F} \ln \left( \frac{k_i a_i'}{\frac{[R^-]}{z_i}} - \frac{[L]_{\text{tot}}}{n} \right) \right) \frac{1}{\gamma_i} \quad (4-35) \]

According to the separate solution method \(^7^9\), the **limiting case a** fulfills the conditions for measuring the pure primary ion whereas **limiting case b** should be applied to the measurement of any interfering ion. Referring to the emf-response, this guarantees a sufficient discrimination between I and J.

To obtain the optimum selectivity, the maximum emf-difference has to be determined with respect to \([R^-]/[L]_{\text{tot}}\):

\[ E_i - E_j \approx \frac{RT}{z_i F} \ln \left( \frac{k_i \beta_{a,i} [L]_{\text{tot}} - \left( \frac{n}{z_i} \right) [R^-]^{z_i}}{z_i} \right) \frac{a_i'}{\gamma_i \gamma_{u,a}} \]

\[ - \frac{RT}{z_j F} \ln \left( \frac{k_j a_j'}{\frac{[R^-]}{z_j}} - \frac{[L]_{\text{tot}}}{m} \right) \frac{1}{\gamma_j} \quad (4-36) \]

For this mathematical procedure, the following substitution is introduced in Eq. 4-36:

\[ \frac{[R^-]}{[I]_{\text{tot}}} = x \quad ([R^-] \text{ is assumed to be constant}) \quad (4-37) \]

From the condition of maximum selectivity, i.e. \( \delta(F_i - F_j) / \delta x = 0 \), the following general solution is obtained for the optimal value of \( x \):

\[ x^2 \left( \frac{n^2 m}{z_i^2 z_j} + \frac{nm}{z_i z_j^2} - \frac{nm}{z_i z_j^2} \right) + x \left( \frac{n^2}{z_i^2} - \frac{n}{z_i} - \frac{m}{z_j} + \frac{m}{z_j^2} \right) + \frac{1}{z_i} = 0 \quad (4-38) \]
Table 4.1. Optimum molar ratio of borate salt to ligand, in ion-selective membranes based on neutral carriers. Calculations are based on Eq. 4-38.

| $|z_i|$ | $|z_j|$ | N  | m  | $\frac{[R^-]}{[L]_{tot}}$ |
|------|------|----|----|-------------------------|
| 2    | 2    | 1  | 1  | 2.00                    |
| 2    | 2    | 1  | 2  | 1.41                    |
| 2    | 2    | 1.5| 1.5| 1.33                    |
| 2    | 2    | 2  | 2  | 1.00                    |
| 2    | 2    | 2  | 3  | 0.77                    |
| 2    | 2    | 3  | 3  | 0.67                    |
| 2    | 2    | 3  | 4  | 0.54                    |
| 2    | 1    | 3  | 3  | 0.45                    |
| 2    | 1    | 1  | 1  | 1.61                    |
| 1    | 1    | 2  | 2  | 0.73                    |
| 1    | 1    | 1  | 2  | 0.71                    |

The optimum ratio of $\frac{[R^-]}{[L]_{tot}}$ for obtaining the best selectivity for the primary ion over the interfering ion is shown in Table 4.1. According to the model described above for a membrane composition with ideal selectivity, the following relation must hold (see examples in Table 4.1):

$$\frac{[L]_{tot}}{m} < \frac{[R^-]}{[L]_{tot}} < \frac{[L]_{tot}}{n}$$  \hspace{1cm} (4-39)

On the basis of this model, the best selectivity can only be obtained by adjusting the ratio of $\frac{[R^-]}{[L]_{tot}}$ and with the additional conditions that $I^+$ has the higher charge number and/or it is complexed by more ligand than the interfering ion i.e. has a higher stoichiometry. For the Mg$^{2+}$-selective membrane based on ETH$^T$ 5504 and ETH 5506, the optimum ratio of $\frac{[R^-]}{[L]_{tot}}$ for obtaining the best selectivity of Mg$^{2+}$ over Ca$^{2+}$ is 1.5 (which is close to 1.4 in Table 4.1). The stoichiometries of the complexed Mg and Ca-ligand are supposed to be 1:1 and 1:2, respectively [78]. It is worth pointing out that the stoichiometry value calculated from the experimental optimum ratio of
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\[ [R^-]/[L]_{tot} \] is the “mean apparent stoichiometry”. This represents the statistical relationship between cation and ligand in the membrane phase.

4.4.2. The extraction property of the plasticizer

To obtain polymeric electrode membranes with sufficient selectivity to the target ion, the plasticizer is introduced into membrane as the organic solvent for the membrane components. The plasticizer directly affects the extraction properties of the membrane and thus the membrane selectivity. Two main effects are operative: (1) the change in free energy of the solvation of the cation from one solvent to another; (2) the change in interaction of the cation with the medium outside the first solvation shell and the ligand binding shell. Free energies of transfer may be large, especially between the solvents of very different polarities (e.g. water and chloroform). The second contribution is taken into account by using the Born equation (Eq. 4-40), which gives the electrostatic part of the free energy change when transferring an ion from a vacuum to a medium of dielectric constant \( \varepsilon \).

\[
\Delta G_{\text{B}} = -\frac{z^2e^2}{2a} \left[ 1 - \frac{1}{\varepsilon} \right]
\]  \hspace{1cm} (4-40)

\( \Delta G_{\text{B}} \): electrostatic part of the free energy change;
\( \varepsilon \): dielectric constant of the plasticizer;
\( e \): elementary charge (1.6022\times10^{-19} \text{ C});
\( a \): radius of the complexed cation;

\[
a = r_c + s
\]  \hspace{1cm} (4-41)

(ionic radius of the cation \( r_c \) + ligand thickness \( s \))

Based on a model of the electrostatic interaction between a positively charged complex and the membrane solvent, Simon\(^{[81]}\) postulated the impact of the dielectric constant of the membrane plasticizer \( \varepsilon \) on the selectivity of the membrane electrode. This is generally described as:

\[
\frac{\partial \log K_{ij}^{\text{net}}}{\partial (1/\varepsilon)} = \frac{N \cdot e^2}{4\pi \varepsilon_0 \cdot RT \cdot 2r \cdot \ln 10} \cdot z_i \cdot (z_i - z_j)
\]  \hspace{1cm} (4-42)
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where

\( \varepsilon_0 \): electrical field constant \((8.854 \times 10^{-12} \text{A}^2 \text{s}^4 \text{m}^{-3} \text{kg}^{-1})\);

\( 2r_C \): overall diameter of the cationic forms;

\( N \): Avogadro constant \((6.022 \times 10^{23} \text{mol}^{-1})\).

It can be seen from Eq. 4-42 that plasticizers of higher dielectric constant (more polar) will enhance the preference of divalent over monovalent cations of the same radius. For example, the selectivity coefficient of the \( \text{Mg}^{2+} \)-selective electrode against \( \text{Na}^+ \), \( \log K_{\text{Mg,Na}}^{\text{pot}} \), can be effected by using different dielectric plasticizers. The experimental results in Figure 4.9 show the dependence of the selectivity of \( \text{Mg}^{2+} \) against \( \text{Na}^+ \) versus the polarity of plasticizers.

\[
\Delta \log K_{\text{Mg,Na}}^{\text{pot}} = \left( \frac{1}{\varepsilon} \right) \frac{N e^2}{RT \ln 10} \left( \frac{2z_{\text{Na}}^2}{2r_{\text{NaL}}} + \frac{z_{\text{Mg}}^2}{2r_{\text{MgL}}} \right)
\]

(4-43)

where

\( z_{\text{Na}} \): charge of \( \text{Na}^+ \);

\( z_{\text{Mg}} \): charge of \( \text{Mg}^{2+} \);

\( r_{\text{NaL}} \): radius of the \( \text{Na}^+\)-ligand complex;

\( r_{\text{MgL}} \): radius of the \( \text{Mg}^{2+}\)-ligand complex.
Figure 4.9. Dependence of the selectivity of Mg\(^{2+}\) versus the polarity (dielectric constant \(\varepsilon\)) of plasticizers. Each mean selectivity coefficient was obtained from 6 measurements. The ligand is ETH 5506 and the dielectric constants \(\varepsilon\) of the tested plasticizers were measured in our laboratory (see section 9.5): \(\varepsilon = 3.9\) (DOS); 7.9 (CP chloroparafin); 13.4 (ETH 8045); 23.9 (o-NPOE).

In the case of the primary and the interfering ions having the same charge, e.g. Mg\(^{2+}\) and Ca\(^{2+}\), the dependence on the dielectric constant of the membrane was derived on the basis of the Born equation [80]:

\[
\Delta \log K_{\text{Mg,Ca}}^{\text{pot}} = \left( \frac{1}{\varepsilon_r} - \frac{1}{\varepsilon_0} \right) \frac{2Ne^2}{4\pi\varepsilon_0 kT \ln 10} \left( \frac{1}{r_{\text{Ca}}} + \frac{1}{r_{\text{Mg}}} \right)
\]

Eq. 4-44 suggests an increase of the discrimination of large ion-ligand complexes when \(\varepsilon\) increases [82,83]. Since Ca\(^{2+}\) tends to form a more voluminous (spatial) ion-ligand complexes than Mg\(^{2+}\) due to its higher complex stoichiometry and its large ionic radius, plasticizers with high dielectric constants should improve the preference of the membrane for Mg\(^{2+}\) over Ca\(^{2+}\). For example, an increase of \(\varepsilon\) from 4 to 18
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theoretically induces a change of the selectivity coefficient $\Delta \log K_{\text{Mg,Ca}}^{\text{tot}} = -1.32$ for $r_{\text{Ca}} = 1.9$ nm and $r_{\text{Mg}} = 1.5$ nm (total volume assumption) [81].

![Structure of o-NPOE](image)

$o$-NPOE (2-nitrophenyl ether)

![Structure of ETH 5373](image)

ETH 5373 (3,7,11,15-tetramethylhexadecyl-2-nitrophenyl-ether)

![Structure of ETH 4314](image)

ETH 4314 (2-nitrophenyl-2-phenyldodecyl ether)

![Structure of ETH 8045](image)

ETH 8045 ([12-(4-ethylphenyl)dodecyl]2-nitrophenyl ether)

![Structure of DOS](image)

DOS (Bis(2-ethylhexyl) sebacate)

Figure 4.10. Structures of plasticizers frequently used for Mg$^{2+}$-selective electrodes
In order to guarantee a good membrane performance, an adequate plasticizer must show no crystallization in the membrane phase and no exudation. Ion-selective membranes show pronounced variations in selectivity when using plasticizers of varying polarity, constitution and lipophilicity as solvents (see Figure 5.9) \cite{84-86}. Different plasticizers will affect the lipophilicity of the PVC membrane, which in turn alters the partition coefficient of the different species. The plasticizers with higher hydrophilicity, e.g. o-NPOE, have higher partition coefficients. When comparing the different plasticizers (ETH 8045, log$P_{\text{TLC}}$=12.8; and o-NPOE, log$P_{\text{TLC}}$=5.8 \cite{81}), cooperative extraction effects were observed for the membrane containing the matching plasticizer and ligand regarding their lipophilicities. For example, ETH 5506 (log$P_{\text{TLC}}$=12.8) favors the more lipophilic plasticizer like ETH 8045 while the less lipophilic ligand (K22B5, log$P_{\text{TLC}}$=3.94) shows a better performance with less lipophilic plasticizer like o-NPOE (see Figure 5.9). This is because the selectivity performance of ligands with specific lipophilicity is enhanced by the membrane solvent lipophilicity.

It can be concluded that plasticizers need to fulfill four principal criteria, namely, high lipophilicity, solubility in the polymeric membrane (no crystallization), no exudation and the ability to enhance selectivity behaviour of the membrane.

**4.4.3 Intrinsic selective behaviour of the ionophores**

To improve the selectivity, the ligand should ideally form complexes which must be as stable as possible for the primary ion ($\beta_{\text{m}}$) and as weak as possible for the interfering ion ($\beta_{\text{n}}$) (see Eq. 4-35). Subsequently, extraction of analyte into the membrane phase is increased. If the sample is aqueous, the extracting phase will generally be a less polar organic phase.

The ligand consists of polar complexing groups and an apolar shell. The apolar shell contributes to the mobility of the complex in an apolar membrane environment and increases the stability of membrane components (ensuring a long lifetime of the electrode). In most cases, a hydrophilic charged ion is enclosed in the interior of the complex, whereas the hydrophobic surface is exposed to the apolar environment. The coordination sites of a ligand must be able to compete with the solvent molecules in
the coordination sphere of the primary ion. For polar coordinating groups, an increase in the dipole moment and polarizability of the binding sites favours the extraction of ions, especially with a higher charge number [1,87].

4.5 Determination of the selectivity coefficients

Based on the Nicolsky-Eisenman equation (Eq. 4-21), $K_{i,j}^{\text{pot}}$ is the potentiometric selectivity coefficient of the primary ion $I$ in the presence of the interfering ion $J$. When $K_{i,j}^{\text{pot}}$ is larger than 1, the ISE responds to $J$ more selectively than to $I$. In most cases, $K_{i,j}^{\text{pot}}$ is smaller than 1, which means ISEs respond to $I$ more selectively than to $J$. This Nicolsky-Eisenman equation assumes a Nernstian response not only for $I$ but also for $J$. The selectivity coefficient is usually used in characterizing and comparing different types of electrodes. The International Union of Pure and Applied Chemistry (IUPAC) has issued several recommendations for reporting the selectivity coefficient which have been internationally accepted [47,79,88]. These are the separate solution method (SSM), fixed interference method (FIM) and matched potential method (MPM).

4.5.1 Separate solution method (SSM) [79]

The potential of an electrochemical cell comprising an ion-selective electrode and a reference electrode is measured in two separate solutions, one containing $I$ with the activity $a_i$ (but no $J$), the other containing $J$ with the activity $a_j=a_i$ (but no $I$). If the measured values are $E_i$ and $E_j$, respectively, the value of $K_{i,j}^{\text{pot}}$ may be calculated from the equation:

$$\log K_{i,j}^{\text{pot}} = \frac{E_j - E_i}{2.303RT/z_iF} + \log a_i - \frac{z_i}{z_j} \log a_j$$

$$= \frac{E_j - E_i}{2.303RT/z_iF} + (1 - \frac{z_i}{z_j}) \log a_i \quad (4-45)$$

The SSM is recommended only if the electrode exhibits a Nernstian response to $I$ and $J$. This method is ideal for routine characterizations of ISEs because of its experimental simplicity. But the inaccuracies of this method arise when the electrode
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does not respond with a Nernstian slope to the interfering ions and/or I and J have different charge numbers [89].

4.5.2 Fixed interference method (FIM) [79]

The potential of an electrochemical cell comprising an ion-selective electrode and a reference electrode is measured in solutions of a constant level of interference, $a_j$, and varying activity of the primary ion, $a_i$. The potential values obtained are plotted vs. the activity of the primary ion (Figure 4.11). The value of $a_i$ can be determined in the graph by intersecting the extrapolation of the linear portions of the curve (Figure 4.11, $a_i=a_i(0)$). The selectivity coefficient $K_{i,j}^{pot}$ is calculated from the equation:

$$K_{i,j}^{pot} = \frac{a_i}{(a_j)^{\frac{z_i}{z_j}}}$$  \hspace{1cm} (4-46)

where both $z_i$ and $z_j$ have the same signs, positive or negative (Figure 4.11).

![Figure 4.11. Determination of selectivity coefficient by FIM.](image)

Again, the discrepancies in this method arise due to the assumption that the electrode functions for both the primary ion and the interfering ions exhibit Nernstian slopes.
The selectivity obtained with FIM may vary with the concentrations of the interfering ions. However, in comparison to SSM, the FIM corresponds to a more practical condition of the measurements. In this work, the SSM was used exclusively to provide a suitable selectivity comparison for the different electrodes presented here.

It has been reported by numerous researchers that some discrepancies were found among selectivity coefficients determined by different methods with otherwise identical conditions [71, 90-93]. This suggests that the selectivity coefficient cannot be treated as a physical constant but a value which can change according to experimental conditions. This is based on the fact that the potentiometric behaviour of the interfering ions often deviates from a Nernstian response and exhibit sub-Nernstian response (sometimes even more complicated response patterns are obtained). However, the Nicolsky-Eisenman equation assumes a Nernstian response for the interfering ions. Another discrepancy in selectivity coefficients determined by the Nicolsky-Eisenman equation-based methods (SSM, FIM) arises from the power term \( a_j^{z_j/z_i} \) of the Nicolsky-Eisenman equation. If the values of \( z_i \) and \( z_j \) differ from each other, the selectivity coefficients obtained cannot be applied to the real situation. The best way to report the selectivity coefficients is discussed in section 4.5.5.

4.5.3 Matched potential method (MPM)

In order to determine a selectivity coefficient without the problems arising from the methods based on the Nicolsky-Eisenman equation (SSM and FIM), the matched potential method (MPM) was recently recommended by IUPAC [47, 94, 95]. According to these recommendations, a solution with a specific activity of primary ion \( I \) is used as the reference solution in MPM (see Figure 4.12). The activity \( a_i \) is assumed to be zero (point A, with \( a_i^A = 0 \)). While the primary ion concentration is increased step by step, the potential change is measured and plotted against \( a_i \) (curve \( \alpha_i \)). Another curve \( \alpha_{i+j} \) is obtained from the potential change by stepwise addition of the interfering ion \( J \) to the same reference solution as in curve \( \alpha_i \). When the change in emf (\( \Delta \text{emf} \)) on curve \( \alpha_{i+j} \) matches that on curve \( \alpha_i \), the ratio between the activities or concentrations of \( I \) (point C) relative to \( J \) (point B) denoted the selectivity coefficient.
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$K_{i,j}^\text{pot}$. Based on IUPAC recommendations, the selectivity coefficient is obtained based on the following equation.

$$K_{i,j}^\text{pot} = \frac{\Delta a_i}{a_j^B} \quad \text{with} \quad \Delta a_i = a_i^c - a_i^A$$  \hspace{1cm} (4.47)

Figure 4.12. Principle of the determination of selectivity coefficients by the MPM: If $\Delta a_i$ and $\Delta a_j$ lead to the same $\Delta\text{emf}$ then $K_{i,j}^\text{pot}$ is defined as $\Delta a_i / \Delta a_j$; $a_i$, $a_j$ correspond to the dimensionless molal activity; $\tilde{m}$, denotes the active molality in 1 mol kg$^{-1}$ H$_2$O.

The MPM is supposed to be more relevant to the practical applications in case where a primary ion and an interfering ion of unequal charge number are involved, and where the Nernstian condition is not satisfied for either the interfering or primary ion. However, new problems emerge when applying the matched potential method, and they will be discussed in section 4.5.5.

4.5.4 Alternative methods

As well as the IUPAC recommended methods for determining the selectivity coefficients, many alternative methods have been proposed corresponding to the different applications of the electrodes, namely the varying interference method (VIM) \cite{1}, the continuous variation method, the specific application method (SAM)
and the least squares regression method. In SAM\cite{96}, the deviation from the ideal Nernstian response is corrected by a factor, equivalent to the selectivity coefficient which can involve a single specific ion as well as the total of all interfering species. The Nernstian response function is approximated by an iterative process varying the selectivity coefficient, and applying the least square method to minimize the deviation of the fitted values from the experimental data.

Nonetheless, discrepancies between a generally valid mathematical description of the selectivity coefficient, and a generally valid practical procedure remain. Most of the algorithms are still based on the Nicolsky-Eisenman equation since this equation simply describes a linear response function on a logarithmic scale, accounting for interference by a weighting factor denoted as a selectivity coefficient. In an attempt to describe more meaningful selectivity coefficients, several practical and theoretical models for describing the response potential have been proposed. Cattrall et al\cite{90} modified the Nicolsky-Eisenman equation regardless of the charge number of ions. Bagg et al\cite{97,98} reported a modified Nicolsky-Eisenman equation based on theoretical considerations. Maccà and Cakrt\cite{99} determined the selectivity coefficient by means of data linearization techniques. Recently Bakker et al\cite{71} proposed a self-consistent model describing the response behaviour of the electrode membranes in mixed solutions of unequally charged ions.

4.5.5 Discussion on reporting the selectivity coefficient

Both IUPAC-recommended methods, SSM and FIM, are based on the Nicolsky-Eisenman equation. The FIM was recommended for the selectivity pattern of an ion-selective electrode to be evaluated in mixed solutions; the SSM was the procedure of use to yield information on selectivity coefficients for different electrodes and membranes under identical conditions, independent of the final application. The SSM-selectivity pattern proved to be convenient in order to describe new designs of electrodes, and of ion-selective carriers despite the drawbacks described by several authors\cite{47}. Furthermore, the selectivity coefficient and the Nicolsky-Eisenman equation were used to estimate the required selectivity coefficients necessary for specific applications of the electrodes. However, results can be misleading\cite{100-103}. 
From our own experience, the following inconsistencies of the theoretical response function of magnesium-selective electrodes were found:

1. The definition of the selectivity coefficient is based on the assumption of thermodynamic equilibrium between the bulk of the ion-selective sensing membrane and the modal activity of the analyte in the sample solution. Relying on short response times of < 2 min, this assumption is in most cases only fulfilled for a small boundary layer between sample solution and the bulk of the ion-selective membrane, not so for the membrane bulk as such [104]. At the liquid junction between reference electrode and sample, a variable steady-state potential is established [105].

2. If the molal activity of an ion varies on either of the two membrane boundaries by several orders of magnitude, the bulk membrane is involved in an activity gradient that can be the source of a diffusion potential. In this case, the membrane electrode shows an emf-drift as long as the new equilibrium is not established. Moreover, the potentiometric electrode membrane is generally not exposed to symmetric condition in view of the molal activity of the primary and interfering ions in contact with the membrane boundaries [104,106,107]. The description and definition of the selectivity coefficient can only be interchangeable for the analyte and the interfering ions [73], if the thermodynamic equilibrium or steady state comprises all boundaries as well as the membrane bulk. In addition, local variations e.g. of the liquid-junction potential must be accounted for, especially for large deviations from equilibrium conditions e.g. by changes in the molal activity by orders of magnitude.

3. Due to the assumptions described under 1, selectivity coefficients as described by SSM vary under several conditions, e.g. as a function of the series of consecutive ions tested and as a function of the emf differences between consecutive solutions. The SSM procedure provides an estimate of the selectivity of an electrode provided that that steady-states are established throughout the potentiometric cell. In practice, however, a gradient between the two membrane boundaries is mostly observed. Therefore, the theoretical description as presented in [71] only holds for the membrane boundary condition, assuming a Nernstian response for all ions, and does not correspond to other boundary conditions of the potentiometric cell.
4. The selectivity coefficients do not only depend on the charge numbers of the primary and interfering ions, but also on the ligand concentration and stoichiometry of these ions in the ion-ligand complexes. The competition between ions of a mixed solution resulted in a sigmoid response function rather than a Nernstian function in the case of the magnesium-selective electrode [104]. For this example, the description of the selectivity coefficient was referred to the concentration of anionic sites within the membrane and to the ligand concentration [1,104]. The selectivity coefficient between magnesium and calcium ions proved to be different from the SSM estimates. This means that any realistic estimate of a specific selectivity coefficient must be derived from a realistic set-up. Discrepancies between SSM experiments and realistic estimations of selectivity behaviour are described in [96].
4.6 Newly proposed method for reporting selectivity coefficient: Consistent Separate Solution Method, SSM$_{\text{cons.}}$

With a mathematically modification of the Nicolsky-Eisenman equation coupled with a change in the measuring procedure, the SSM-approach was found to give selectivity coefficients that were consistent under various conditions $[72]$. The method circumvents the problems caused by the ion charge number in the unmodified Nicolsky-Eisenman equation.

According to IUPAC, the emf values ($E_i$ and $E_j$) of an electrode in pure solution of each $I$ and $J$ are used for calculations. The corresponding electrode potentials are:

\begin{align*}
E_i &= E^o + \frac{RT}{z_i F} \ln(a_i) \\
E_j &= E^o + \frac{RT}{z_i F} \ln(K_{ij}^{\text{pot}} a_j^{z_j}) = E^o + \frac{RT}{z_i F} \ln(K_{ij}^{\text{pot}}) + \frac{RT}{z_i F} \ln(a_j^{z_j}) \\
\end{align*}

By subtracting Eq. 4-48 from Eq. 4-49, the definition of the SSM-selectivity coefficient as recommended by IUPAC is obtained:

\begin{align*}
\Delta E &= E_j - E_i = \frac{RT}{z_i F} \ln(K_{ij}^{\text{pot}}) + \ln(a_j^{z_j}) - \ln(a_i) \\
&= \frac{2.303 \cdot RT}{z_i F} \left[ \log K_{ij}^{\text{pot}} + \log(a_j^{z_j}) - \log(a_i) \right] \\
&= \log K_{ij}^{\text{pot}} = \frac{E_j - E_i}{2.303 \cdot RT \cdot z_i F / z_j} + \log(a_i) - \frac{z_i}{z_j} \log(a_j) \\
\end{align*}
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Assuming that the solutions of the primary ion and the interfering ion have the same molal activity \( a \), the following relationship holds.

\[
a = a_i = a_j \Rightarrow \log K_{i,j}^{\text{pot}} = \frac{E_j - E_i}{2.303RT/\mathcal{F}} + \left( \frac{z_i}{z_j} \right) \log(a)
\]  

(4-51)

Based on this formula Eq. 4-51, two cases can be distinguished:

- If \( z_i = z_j \), then \( \log K_{i,j}^{\text{pot}} = \frac{E_j - E_i}{2.303RT/\mathcal{F}} \), i.e., \( K_{i,j}^{\text{pot}} \) correctly describes the selectivity of the electrode for the primary ion over the interfering ion.

- If however, \( z_i \neq z_j \), it can be seen from Eq. 4-51 that \( \log K_{i,j}^{\text{pot}} \) depends on the charge number of the primary and the interfering ions. This charge-dependence of \( \log K_{i,j}^{\text{pot}} \) leads to the inconsistency: treating the primary ion as the interfering ion and vice versa gives different selectivity coefficients while only a change of sign would be expected. In our experiments with the Mg\(^{2+}\)-selective electrode we measured e.g. \( \log K_{\text{Mg,K}}^{\text{pot}} = -2.9 \), but \( \log K_{\text{K,Mg}}^{\text{pot}} = 1.4 \) (Table 4.2).

This problem can be overcome as follows. Subtracting Eq. 4-48 from Eq. 4-49, which results in

\[
E_j - E_i = \frac{RT}{z_j \mathcal{F}} \left[ \ln K_{i,j}^{\text{pot}} + \frac{z_i}{z_j} \ln(a_j) - \ln(a_i) \right], \text{ i.e.}
\]

\[
E_j - E_i = \frac{RT}{z_j \mathcal{F}} \ln(K_{i,j}^{\text{pot}}) + \frac{RT}{z_i \mathcal{F}} \ln(a_j) - \frac{RT}{z_i \mathcal{F}} \ln(a_i)
\]  

(4-52)

Eq. 4-52 shows the contribution of the primary as well as the interfering ion to the potential difference, \( \Delta \text{emf} \). The latter two terms depend on the charge of the respective ions. A charge dependence of the selectivity coefficient term is not feasible since \( K_{i,j}^{\text{pot}} \) represents the correction factor to compensate for the emf-differences. This charge dependence can be eliminated as follows:
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\[ E_j - E_i = \frac{RT}{F} \ln(K_{i,j}^{\text{cons}}) + \frac{RT}{\varepsilon_j F} \ln(a_j) - \frac{RT}{\varepsilon_i F} \ln(a_i) \]  \hspace{1cm} (4-53)

The Eq. 4-52 and Eq. 4-53 results in the correlation between \( K_{i,j}^{\text{pot}} \) and \( K_{i,j}^{\text{cons}} \):

\[ K_{i,j}^{\text{pot}} = \left( K_{i,j}^{\text{cons}} \right)^{\gamma_i} \]  \hspace{1cm} (4-54)

Based on Eq. 4-53 and the Nicolsky-Eisenman equation, the appropriate electrode response function is:

\[ E_i = E^\circ + \frac{RT}{F} \ln\left(a_i^{\gamma_i} + K_{i,j}^{\text{cons}} \cdot a_j^{\gamma_j} \right) \]  \hspace{1cm} (4-55)

Furthermore, if \( a_i = a_j = 1 \) and \( \log a_i = \log a_j = 0 \) from Eq. 4-53, the remaining terms in this formula, that are charge dependent, are eliminated.

\[ E_j^{a_i=1} - E_i^{a_i=1} = \frac{RT}{F} \ln\left(K_{i,j}^{\text{cons}} \right) \]

\[ \Rightarrow \ln\left(K_{i,j}^{\text{cons}} \right) = \frac{E_j^{a_i=1} - E_i^{a_i=1}}{RT/F} \]

\[ \Rightarrow \log\left(K_{i,j}^{\text{cons}} \right) = \frac{E_j^{a_i=1} - E_i^{a_i=1}}{2.303 \cdot RT/F} \]  \hspace{1cm} (4-56)

The selectivity coefficient so defined is independent of the charge number of the ions involved. The agreement of these calculations, with the experimental data is demonstrated in Table 4.2.

In the following, the validity of this algorithm is demonstrated by the results from magnesium-selective measurements based on the ionophore ETH 5506. In order to eliminate the charge terms, the emf values at \( a_i = 1 \) and \( a_j = 1 \) were measured. Owing to the solubility of some salts, it proved very difficult or even impossible to prepare solutions of an active molality equal 1 mol·kg\(^{-1}\).
4. Theory of ion-selective electrodes

Table 4.2. Comparison of selectivity coefficients obtained with two methods by using Mg$^{2+}$-selective electrode (ionophore: ETH 5506; plasticizer: o-NPOE).

<table>
<thead>
<tr>
<th>Primary ion</th>
<th>Mg$^{2+}$</th>
<th>Ca$^{2+}$</th>
<th>Mg$^{2+}$</th>
<th>K$^+$</th>
<th>Mg$^{2+}$</th>
<th>Na$^+$</th>
<th>Mg$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interfering ion</td>
<td>log $K_{i,j}^{\text{pot}}$</td>
<td>SSM (log $K_{i,j}^{\text{PotP}}$)</td>
<td>SSM (log $K_{i,j}^{\text{tot}}$)</td>
<td>SSM (log $K_{i,j}^{\text{cor}}$)</td>
<td>SSM (log $K_{i,j}^{\text{cor}}$)</td>
<td>SSM (log $K_{i,j}^{\text{corr}}$)</td>
<td>SSM (log $K_{i,j}^{\text{corr}}$)</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>-1.9</td>
<td>1.9</td>
<td>-2.9</td>
<td>1.4</td>
<td>-3.6</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>-1.0</td>
<td>1.0</td>
<td>-1.5</td>
<td>1.5</td>
<td>-1.8</td>
<td>1.8</td>
<td></td>
</tr>
</tbody>
</table>

4.7 The required selectivity coefficient for clinical and biological uses

When ion-selective electrodes are applied to the measurements in extracellular fluid, an adequate selectivity of the primary ion over the interfering ions has to be achieved. Based on the conventional Nicolsky-Eisenman equation, the required selectivity coefficients are calculated assuming an allowable error of 1% (0.01) in the worst case of a maximum quantity of interfering ion, $a_{j,\text{max}}$, and a minimum quantity of primary ion, $a_{i,\text{min}}$, within the physiological range by the following equation [108]:

$$K_{i,j}^{\text{pot \ required}} \leq 0.01 \cdot a_{i,\text{min}} / a_{j,\text{max}}$$ (4-57)

or

$$\log(K_{i,j}^{\text{pot \ required}}) \leq \log\left(0.01 \cdot a_{i,\text{min}} / a_{j,\text{max}}\right)$$ (4-58)

$z_i, z_j$: charges of the primary I and the interfering ion J, respectively.

When the electrode is calibrated using physiological background solutions, the variations of the interfering ion around its medium activity value in the sample has to be considered. The difference between the activity of the calibrator $a_{j,\text{cal}}$ and the unknown solution $a_j (a_{j,\text{cal}} - a_{j,\text{max}})$ contributes to the actual interference. Eq. 4-58 can then be changed to:

$$\log(K_{i,j}^{\text{pot \ required}}) \leq \log\left[0.01 \cdot a_{i,\text{min}} / (a_{j,\text{cal}} - a_{j,\text{max}})\right]$$ (4-59)
If the primary and the interfering ion have different charge numbers \((z_i \neq z_j)\), the required selectivity coefficients based on the conventional Nicolsky-Eisenman equation become inexact due to discrepancies described above.

From Eq. 4-55, which defines a charge-independent selectivity coefficient \( \log K_{\text{cons}}^{i,j} \), the calculation of the required selectivity coefficient, \( \log(K_{\text{cons}}^{i,j})_{\text{required}} \), can be carried out for a given allowed relative error, 1% (0.01), for the measurement in physiological solutions.

\[
\log(K_{\text{cons}}^{i,j})_{\text{required}} \leq \log \left( 0.01 \cdot \frac{a_{i,\text{min}}^{1/z_i}}{a_{j,\text{max}}^{1/z_j}} \right) \quad (4-60)
\]

With physiological background calibration, the charge independent required selectivity coefficient is:

\[
\log(K_{\text{cons}}^{i,j})_{\text{required}} \leq \log \left( 0.01 \cdot \frac{a_{i,\text{min}}^{1/z_i}}{a_{j,\text{cal}}^{1/z_j} - a_{j,\text{cal}}^{1/z_j}} \right) \quad (4-61)
\]

For detecting the main cations in samples such as serum or plasma, e.g. \( \text{Na}^+, \text{K}^+, \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \), the “consistent required selectivity coefficients ( \( \log(K_{\text{cons}}^{i,j})_{\text{required}} \) )” for each cation was calculated according to Eq. 4-60 and 4-61. A comparison of \( \log(K_{\text{cons}}^{i,j})_{\text{required}} \) and \( \log(K_{\text{pot}}^{i,j})_{\text{required}} \) is shown in Appendix 10.5.

A maximum error of 1.0% is tolerated in the determination of analyte ion activity. As \( \log(K_{\text{pot}}^{i,j})_{\text{required}} \) based on conventional Nicolsky-Eisenman equation can result in a misleading interpretation of the required selectivity due to its charge-dependence, the charge-independent \( \log(K_{\text{cons}}^{i,j})_{\text{required}} \) gives a more precise interpretation of the requirement of the electrode to discriminate the primary ion against the co-existing secondary ion. It can be seen from the calculated results in Appendix 10.5, that for the monovalent ion, e.g. \( \text{K}^+, \text{Na}^+ \), the required selectivity coefficients obtained from both methods are identical while for the divalent primary ions, e.g. \( \text{Mg}^{2+}, \text{Ca}^{2+} \), \( \log(K_{\text{cons}}^{i,j})_{\text{required}} \) is different from \( \log(K_{\text{pot}}^{i,j})_{\text{required}} \).
5. Investigation of Mg\textsuperscript{2+}-selective ionophores

5.1 Characteristics of alkali and alkaline-earth cations (AC and AEC)

The interaction between cations and the ligand binding sites is the main force for complexation, which can be divided into several energy contributions, mostly of an electrostatic nature. The position of an element in the periodic table determines its interaction with the ligand regarding its charge, radius, polarity as well as the polarizability. Alkali and alkaline-earth cations (AC and AEC) generally form strong ionic bonds with ligand and are sorted as hard or intermediate acids by Hard and Soft Acid and Base principle (HSAB)\textsuperscript{[109,110]} in which a classification of hard and soft ions is evaluated by their surface charge density (zÅ\textsuperscript{-2}, z=1 or 2) (see Table 5.1).

Table 5.1. Alkali and alkaline-earth cations

<table>
<thead>
<tr>
<th>Cation</th>
<th>Ionic radius r\textsubscript{1} (Å)</th>
<th>Hydration number</th>
<th>Surface charge density (z Å)</th>
<th>Polarizability (Å\textsuperscript{3})</th>
<th>(\Delta G\text{H}_{\text{Hydration}}) (kJmol\textsuperscript{-1}, 25°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li\textsuperscript{+}</td>
<td>0.76</td>
<td>6</td>
<td>0.13</td>
<td>0.03</td>
<td>509.96</td>
</tr>
<tr>
<td>Na\textsuperscript{+}</td>
<td>1.02</td>
<td>6</td>
<td>0.085</td>
<td>0.3</td>
<td>411.73</td>
</tr>
<tr>
<td>K\textsuperscript{+}</td>
<td>1.38</td>
<td>6</td>
<td>0.045</td>
<td>1.1</td>
<td>336.49</td>
</tr>
<tr>
<td>Rb\textsuperscript{+}</td>
<td>1.49</td>
<td>6</td>
<td>0.035</td>
<td>1.9</td>
<td>315.59</td>
</tr>
<tr>
<td>Cs\textsuperscript{+}</td>
<td>1.65</td>
<td>6</td>
<td>0.03</td>
<td>2.9</td>
<td>284.20</td>
</tr>
<tr>
<td>Be\textsuperscript{2+}</td>
<td>0.34</td>
<td>4</td>
<td>1.37</td>
<td>0.008</td>
<td>2432</td>
</tr>
<tr>
<td>Mg\textsuperscript{2+}</td>
<td>0.72</td>
<td>6</td>
<td>0.26</td>
<td>0.2</td>
<td>1897.72</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+}</td>
<td>1.12</td>
<td>8</td>
<td>0.14</td>
<td>0.9</td>
<td>1584.22</td>
</tr>
<tr>
<td>Sr\textsuperscript{2+}</td>
<td>1.27</td>
<td>8</td>
<td>0.10</td>
<td>1.28</td>
<td>1421.2</td>
</tr>
<tr>
<td>Ba\textsuperscript{2+}</td>
<td>1.42</td>
<td>8</td>
<td>0.08</td>
<td>2.5</td>
<td>1312.52</td>
</tr>
</tbody>
</table>
It is obvious that due to the high surface charge density of the magnesium ion of 0.26 \( z = 2 \) for an octahedral coordination sphere, magnesium hydrate is more stable than calcium hydrate as well as hydrates of other cations (AC and AEC). The free energy of hydration, -1.897 kJ mol\(^{-1}\), of Mg\(^{2+}\) has to be compensated for by the sum of the free energy of interaction with the ligand and the free energy of transfer to the membrane solvent (plasticizer).

### 5.2 Development of Mg\(^{2+}\)-selective ionophores

Because of its high surface charge density and free energy of hydration, Mg\(^{2+}\) is preferentially complexed by ligands with atoms that act as electron pair donors (EPD), e.g. oxygen and nitrogen atoms. One of the primary goals of the ionophore development was to obtain good selectivity against the appropriate background ions. In the recent two decades, Mg\(^{2+}\)-selective ionophores have been intensively developed. From the basic considerations discussed previously [1, 114], electrically neutral lipophilic amide derivatives were considered to be attractive candidates for designing Mg\(^{2+}\)-selective ionophores. A set of amides were synthesised and investigated, whereof the di- and tripodal malondiamides showed the most attractive features [115-118]. The discrimination against calcium, potassium and sodium ions by these ionophores was of primary interest. Bis(malondiamides) were the first compounds which showed a pronounced preference for magnesium ions [35, 118, 119]. The membrane electrodes containing these ligands achieved selectivity in the range of \( \log K_{\text{Mg,Ca}}^{\text{red}} < -0.1 \) (SSM) in the presence of 120 mol\% of lipophilic borate salt in the solvent polymeric membranes.

Tris(malondiamides) were expected to improve the discrimination of alkali over alkaline earth ions since Mg\(^{2+}\) is typically involved in octahedral coordination and assumed to form 1:1 complexes with tripodal ligands. Using ionophores of this class, e.g. ETH 7025, electrodes discriminated against Ca\(^{2+}\) by more than a factor of ten (\( \log K_{\text{Mg,Ca}}^{\text{red}} < -1 \), SSM). Attaching three secondary malondiamide units to a benzene core e.g. in ETH 3832 [120] (see Figure 5.1), significantly improved the selectivity for magnesium ion over calcium ions [117, 121]. The more lipophilic adamantylamide derivatives, e.g. ETH 5506, showed improved selectivity of Mg\(^{2+}\) against Ca\(^{2+}\), K\(^+\).
and Na⁺ (log $K_{\text{Mg,Na}}^{\text{pot}} = -1.8$; log $K_{\text{Mg,K}}^{\text{pot}} = -2.9$; log $K_{\text{Mg,Ca}}^{\text{pot}} = -4.1$, SSM) for physiological applications [72]. Recently, malondiamide derivatives of diazacrown ethers were synthesized and studied by K. Suzuki et al [36]. Compared to ETH 5506 and ETH T 5504, K22B5 shows even greater Mg²⁺ selectivity against Ca²⁺ (log $K_{\text{Mg,Ca}}^{\text{pot}} = -2.5$, SSM) but unfortunately poor discrimination for monovalent cations (log $K_{\text{Mg,K}}^{\text{pot}} = -1.5$; log $K_{\text{Mg,Na}}^{\text{pot}} = -3.2$, SSM).
5. Investigation of Mg$^{2+}$-selective ionophores

ETH 5214

ETH 4030

ETH 7025

ETH 3832
5. Investigation of Mg\(^{2+}\)-selective ionophores

Figure 5.1. Structures of the Mg\(^{2+}\) selective ionophores
5.3 Characterization of the new Mg$^{2+}$-selective ionophores

The discrimination pattern of magnesium-selective electrodes against alkali and alkaline earth metal ions varies significantly with the composition of the polymeric bulk membranes. In this respect, the type and amount of lipophilic anions and the type and lipophilicity of the plasticizer decisively influence the selectivity. The different types of ionophores form complexes with Mg$^{2+}$ in their specific stoichiometry. From theoretical considerations involving the assumed stoichiometry and the charge number of interacting ions, the optimum borate/ligand ratio within a membrane can be estimated (see section 4.4.1). Correspondingly, the inverse procedure is feasible. Each ligand is titrated by varying the molar ratio between ligand and borate and the selectivity coefficients ($\log K_{\text{Mg,SSM}}^{\text{ptf}}$) against the relevant interfering ions are determined for each membrane composition. Accordingly, the optimum borate ratio for each ionophore may be found, and the apparent stoichiometry of the ion-ligand complex can be derived based on the mentioned theoretical model (see section 4.4.1). Results of such measurements are presented in the following sections.

5.3.1 The monopodal ionophores ETH$^T$ 2001, ETH$^T$ 2002 and ETH$^T$ 2003
5. Investigation of Mg$^{2+}$-selective ionophores

Figure 5.2. ETH$^T$ 2001, ETH$^T$ 2002 and ETH$^T$ 2003

ETH$^T$ 2001, ETH$^T$ 2002 and ETH$^T$ 2003 are malonic diamide derivatives with adamantanyl groups as lipophilic substituents. These compounds are regarded as the "monomers" of the oligopodal ligands, and are supposed to coordinate to Mg$^{2+}$ by their carbonyl oxygen atoms. The ligands differ in their substitution at the two amide nitrogens, which changes the electron density of the amide group and the ketone moiety respectively ($\beta$-diketone section). These ionophores therefore show different conformations according to MM2 calculations [122].

The dependence of the selectivity on the ligand / borate ratio is illustrated for ETH$^T$ 2001, ETH$^T$ 2002 and ETH$^T$ 2003 in Figure 5.3. It is obvious from the figure that an optimum selectivity is obtained for 20% - 40 mol% of KTpClPB relative to the three different ligands in the membranes. According to Table 4.1, this ratio implies that the stoichiometry of complex between the ligand and Mg$^{2+}$ is about 3:1, and between the Ca$^{2+}$ and the ligand is about 4:1. ETH$^T$ 2003 seems to favor higher stoichiometry to both alkali and alkaline earth ions owing to its non-symmetric structure. With higher concentrations of borate, the membrane behaves as a pure cation-exchange system in which the membrane has a higher affinity to monovalent cations than divalent ones in accordance with the so-called "Hoffmeister series" [1].
5. Investigation of Mg$^{2+}$-selective ionophores

a)

b)
5. Investigation of Mg\textsuperscript{2+}-selective ionophores

c)

Figure 5.3. Selectivity of ionophores a) ETH\textsuperscript{T} 2001, b) ETH\textsuperscript{T} 2002 and c) ETH\textsuperscript{T} 2003 with the variation of borate content in the membrane composition. For each ionophore, 6 membranes were prepared with different borate/ionophore ratios; and 6 measurements of each membrane were carried out (n=6). The selectivity coefficients of each membrane electrodes are listed in Appendix 10.6.

When comparing the experimental lipophilicities of ETH\textsuperscript{T} 2002 (log\textsubscript{TLC}=1.3) and ETH\textsuperscript{T} 2003 (log\textsubscript{TLC}=5.45), a significant difference is seen. ETH\textsuperscript{T} 2002 is less lipophilic by a factor of 10\textsuperscript{4} which may indicate the existence of strong intramolecular association (see Table 5.2). Intramolecular interaction was assumed for asymmetric monopodal malondiamides of the type ETH\textsuperscript{T} 2003 derivated by lipophilic alkyl residues instead of adamantyl. IR (Infrared spectroscopy) studies at different concentrations of ETH\textsuperscript{T} 2003 in CCl\textsubscript{4} showed that the carbonyl groups in the ligand participate in hydrogen bonding [123].
In conclusion, the results agree with a conformation that is at least favorable for the rejection of alkali ions (Figure 5.4). The significance of the results refers to Table 5.2.

5.3.2 Structural analysis of Mg$^{2+}$-complexes with the Cambridge Structural Database

The structural analysis of Mg$^{2+}$-complexes by surveying the crystal structural database helps in the understanding of the complexation procedure between Mg$^{2+}$ and ligands. Derived from crystal structures (Cambridge Structural Database), Mg$^{2+}$ complexes show a C=O...Mg$^{2+}$ distance of $\gamma = 206\pm20$ (±1SD) pm. The binding angle $\alpha$ between C···O···Mg$^{2+}$ is specified with $\alpha = 136\pm7$ degrees and the torsion angle (N, C, O, Mg$^{2+}$) varies largely with the coordinating species. For diamides with octahedral co-ordination geometry, $\gamma = 166$ to 178 pm and $\beta = 12.5$ to 23 degrees (13 different complexes, 33 entries, see Figure 5.5). Unfortunately malonyl diamides are not covered by this database. From the theoretical point of view, it can be derived that the magnesium ions coordinate in octahedral complexes. This means that, ideally, the coordination site is formed by 3 malondiamide “monomers” opposed to only one tripodal ligand. This is supported by the experiments in section 5.3.1. It is assumed
that this effect is also mirrored by the selectivity pattern e.g. to Ca\(^{2+}\) and the borate ratio relative to the ligand (at least in cases where the competition of an ion for the ligand is the major factor influencing the selectivity coefficient). MM2 calculations [122] have shown that the dihedral distortion angles for the symmetric monomers are closer to an E-conformation referring to the diamide-oxygens (-110° distortion between the two planes exposing the carbonyl groups, -N-C=O-C- and -C-C=O-N-). The asymmetric monomer ETH\(^{T}\) 2003 shows a more favorable distortion angle of +127° between the two planes which results in a closer distance between the two co-ordinating oxygen atoms of 429 pm as opposed to 446 pm for the symmetric ligands. This conformation is closer to the Z-conformation referring to the diamide carbonyl groups.

\[ \text{Figure 5.5. Crystal structure of Mg}^{2+}\text{-Amide complex} \]
\[ \gamma: \text{distance of O}...\text{Mg}^{2+}; \]
\[ \alpha: \text{binding angle between C}...\text{O}...\text{Mg}^{2+}; \]
\[ \beta: \text{torsion angle (N}...\text{C}...\text{O}...\text{Mg}^{2+}) \]
5.3.3 Bipodal azacrown-ionophores ETH\textsuperscript{T} 2022 and K22B5

ETH\textsuperscript{T} 2022 and K22B5 are malondiamide derivatives of a diazacrown ether. Substitution of the amide nitrogens by two adamantyl groups results in two different bipodal ionophores where the former has two tertiary malondiamide nitrogens, and the latter has secondary ones (see Figure 5.1). Selectivity patterns are given in Figure 5.6. The best selectivity against the main interfering ion Ca\textsuperscript{2+} is obtained for an optimum ratio of borate to ligand of 100 mol % for K22B5 and 120-140 mol % for ETH\textsuperscript{T} 2022 where o-NPOE and ETH 8045 are applied as a plasticizer. Referring to the theoretical model (see Table 4.1), a higher stoichiometry of the cation-ligand complex is expected for K22B5 than for ETH\textsuperscript{T} 2022. K22B5 is likely to form 2:1 complexes with Mg\textsuperscript{2+} and Ca\textsuperscript{2+} while ETH\textsuperscript{T} 2022 tends to coordinate with Mg\textsuperscript{2+} in a 3:2 stoichiometry. The discrimination of Ca\textsuperscript{2+} is more pronounced for K22B5 than for ETH\textsuperscript{T} 2022. This implies that the replacement of the methyl groups by the hydrogen atoms leads to an improvement of the selectivity for Mg\textsuperscript{2+} against Ca\textsuperscript{2+}. Diazacrown derivatives show much improved “overall” selectivity for Mg\textsuperscript{2+} over Ca\textsuperscript{2+} than the stretched malondiamide monomers, e.g. ETH\textsuperscript{T} 2001 and ETH\textsuperscript{T} 2002 (see Table 5.2). The same holds true for tripodal ligands. This fact implies that the azacrown moiety could be involved in complexation of Mg\textsuperscript{2+}. Again the derivative with secondary amide nitrogen moieties shows a better selectivity over the tertiary derivatives.

a)
5. Investigation of Mg$^{2+}$-selective ionophores

b)

![Graph showing selectivity variation of membranes containing ionophores](image)

Figure 5.6. Selectivity (SSM) variation of membranes containing ionophores

a) K22B5, b) ETH$^T$ 2022 as a function of the borate content (KTpClPB). Plasticizer is o-NPOE. For each ionophore, 4 membranes were prepared with different borate/ionophore ratios; and 6 measurements of each membrane were carried out (n=6). The selective coefficients of each membrane electrodes are listed in Appendix 10.6.

5.3.4 Tripodal malondiamides ETH 5506, ETH$^T$ 5504 and ETH 3832

Tris(malondiamide) derivatives, ETH 5506, ETH$^T$ 5504 and ETH 3832, possessing six coordinating groups (amide groups, carbonyl oxygen) preferably form 1:1 complexes with Mg$^{2+}$. An increase in lipophilicity of the ionophores was achieved by introducing a benzene ring and connecting three malondiamide units to the core moiety. Compared to the monopodal ionophores, tripodal ionophores showed an enhanced selectivity for Mg$^{2+}$ over Ca$^{2+}$. Interestingly the discrimination of calcium is improved by about a factor of three logarithm units (see Table 5.2). It has to be assumed that the fixed conformation has no effect on the stability of the complex but has a statistical kinetic effect. Only the statistical probability of co-ordination of the three diamides at the same time is a factor of three larger. Opposite, for monopodal ligands only two coordinating sites at a time are assumed to be occupied by the ligand, the remaining four coordinating sites are occupied by water (or plasticizer).
5. Investigation of Mg\(^{2+}\)-selective ionophores

As previously reported \[^{123}\] , membranes containing 150 mol\% KTpClPB relative to the ligand showed the optimum discrimination for Mg\(^{2+}\) against Ca\(^{2+}\) and Na\(^{+}\). This corresponds to apparent stoichiometric ratios of ligand to Mg\(^{2+}\), Ca\(^{2+}\) and Na\(^{+}\) of 1:1, 2:1 and 3:2, respectively (see Table 4.1).

In ETH 5506 and ETH\(^T\) 5504, adamantyl groups are attached to the malonyl-diamide groups. Adamantyl groups render the ligands highly lipophilic and account for the slightly better selectivity for Mg\(^{2+}\) against Ca\(^{2+}\) compared to ETH 3832. ETH 5506 and ETH\(^T\) 5504 differ from each other in their structure by the length of the methylene bridge between the benzene core and the malondiamide groups (four methylene moieties in ETH\(^T\) 5504 and five in ETH 5506). ETH 5506 and ETH\(^T\) 5504 show very similar “overall” selectivity coefficients (see Figure 5.7). This result corresponds to the previous studies on Mg\(^{2+}\) ionophores \[^{123}\]. For tris(malondiamide) ligands with side chain length longer than four methylene groups, the Mg\(^{2+}\)-ligand complexes showed a small difference in their interaction energy relative to the respective Ca\(^{2+}\)-ligand complexes. When the side chain length was longer than four methylene groups, such ionophores showed similar discrimination of interfering ions. However, too high a lipophilicity of long methylene side chains in tris(malondiamide) ligands slowed down the complexation process \[^{124}\]. This might be the reason why the behaviour of potentiometric magnesium-selective microelectrodes based on ETH\(^T\) 5504 is more favorable than ETH 5506 \[^{57}\].
5. Investigation of Mg\(^{2+}\)-selective ionophores

Figure 5.7. A comparison of selectivity coefficients (SSM) of electrode containing the ionophores ETH\(^{T}\) 2022, K22B5, ETH 5506, ETH\(^{T}\) 5504 and ETH\(^{T}\) 3832 with their optimum borate contents in membrane compositions. Plasticizer in all membranes was o-NPOE. The mean value of each selectivity coefficient was calculated based on 6 measurements (see Table 5.2).

K22B5 shows higher Ca\(^{2+}\) discrimination but reduced discrimination of monovalent cations compared to ETH 5506 and ETH\(^{T}\) 5504. If the two types of ligands are used for measurement in physiological samples, they fulfill the theoretically requirements for selectivity coefficients [1] (required selectivity coefficients with background calibration, SSM, log\( K_{\text{Mg,Ca}}^{\text{pot}} \) = -1.64; log\( K_{\text{Mg,K}}^{\text{pot}} \) = -0.46; log\( K_{\text{Mg,Na}}^{\text{pot}} \) = -2.75). It is worth mentioning that the selectivity coefficients obtained with SSM normally overestimate the ability to discriminate between primary and interfering ions by the ion-selective membranes. The measured selectivity pattern based on SSM is not comparable to conditions in mixed solutions [86]. On the other hand, the relatively low lipophilicity of K22B5 (log\( P_{\text{TLC}} \)=3.94) does not meet the required lipophilicity of an ionophore for physiological measurements [73]. The required lipophilicity of an ionophore in continuous measurements of serum/plasma is log\( P_{\text{TLC}} \)=8.4 (30 days, 24 h).
5.3.5 Influence of membrane composition: plasticizers

In order to guarantee an "adequate membrane performance", the plasticizer must show sufficient lipophilicity, no crystallisation in the membrane phase and no exudation. Mg$^{2+}$ selective membranes incorporating ionophores of different structures showed pronounced variations (see Figure 5.8) in the selectivity coefficients when using plasticizers of varying polarity, composition and lipophilicity as solvents. When plasticizers of different lipophilicities such as e.g. ETH 8045 and $\alpha$-NPOE (ETH 8045, log $P_{TLC}$=12.8; $\alpha$-NPOE, log $P_{TLC}$=5.8) were employed, shifts in the selectivity coefficients were observed (see Figure 5.8). The highly lipophilic ionophores, ETH 5506 and ETHT 5504, in the membrane plasticized with ETH 8045 showed better selectivity than in the membrane plasticized with $\alpha$-NPOE. On the other hand, the more fixed conformation of azacrowns, e.g. K22B5, showed a less pronounced but opposite behaviour where the more lipophilic plasticizer seemed to reduce the extraction of Mg$^{2+}$. These results indicate that the extraction mechanism of the two classes of ligand is different.

Figure 5.8. Influence of plasticizers on selectivity of membrane electrodes containing different ionophores. In the membranes containing ETHT 5504 and ETH 5506, 150 mol% of KTpClPB relative to ligand was added. In the membrane containing K22B5, 100 mol% of KTpClPB relative to ligand was added.
O’Donell et al. confirmed by $^{13}$C NMR technique that ionophores substituted by adamantyl groups are forced into a fixed conformation as compared to e.g. ETH 7025 and ETH 3832 [116]. A lipophilic environment may energetically favour this effect. The preformation of the ligand sphere is less important for diazacrown derivatives. The plasticizer cannot influence the extraction behaviour to the same extent. The reduction in ionic strength owing to the increased lipophilicity may also not be primarily responsible for this behaviour.

5.3.6 **Comparison of lipophilicities**

The major factor limiting the lifetime of ion-selective membranes in potentiometric measurements is the loss of components, specifically of ionophore and plasticizer, into the bathing aqueous solution. For continuous monitoring with solvent polymeric membranes, the ionophore has to be lipophilic enough to guarantee a long and stable response of the ion-selective electrode. Loss of plasticizer is most critical [125,126]. The required lipophilicities for membrane components to obtain sensors of a specified lifetime have been previously calculated [126]. For example, in continuous-flow use with a lifetime of over 30 days and 24 h use per day, the required lipophilicity values log $P_{TLC}$ of ionophores are $> 2.3$ and $> 8.4$ for continuous measurements in urine and serum, respectively. The lipophilicities, log $P_{TLC}$, of the membrane components investigated in thesis are shown in Figure 5.9.
5. Investigation of Mg$^{2+}$-selective ionophores

Figure 5.9. Lipophilicity of the ionophores and plasticizers as determined by their TLC retention, $R_f$. The TLC system is calibrated with a set of reference compounds of known lipophilicities (see section 9.4). The mean value of logP$_{TLC}$ of each were calculated on the basis of 6 measurements ($n=6$) (Table 5.2).

Tris(malondiamide) derivatives show a sufficient lipophilicity (logP$_{TLC}$: ETH 5506, 9.75; ETH$^T$ 5504, 8.29 and ETH 3832, 8.60) for using the ion-selective membranes in physiological samples such as urine, serum or whole blood. Although K22B5 exhibited an improved Mg$^{2+}$ selectivity against Ca$^{2+}$ as compared to ETH 5506 and ETH$^T$ 5504, it does not meet the required lipophilicity value for measurement in serum.
5. Investigation of Mg$^{2+}$-selective ionophores

5.3.7 Conclusion

In summary, a study of Mg$^{2+}$-selective ionophores of different structures is reported here. SSM-selectivity data are used together with a theoretical model to interpret differences in the Mg$^{2+}$ recognition process of different ionophores. The selectivity coefficients and lipophilicities of the electrode membranes based on these ionophores are summarized in Table 5.2.

Table 5.2. Selectivity coefficients a) log $K_{Mg}^{pot}$ (mean ± 2SD, n=6), of the electrodes b) based on the studied ionophores and the lipophilicities (log $P_{TLC}$) of the ionophores. The optimum quantity of KTpClPB was added to the membrane for each ionophore.

<table>
<thead>
<tr>
<th>Ionophore</th>
<th>Log $P_{TLC}$</th>
<th>Plasticizer</th>
<th>KTpClPB mol % to L</th>
<th>KTpClPB</th>
<th>log $K_{Mg}^{pot}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETH$^T$ 2001</td>
<td>5.53 ±0.60</td>
<td>o-NPOE</td>
<td>40</td>
<td></td>
<td>-0.7 ±0.08</td>
</tr>
<tr>
<td>ETH$^T$ 2002</td>
<td>1.30 ±0.77</td>
<td>o-NPOE</td>
<td>40</td>
<td></td>
<td>-0.6 ±0.05</td>
</tr>
<tr>
<td>ETH$^T$ 2003</td>
<td>5.45 ±0.59</td>
<td>o-NPOE</td>
<td>40</td>
<td></td>
<td>-0.6 ±0.08</td>
</tr>
<tr>
<td>ETH$^T$ 2004</td>
<td>4.44 ±0.65</td>
<td>o-NPOE</td>
<td>120</td>
<td></td>
<td>-1.7 ±0.13</td>
</tr>
<tr>
<td>K22B5</td>
<td>3.94 ±0.59</td>
<td>o-NPOE</td>
<td>100</td>
<td></td>
<td>-2.6 ±0.12</td>
</tr>
<tr>
<td>ETH 8045</td>
<td>100</td>
<td></td>
<td>-2.2 ±0.08</td>
<td></td>
<td>-2.1 ±0.13</td>
</tr>
<tr>
<td>ETH 5506</td>
<td>8.48 ±0.98</td>
<td>o-NPOE</td>
<td>155</td>
<td></td>
<td>-1.9 ±0.06</td>
</tr>
<tr>
<td>ETH 8045</td>
<td>155</td>
<td></td>
<td>-1.9 ±0.06</td>
<td></td>
<td>-3.3 ±0.09</td>
</tr>
<tr>
<td>ETH$^T$ 5504</td>
<td>7.54 ±0.82</td>
<td>o-NPOE</td>
<td>155</td>
<td></td>
<td>-1.8 ±0.04</td>
</tr>
<tr>
<td>ETH 8045</td>
<td>155</td>
<td></td>
<td>-1.9 ±0.05</td>
<td></td>
<td>-3.3 ±0.13</td>
</tr>
<tr>
<td>ETH 3832</td>
<td>7.37 ±0.85</td>
<td>o-NPOE</td>
<td>155</td>
<td></td>
<td>-1.3 ±0.18</td>
</tr>
</tbody>
</table>

a) Selectivity coefficients are determined by SSM (separate solution method).
b) Each ionophore was incorporated together with the optimum amount of borate, KTpClPB, into a PVC membrane electrode.

Of all the studied ionophores, K22B5, ETH 5506 and ETH$^T$ 5504 showed a sufficient selectivity for Mg$^{2+}$ against the main interfering cations for serum/plasma measurement. Despite the fact that the selectivity of K22B5 for Mg$^{2+}$ against Ca$^{2+}$ is...
5. Investigation of Mg$^{2+}$-selective ionophores

the largest one ($\log K^\text{ref}_{\text{Mg,Ca}} = -2.6$), this ligand is not recommended for continuous monitoring of blood plasma and serum due to its low lipophilicity and, therefore, short lifetime of the electrode. Both ETH 5506 and ETH$^\text{T}$ 5504 exhibited the same Mg$^{2+}$ recognition ability against interfering cations and can be applied to magnesium selective electrodes in monitoring clinical samples. Although these results are encouraging, it must be pointed out that a direct transfer of selectivity data from SSM to conditions in mixed solutions is not feasible. A re-evaluation of the electrode response in mixed solutions and in the dedicated dynamic range e.g. by the SAM [1,73] or MPM [47,72] is necessary. ETH$^\text{T}$ 5504 has shown to be more suitable for use with microelectrodes [57].
6. Impedance study of Mg$^{2+}$-selective membranes

6.1. Introduction

Describing the response mechanism of ion-selective electrodes (ISEs) is a very intriguing problem. Solving it should help to elucidate why and how these membranes exhibit Nernstian behaviour and ion selectivity. Understanding the key potential-forcing interfacial processes such as ion-transfer kinetics and surface layer formation should enable new and better electrodes to be developed. Impedance spectroscopy has lately become a popular tool for the study of membrane processes. Buck et al. carried out theoretical studies of ion transfer across membranes under various conditions and set up corresponding models [127,128]. Armstrong et al [129,130], Cammann et al [131,132] and others [128,133,134] have also reported research results in this area. An overview on different membrane models is given in Ref [1].

The behaviour of the electrode membrane depends on its dielectric properties (bulk membrane resistance, interfacial charge-transfer resistance and the membrane permittivity) and on its basic chemistry. The selectivity of an electrode membrane can be investigated by means of impedance analysis as an alternative to classical emf measurements under realistic conditions, unlike the traditional calorimetric methods or transport studies on the membrane [135,136]. Emf- and impedance measurements are not independent of each other, and, therefore, they may not be referred to as "orthogonal methods" [41]. Rather the two methods are complementary and allow elucidation of different aspects of ion exchange and extraction mechanisms. Whereas investigations by emf measurements involves the whole potentiometric cell design including the liquid junction potential, impedance analysis allows distinction between the membrane bulk resistance and the charge-transfer at the boundaries. Additives such as lipophilic ionic sites exert an influence on ion extraction properties of Mg$^{2+}$-selective electrodes [78,104]. They are supposed to decrease the bulk membrane resistance and the interfacial charge-transfer barrier. The mechanisms of borate catalysis and the form of its active contribution [104] are analyzed here by evaluating the interfacial charge-transfer impedance.
In addition, the permittivity, $\varepsilon$, and the lipophilicity, log $P_{\text{TLC}}$, of plasticizers such as ETH 8045 and o-NPOE (for chemical names see section 10.3) were shown to influence the selectivity and sensitivity of the electrode membrane [81,108,121]. Surprisingly, the more lipophilic plasticizer ETH 8045 was more effective than o-NPOE. It was assumed that the relatively high permittivity of o-NPOE ($\varepsilon$ = 23.9) was considerably reduced in the more lipophilic homologous ETH 8045. The influence of the lipophilicity on the permittivity of an analogue plasticizer, and the influence of $\varepsilon$ of the plasticizer on the bulk membrane resistance was investigated and discussed in this work. The results of the impedance analysis of the Mg$^{2+}$-selective membrane, based on the ionophore ETH 5506, are reported here, and correlations between its behaviour and properties are interpreted.

Any electrochemical cell can be represented in terms of an equivalent electrical circuit that comprises a combination of resistance and capacitance (Figure 6.1). This circuit should contain at the very least components to represent:

- a double layer: a pure capacitor of capacity $C_d$;
- an impedance of the faradaic process $Z_f$ ($Z_f = R_{ct} + Z_w$), which comprises charge-transfer impedance, $R_{ct}$, and an impedance that measures the difficulty of mass transport of the electroactive species, called the Warburg impedance, $Z_w$;
- a uncompensated resistance, $R_{ct}$, which is, usually, the solution resistance between active and reference electrodes.

The combination of these elements is shown in Figure 6.1, with $Z_f$ parallel to $C_d$. For kinetically favored reactions $R_{ct} \rightarrow 0$ and $Z_w$ predominates, and for difficult reactions $R_{ct} \rightarrow \infty$ and $R_{ct}$ predominates. This is called the Randles circuit [137].
6. Impedance of Mg$^{2+}$-selective membranes

Figure 6.1. Equivalent electrical circuit of an electrochemical cell for a simple electrode process. $R_\Omega$ is the solution resistance, of the contacts and electrode materials, $Z_I$ the impedance of the electrode process, and $C_d$ the double layer capacity.

The Faradaic Impedance $Z_f$ for a simple electrode process is expressed as $[137]$:

$$Z_f = Z' + iZ''$$

(6.1)

$Z'$ is the real part (in-phase) and $Z''$ is the imaginary part (out-of-phase):

$$Z' = R_{ct} + \sigma \omega^{-1/2}$$

(6.2)

$$Z'' = -\sigma \omega^{1/2}$$

(6.3)

with $\omega$ the perturbation frequency (rad s$^{-1}$) and $\sigma$ the Warburg coefficient ($\Omega$ cm$^2$s$^{-1}$).

For the electrochemical cell in Figure 6.1, the experimental impedance is always obtained as if it were the result of a resistance $Z_f$ and capacitance $C_d$ in parallel. The complex impedance $Z(\omega)$ can be described as:

$$Z(\omega) = R_\Omega + \frac{1}{\frac{1}{R_{ct}} + i\omega C_d}$$

(6.4)

where

- $R_\Omega$: electrolyte resistance
- $R_{ct}$: charge-transfer resistance at the membrane boundaries
- $C_d$: double-layer capacitance at the membrane boundaries
- $\omega$: alternative current angular frequency of the electrical circuit

Regarding the full Randles equivalent circuit for this simple charge-transfer reaction, the complex impedance can be split into the real part (in-phase) and imaginary part (out-of-phase) components $[137]$.
6. Impedance of Mg$^{2+}$-selective membranes

\[
Z' = R_\Omega + \frac{R_{el} + \sigma \omega^{1/2}}{(\sigma \omega^{-1/2} C_d + 1)^2 + \sigma^2 C_d^2 (R_{el} + \sigma \omega^{-1/2})^2}
\]

(6-5)

\[
Z'' = \frac{\omega C_d (R_{el} + \sigma \omega^{-1/2})^2 + \sigma^2 C_d^2 + \sigma \omega^{-1/2}}{(\sigma \omega^{-1/2} C_d + 1)^2 + \sigma^2 C_d^2 (R_{el} + \sigma \omega^{-1/2})^2}
\]

(6-6)

Both components can be represented as a complex plane plot of $Z'$ vs $-Z''$ as shown in Figure 6.2 (Sluyters or Cole-Cole plot). The electric properties of the measured system can be obtained from this complex plane plot.

![Cole-Cole plot for an impedance spectrum](image)

Figure 6.2 Cole-Cole plot for an impedance spectrum

In the low-frequency part, the plot is a straight line of unit slope. The line corresponds to a reaction controlled solely by diffusion, and the impedance is the Warburg impedance, $Z_W$. In the high-frequency part, the plot is a semicircle which is purely kinetically controlled and $R_{el} >> Z_W$. The semi-circle plot can be expressed as:

\[
\left( Z' - R_\Omega - \frac{R_{el}}{2} \right)^2 + (Z'')^2 = \left( \frac{R_{el}}{2} \right)^2
\]

(6-7)
where the radius is $R_{ct}/2$ with intercepts on the $Z'$-axis of $R_\Omega$ ($\omega \to \infty$) and of $R_\Omega + R_{ct}$ ($\omega \to 0$).

In an electrochemical cell which involves several steps, a succession of semicircles side by side is obtained, corresponding to RC parallel combinations in series and with different RC time constants, from which it is possible to deduce the corresponding parameters. Practically the impedance spectrum of the membrane can be measured in a symmetric electrochemical cell arrangement (Figure 6.3). Two half-cells are filled with the bathing electrolyte solution. After at least 4 hours, the ac impedance is measured from 50 kHz to 0.01 Hz. The impedance spectrum contains (1) a bulk membrane impedance (bulk membrane resistance), (2) a charge-transfer impedance (charge-transfer resistance) and (3) a mass diffusion impedance (Warburg impedance). If the speed of the charge-transfer is very fast at the interface, the second semicircle (charge-transfer resistance) is either very small or cannot be recognized due to the resolution limit. However, a range value can be estimated. With the help of such impedance analysis, the important physical properties of the membrane can be obtained.

Figure 6.3 Electrochemical cell for impedance measurement
The electrical equivalent circuit corresponding to a membrane in a symmetrical PTFE cell (See Figure 6.3) is given as in Figure 6.4.a). It consists of the bathing solution resistance, $R_a$, the bulk membrane impedance, $Z_b$ ($R_b$), the impedance of faradaic processes, $Z_f$ which incorporates the interfacial charge-transfer resistance, $R_{ct}$, and the Warburg impedance (mass diffusion impedance), $W$. $C_g$ and $C_d$ represent the geometric capacitance and the double layer capacitance, respectively.

a)

b)

Figure 6.4. a. An equivalent circuit to the electrode process system. $R_a$: bathing solution resistance; $Z_b$: impedance of bulk membrane; $Z_f$: impedance of the electrode process; $W$: Warburg Impedance; $C_g$: geometric capacitance; $C_d$: double layer capacitance.

b. Impedance plane plot corresponding to the components in Figure 6.4.a simplified to illustrate the 3 components, namely bulk membrane impedance, charge transfer impedance and Warburg impedance.

The impedance spectrum in Figure 6.4.b. consists of two semicircles, where the first semicircle corresponds to the bulk membrane resistance, $R_b$; the second semicircle in the medium frequency range corresponds to the interfacial charge-transfer resistance at the membrane boundaries, $R_{ct}$. It is correlated directly with the potentiometric selectivity of the ISE membrane under study. If the speed of the charge-transfer is very fast at the interface, the second semicircle (charge-transfer resistance) will also
6. Impedance of Mg\textsuperscript{2+}-selective membranes

very fast at the interface, the second semicircle (charge-transfer resistance) will also be very small or even unrecognizable due to the resolution limit. However, a range value can be estimated. The straight line of the unit slope corresponds to the resistance of the mass diffusion process within the membrane phase.

The permittivity $\varepsilon$ of substance is an intrinsic passive electrical property. For a specimen held between two parallel electrodes of area $A$ separated by a distance $d$, the measured capacitance $C$ is related to the permittivity by the following equation \cite{138},

$$C = \varepsilon \cdot \varepsilon_0 (A/d) \quad (6-8)$$

$\varepsilon_0$ is the capacitance of a cell of unit dimensions containing a vacuum, equal to 8.854 $\times 10^{-14}$ F cm$^{-1}$, so that any matter existing between the electrodes will have the effect of raising the capacitance by a factor $\varepsilon$, a factor which was formerly called the dielectric constant, but (since it is not constant) is more properly referred to as the permittivity. From the measured capacitance $C$ and the cell constant ($d/A$), the permittivity $\varepsilon$ can be obtained. The permittivity of the bulk membrane and membrane components (e.g. plasticizers) affects the selectivity patterns of selective membranes \cite{81}.

6.2 Dielectric properties of the membranes and membrane composition

Bulk membrane resistance is an important electric property of an ISE. It is related to the ion transport behaviour within the membrane phase \cite{51}. In practice, a low bulk membrane resistance indicates a fast equilibration of the ion uptake or exchange process. The bulk membrane resistance of an ISE is mainly related to the membrane's composition (the type of ionophore and additives), its thickness, and the temperature. Impedance spectra can be used to determine bulk membrane resistance and to investigate the charge-transfer process at the boundaries.

In the membrane phase, the diffusion of the charged ion-ligand complex contributes to the reduction of the membrane resistance. Lipophilic anionic sites compensate the charge number of the ion-ligand complexes within the membrane. The addition of
6. Impedance of \( \text{Mg}^{2+} \)-selective membranes

Lipophilic anionic site to a membrane reduces the bulk membrane resistance significantly [78].

In order to study the contribution of each membrane component (selective ligand, lipophilic ionic sites, and plasticizer) to the membrane resistance, five membranes were prepared with different compositions (Table 6.1). The results in Table 6.1 were obtained from the impedance spectra in Figure 6.4 and Figure 6.5, which were measured with a bathing solution of 0.1 M \( \text{MgCl}_2 \).

Table 6.1 Membrane composition and electrical properties.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>KTPCIPB (mg)</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETH\textsuperscript{T} 5506 (mg)</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETH 8045 (mg)</td>
<td>118</td>
<td>118</td>
<td>118</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>( \alpha )-NPOE (mg)</td>
<td>56</td>
<td>56</td>
<td>56</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>PVC (mg)</td>
<td>30.5±2.5</td>
<td>2450±250</td>
<td>4300±400</td>
<td>670±20</td>
<td>34.7±1.9</td>
</tr>
<tr>
<td>( R_{\text{bulk}}, \text{k}\Omega ) \textsuperscript{(a)}</td>
<td>824</td>
<td>130</td>
<td>98</td>
<td>122</td>
<td>779</td>
</tr>
<tr>
<td>( C_{\text{bulk}}, \text{pF} ) \textsuperscript{(b)}</td>
<td>43±1.7</td>
<td>10.2±1.4</td>
<td>8.7±1.1</td>
<td>10.1±0.4</td>
<td>41.2±1.3</td>
</tr>
<tr>
<td>( C_{\text{bulk}} ) \textsuperscript{(c)}</td>
<td>-1.9</td>
<td>-0.4</td>
<td>-0.86</td>
<td>-0.38</td>
<td>-0.89</td>
</tr>
<tr>
<td>( K_{\text{Mg,Na}}^{\text{pot}} ) \textsuperscript{(d)}</td>
<td>-3.9</td>
<td>-3.6</td>
<td>-0.65</td>
<td>-0.05</td>
<td>1.10</td>
</tr>
</tbody>
</table>

(a) \( R_{\text{bulk}} \): resistance (n=4);
(b) \( C_{\text{bulk}} \): capacitance;
(c) \( \varepsilon \): permittivity of membrane at \( \omega=10\ \text{kHz} \);
(d) \( \varepsilon_{\text{ETH 8045}}=13.5\pm0.4, \ n=4, \varepsilon_{\alpha \text{-NPOE}}=23.9\pm0.3, \ n=4 \)
Figure 6.5.a. Comparison of impedance spectra from membranes of different compositions. The measurement points start with 50 kHz on the left side and end with 0.01 Hz on the right side. The membranes had a thickness of 150±20 µm. The bathing solution contained 0.1 M MgCl₂; the membrane composition is listed in Table 6.1. (✧) Membrane 1; (□) Membrane 2; (Δ) Membrane 3; (○) Membrane 4; (♦) Membrane 5;
b. Enlarged plot of Membrane 1 and Membrane 5.
From the results presented in Table 6.1, the following conclusions can be drawn:

- The presence of ionic sites (KTpClPB) in a neutral ligand-based ion-selective membrane significantly reduces the membrane resistance ($R_{\text{bulk, Membrane 1}}$: 28-33 kΩ; $R_{\text{bulk, Membrane 2}}$: 2200-2700 kΩ).

- It can be seen from the spectra of Membrane 2 (without anionic sites, with ETH 5506) and Membrane 3 (without anionic sites, without ligand) that the neutral ion-selective ligand contributes significantly to the reduction of the membrane resistance if the membrane resistance is high ($R_{\text{Membrane 2}}$: 2200-2700 kΩ; $R_{\text{Membrane 3}}$: 3900-4700 kΩ). In contrast, if the membrane resistance is low ($R_{\text{Membrane 1}}$: 28-33 kΩ; $R_{\text{Membrane 5}}$: 32.8-36.6 kΩ), the ionophore shows only a minor effect on the resistance. In both cases the relative permittivity is increased by approximately 1.8 units by incorporating the ionophore. In a membrane phase, the existence of charged ion-ligand complexes lowers the bulk membrane resistance and increases the permittivity.

- The permittivity of a membrane depends on its composition. Membrane 1 and 5 had a much higher permittivity ($\varepsilon_r$ Membrane 1: 41.3-44.7, $\varepsilon_r$ Membrane 5: 39.9-42.5) than both Membrane 2 ($\varepsilon_r$ Membrane 2: 8.8-11.6) and Membrane 3 ($\varepsilon_r$ Membrane 3: 7.6-9.8) since Membrane 1 and 5 contained anionic sites in addition to the neutral ionophore which were not present in Membrane 2 and Membrane 3. Since KTpClPB is a rather symmetric molecule and the charge is shielded by the phenyl moieties, a considerable influence on the relative permittivity is surprising.

- The permittivity of the plasticizer influences the bulk membrane resistance. Membrane 3 contained the less polar plasticizer ETH 8045 ($\varepsilon=13.5\pm0.4$) and had a higher membrane resistance than Membrane 4 with the more polar plasticizer o-NPOE ($\varepsilon=23.9\pm0.3$). The lipophilic side chain of ETH 8045 (12-(4-ethylphenyl)dodecylether) reduces the permittivity to 56% of o-NPOE (2-nitrophenyl octylether).
6.3 Charge-transfer resistance at the boundaries and the effect of lipophilic anionic sites

In Figure 6.6, the charge-transfer process (second semicircle of the spectrum) at the membrane/solution interface was investigated by using a diluted solution of 0.01 M MgCl₂. The charge-transfer resistance, \( R_{ct} \), of the two membranes (Membrane 1 and Membrane 2) with and without anionic sites was compared. In Membrane 1 (with KTpClPB) the charge-transfer resistance (second semicircle) is too low to be recognized, while in Membrane 2 (without KTpClPB) the second semicircle is clearly visible and the charge-transfer resistance is estimated as 150 kΩ.

The results show that the potentiometric response to the primary ion in aqueous solution is mainly achieved by the charge-transfer process at the membrane/solution interface. The charge-transfer resistance depends largely on the interfacial complexation between the ligand and the ion. The incorporated cation-exchanger sites (KTpClPB) catalyse the interfacial charge-transfer process and lower the barriers for the cation exchange reaction at the membrane/solution interface. These results mirror the selectivity pattern of the membranes. Membrane 2 shows a loss in the selectivity for Mg²⁺ (see Table 6.1) due to the high interfacial charge-transfer resistance.

a)
6. Impedance of Mg\(^{2+}\)-selective membranes

b)

![Impedance plots](image)

Figure 6.6. Impedance plots for membranes with- and without borate (KTPbClPB).

a) Impedance spectrum of Membrane 1 (with borate).
b) Impedance spectrum of Membrane 2 (without borate).

The bathing solution contained 0.01 M MgCl\(_2\).

6.4 Estimating the selectivity pattern from the charge-transfer resistance

An ion-selective membrane prefers the primary ion rather than the interfering ions because the ion-selective ligand has a specific affinity for the primary ion at the membrane/solution interface. The membrane has a lower charge-transfer resistance in contacting the primary ion than the interfering ion in agreement with the membrane selectivity [131].

When the Mg\(^{2+}\)-selective membrane contacts the primary ion Mg\(^{2+}\), its charge-transfer process is kinetically controlled and the interfacial charge-transfer resistance is very low. Compared to the first semicircle of the bulk membrane resistance, the second semicircle in the plane plot is small or even unmeasurable due to the low resolution of the measurement system. However, a range value can still be estimated.
6. Impedance of Mg$^{2+}$-selective membranes

b)
6. Impedance of Mg$^{2+}$-selective membranes

In Figure 6.7, a series of impedance spectra are shown for the Mg$^{2+}$-selective membrane in different bathing solutions (0.1 M MgCl$_2$, 0.1 M CaCl$_2$ and 0.1 M NaCl). With a bathing solution of 0.1 M MgCl$_2$ or CaCl$_2$ on either side of the PVC membrane, the second semicircle is not recognizable which indicates a rapid charge-transfer process. In 0.1 M NaCl bathing solution, the second semicircle is observable and the $R_{ct}$ is estimated as $\sim$ 4.5 k$\Omega$. When comparing the three cases (b, c and d), it is apparent that the charge-transfer resistance in case d is much higher than in case b and case c.

$$R_{ct}(Na^+) >> R_{ct}(Ca^{2+}), R_{ct}(Mg^{2+})$$

This result is due to the fact that the ETH 5506-based Mg$^{2+}$-selective membrane has a relatively low discrimination against Ca$^{2+}$ and a high discrimination against Na$^+$ ($\log K_{Mg,Na}^{pot} = -1.9$ and $\log K_{Mg,Na}^{pot} = -3.9$). The similar values of $R_{ct}$ (Mg$^{2+}$) and $R_{ct}$ (Ca$^{2+}$) indicate the relatively high competition of complexation with the ligand between Mg$^{2+}$ and Ca$^{2+}$. However Mg$^{2+}$ is still preferred by the ligand ETH 5506.

6.5 Conclusion
6. Impedance of Mg$^{2+}$-selective membranes

6.5 Conclusion

The correlations between the behaviour of the Mg$^{2+}$-selective membrane and its electric properties have been shown by the method of the impedance analysis. The bulk membrane resistance for the primary ion decreased with the addition of the lipophilic borate. It also decreases with the polarity of the plasticizer, which is the solvent. Moreover, the interfacial charge-transfer resistance was significantly reduced by adding borate into the membrane which enhanced the membrane’s affinity for the primary ion. The Mg$^{2+}$-selective membrane showed a higher charge-transfer resistance for Na$^+$ than for Ca$^{2+}$ and Mg$^{2+}$. This phenomenon explains the order of selectivity of the Mg$^{2+}$-selective membranes.
7. Clinical and biological applications of Mg$^{2+}$-selective electrodes

7.1 Influence of the inner electrolyte on the selectivity

In potentiometry with polymeric ion-selective electrodes (ISEs), the sensing potential of the polymeric membrane, in essence, consists of the diffusion potential within the membrane and phase boundary potentials (Donnan potential) at the two interfaces (Figure 4.6 and Eq. 4-17). Commonly, studies of membrane behaviour are carried out assuming that the activity and composition of the inner electrolyte solution is fixed as a constant. Therefore, the membrane potential $E_M$ linearly responds to the logarithm of the active molality of the primary ion in sample solution. In the following section, the influence of the inner solution on the selectivity of ion selective electrodes was studied. A recent study showed that the detection limit of the selective electrode could be improved to a much lower level by reducing the concentration of the inner electrolyte solution $[139]$. As the selectivity of the electrode is related to its detection limit, the inner electrolyte influences the selectivity of the membrane. Variation of the inner electrolyte affects the behaviour of the electrode membrane, regarding the selectivity of primary ion over interfering ions.

As the diffusion potential within the membrane can be regarded as being in a steady state $[1]$, the response of the electrode membrane is mainly due to the change of the phase boundary potential at the membrane-solution interface $[102,140-142]$. The relationship between the phase boundary potential $E_b$ and the electrolyte active molality in aqueous solution $a_i$ can be deduced on the basis of Eq. 7-1 $[51]$.

$$
E_b = s_i \log \frac{k_i a_i}{\gamma_i c_i} \approx s_i \log \frac{k_i a_i}{c_i} \quad (7-1)
$$

where

- "i" referring to the primary ion I.
- $E_b$ boundary potential arising at the interface of membrane phase and aqueous phase;
- $s_i$ response slope of the membrane electrode to the analyte I;
- $k_i$ partition coefficient of analyte I between membrane phase and aqueous phases;
- $a_i$ molal activity of the analyte I in the aqueous phase;
7. Clinical and biological applications of Mg$^{2+}$-selective electrodes

$c_i$ concentration of the analyte I in the boundary layer of membrane phase;

$\gamma_i$ activity coefficient of I in the membrane phase, which is assumed to be 1.

![Figure 7.1. Scheme of selective complexation at the two interfacial boundaries.](image)

The $a_i^s$ and $a_i^l$ refer to the active molalities of ion I in aqueous phases (sample and inner solution); $c_i^s$ and $c_i^l$ are the concentrations of I at the membrane boundaries contacting the sample solution and the inner solution; $E_f^s$ and $E_f^l$ are the phase boundary potentials at the interfaces between the membrane/sample solution and the membrane/inner solution.

To simply the selectivity study, the charges of both I and J are assumed to be identical ($z_i = z_j$) and the complexation process of the ligand and electrolyte ($\beta_{L_n}$ and $\beta_{L_m}$) within membrane phase is not discussed here. The electrode membrane is assumed to be thin enough that the diffusion potential $E_d$ within the membrane is considered to be constant. The influence of the inner electrolyte on the selectivity is formulated following the procedure of SSM, where two separate sample solutions containing I and J are respectively measured. The selectivity coefficient of the electrode is calculated on the basis of the potential response in the two solutions. Two conditions are considered in the following study:

1. different active molalities of the inner electrolyte
2. different compositions of the inner electrolyte.
7. Clinical and biological applications of Mg\(^{2+}\)-selective electrodes

7.1.1 Different active molalities of the inner electrolyte solution

According to the above assumptions, the selectivity of the electrode membrane can be compared for two cases of different active molalities of the inner electrolyte solution which contains only I. The inner solution in Case A has a higher active molality of I than in Case B \((a_{i(A)}^{r} > a_{i(B)}^{r})\).

In Case A, the active molality of the inner electrolyte is \(a_{i(A)}^{r}\), where the prime "r" denotes the inner reference solution. At the membrane-inner solution interface, the phase boundary potential \(E_{A}^{r}\) is:

\[
E_{A}^{r} = s_{i} \log \frac{k_{i} a_{i(A)}^{r}}{c_{i(A)}^{r}}
\]  

(7-2)

where
\begin{itemize}
  \item \(k_{i}\) partition coefficient of I between the membrane and aqueous phase;
  \item \(c_{i(A)}^{r}\) concentration of analyte I in the membrane boundary layer contacting the inner solution;
  \item \(a_{i(A)}^{r}\) active molality of analyte I in inner solution.
\end{itemize}

If the sample solution contains only I (active molality \(a_{i(A)}^{s}\)) where the prime "s" denotes the sample solution, the local phase boundary potential, \(E_{A}^{s}(i)\) at the membrane-sample interface is:

\[
E_{A}^{s}(i) = s_{i} \log \frac{k_{i} a_{i(A)}^{s}}{c_{i(A)}^{s}}
\]  

(7-3)

where
\begin{itemize}
  \item \(c_{i(A)}^{s}\) concentration of electrolyte I in the membrane boundary layer which contacts the sample solution
  \item \(a_{i(A)}^{s}\) molal activity of electrolyte I in sample solution
\end{itemize}
Based on Eq. 7-2 and 7-3, the potential difference between two membrane surfaces can be described as:

\[
\Delta E_A^i = E_A^i - E_A^s = s_i \left( \log \frac{k_i a_i^r(A)}{c_i^s} - \log \frac{k_i a_i^s(A)}{c_i^r} \right)
\]

\[
= s_i (\log a_i^r(A) - \log a_i^s(A)) + s_i (\log c_i^s(A) - \log c_i^r(A))
\]

(7-4)

If the sample solution contains only \( J (a_{j(A)}^r) \), the local phase boundary potential, \( E_A^s(j) \), at the membrane-sample interface is:

\[
E_A^s(j) = s_i \log \frac{k_i a_{j(A)}^r}{c_{j(A)}^s}
\]

(7-5)

where

- \( s_i \) refers to the interfering ion \( J \).
- \( c_{j(A)}^s \) concentration of \( J \) in the membrane layer at the boundary with the sample solution.
- \( a_{j(A)}^s \) molal activity of \( J \) in the sample solution.

Analogous to Eq. 7-4, the potential difference between the two membrane boundaries is:

\[
\Delta E_A^i = E_A^i - E_A^s(j) = s_i \left( \log \frac{k_i a_{j(A)}^r}{c_{j(A)}^s} - \log \frac{k_j a_{j(A)}^r}{c_{j(A)}^s} \right)
\]

\[
= s_i (\log \frac{k_i}{k_j} + \log \frac{a_{j(A)}^r}{a_{j(A)}^s} + \log \frac{c_{j(A)}^s}{c_{j(A)}^r})
\]

(7-6)

According to the SSM procedure, \( \Delta E_A^j - \Delta E_A^i \) is related to the selectivity of the electrode membrane as follows:
The active molalities of I and J in the two separate sample solutions can be experimentally set as equal, \( a_{\text{FC}} = a_{\text{JC}} \). The selectivity coefficient is calculated according to the SSM procedure:

\[
\log K_{i,j}^{\text{pot}} (A) = \frac{\Delta E_{1}^{i} - \Delta E_{1}^{j}}{S_{i}} = \log \frac{k_{1}}{k_{j}} + \log \frac{c_{i}^{s}}{c_{j}^{s}}
\]  

(7-8)

In Case B, the inner reference solution contains I with active molality, \( a_{j}^{c} \). The selectivity coefficient \( \log K_{i,j}^{\text{pot}} (B) \) can be obtained analogous to Case A:

\[
\log K_{i,j}^{\text{pot}} (B) = \log \frac{\Delta E_{1}^{i} - \Delta E_{1}^{j}}{S_{i}} = \log \frac{k_{1}}{k_{j}} + \log \frac{c_{i}^{s}}{c_{j}^{s}}
\]  

(7-9)

By comparing the two cases (Eq. 7-8 and 7-9), the effect of different active molalities of the inner electrolyte on the selectivity of electrode can be evaluated.

\[
\log K_{i,j}^{\text{pot}} (B) - \log K_{i,j}^{\text{pot}} (A) = \log \frac{c_{i}^{s}}{c_{j}^{s}} - \log \frac{c_{i}^{s}}{c_{j}^{s}}
\]  

(7-10)

Eq. 7-10 shows that the concentration ratio of electrolytes in the membrane affects the selectivity of the electrode.

As the inner solution has sufficient contact with the electrode membrane, the concentration level of the inner electrolyte solution affects the complexation of the ligand sites and the electrolyte ion (only I) in the membrane. In the membrane with the greater active molality of the inner electrolyte, more ligand sites would be occupied by the electrolyte ions from the inner solution. Hence less “free” ligand sites are left in the membrane phase.
With regard to the second term in Eq. 7-10, \( c_{i(B)}^s \) and \( c_{i(A)}^s \) are considered as identical in Case A and Case B (\( c_{i(B)}^s \approx c_{i(A)}^s \)) and \( \log \frac{c_{i(B)}^s}{c_{i(A)}^s} = 0 \). This is because the complexed primary ions in the membrane phase are not able to discriminate between ions from the sample solution or from the inner solution.

With respect to the first term, the inner electrolyte has a lower active molality of I in Case B than in Case A (\( a_{i(B)}^I < a_{i(A)}^I \)) and therefore less ligand sites in the membrane are occupied by the primary ions from the inner solution. When the sample solution containing J is measured, more interfering ions are able to enter the membrane phase for the complexation by ligands at the membrane-sample interface and \( c_{j(B)}^I > c_{j(A)}^I \). Thus, Eq. 7-10 can be changed to:

\[
\log K_{i,j}^{\text{pot}} (B) - \log K_{i,j}^{\text{pot}} (A) = \log \frac{c_{i(B)}^s}{c_{i(A)}^s} - \log \frac{c_{j(B)}^s}{c_{j(A)}^s} > 0 \tag{7-11}
\]

and

\[
\log K_{i,j}^{\text{pot}} (B) > \log K_{i,j}^{\text{pot}} (A) \tag{7-12}
\]

Eq. 7-12 reveals that the membrane electrode with a higher active molality of the inner electrolyte exhibits an improved selectivity of I over J.

Before emf measurements are carried out, the electrode is commonly “pre-conditioned” in a conditioning solution that is usually identical to the inner solution in most cases, [51]. When a concentrated electrolyte solution is used as the inner solution and the conditioning solution (Case A), two surfaces of the membrane are “sufficiently conditioned” and more ligands sites in the membrane form complexes with I. When the sample solution contains only J (but no I), less “free” ligand is available for complexing the interfering ions. This is the reason that the selectivity in Case A is better than in Case B.
7. Clinical and biological applications of Mg\(^{2+}\)-selective electrodes

7.1.2 Different compositions of electrolytes as the inner filling solution

Using different electrolyte compositions as the inner electrolyte solution influences the selectivity of the electrode. In the following, two electrodes containing different types of inner electrolytes (I for Case C, J for Case D) are compared for the variation in selectivity.

In **Case C**, the inner solution contains only I. Analogous to the **Case A**, the selectivity coefficient, \( \log K_{ij}^{\text{ref}}(C) \), is then obtained according to the SSM procedure:

\[
\log K_{ij}^{\text{ref}}(C) = \frac{\Delta E_{i}^{\text{j}} - \Delta E_{i}^{\text{i}}}{s_{i}} = \log k_{i} + \log \frac{c_{i}^{s}}{c_{i}^{j}}
\]  \hspace{1cm} (7-13)

In **Case D**, the inner reference solution contains only J \((a_{j(D)}^{s})\). At the membrane-inner solution interface, the phase boundary potential is:

\[
E_{D}^{s} = \log \frac{k_{j}a_{j(D)}^{s}}{c_{j(D)}^{s}}
\]  \hspace{1cm} (7-14)

Following the SSM procedure, the sample solutions containing only I and J are measured alternatively. The phase boundary potentials, \(E_{D}^{s}(i)\) and \(E_{D}^{s}(j)\), at the membrane-sample interface are:

\[
E_{D}^{s}(i) = \log \frac{k_{i}a_{i(D)}^{s}}{c_{i(D)}^{s}}
\]  \hspace{1cm} (7-15)

and

\[
E_{D}^{s}(j) = \log \frac{k_{j}a_{j(D)}^{s}}{c_{j(D)}^{s}}
\]  \hspace{1cm} (7-16)

In the above two situations (Eq. 7-15 and 7-16), the potential difference between the two membrane borders can be described as:
\[ \Delta E^i_D = E^r_D - E^e_D(i) = s_i \left( \log \frac{k_i a^{(D)}_{j(D)}}{c^{r}_{j(D)}} - \log \frac{k_i a^{s}_{j(D)}}{c^{s}_{j(D)}} \right) \]  
\[ = s_i \left( \log \frac{k_i}{k_j} + \log \frac{a^{r}_{j(D)}}{a^{s}_{j(D)}} + \log \frac{c^{r}_{j(D)}}{c^{s}_{j(D)}} \right) \]  
(7-17)

and

\[ \Delta E^j_D = E^r_D - E^e_D(j) = s_i \left( \log \frac{k_j a^{(D)}_{i(D)}}{c^{r}_{i(D)}} - \log \frac{k_j a^{s}_{i(D)}}{c^{s}_{i(D)}} \right) \]  
\[ = s_i \left( \log \frac{a^{r}_{i(D)}}{a^{s}_{i(D)}} + \log \frac{c^{r}_{i(D)}}{c^{s}_{i(D)}} \right) \]  
(7-18)

According to the SSM, \( \Delta E^j_D - \Delta E^i_D \) is related to the selectivity of the electrode as follows:

\[ \Delta E^j_D - \Delta E^i_D = s_i \left( \log \frac{a^{r}_{i(D)}}{a^{s}_{i(D)}} + \log \frac{c^{r}_{i(D)}}{c^{s}_{i(D)}} \right) \]  
\[ = s_i \left( \log \frac{k_i}{k_j} + \log \frac{a^{r}_{i(D)}}{a^{s}_{i(D)}} + \log \frac{c^{r}_{i(D)}}{c^{s}_{i(D)}} \right) \]  
(7-19)

The active molalities of I and J in two separate solutions can be experimentally set as equal, \( a^{s}_{i(D)} = a^{s}_{j(D)} \). The selectivity coefficient, \( \log K^\text{pot}_{l,j}(D) \), can then be obtained according to the SSM procedure:

\[ \log K^\text{pot}_{l,j}(D) = \frac{\Delta E^j_D - \Delta E^i_D}{s_i} = \log \frac{k_i}{k_j} + \log \frac{c^{r}_{i(D)}}{c^{s}_{i(D)}} \]  
(7-20)

By comparing the two selectivity coefficients \( \log K^\text{pot}_{l,j}(C) \) and \( \log K^\text{pot}_{l,j}(D) \) (Eq. 7-13 and 7-20), the effect of the different inner electrolyte solutions (I and J) on the selectivity of the electrode can be deduced as follows:
7. Clinical and biological applications of Mg$^{2+}$-selective electrodes

\[
\log K_{i,j}^{\text{pot}} (D) - \log K_{i,j}^{\text{pot}} (C) = (\log \frac{k_i}{k_j} + \log \frac{c_{i(D)}^s}{c_{i(C)}^s}) - (\log \frac{k_i}{k_j} + \log \frac{c_{i(C)}^s}{c_{i(C)}^s})
\]

\[
= \log \frac{c_{i(D)}^s}{c_{i(D)}^s} + \log \frac{c_{i(C)}^s}{c_{i(C)}^s} - \log \frac{c_{i(C)}^s}{c_{i(D)}^s} + \log \frac{c_{i(D)}^s}{c_{i(D)}^s}
\]

\[
= \log \frac{c_{i(D)}^s}{c_{i(D)}^s} - \log \frac{c_{i(C)}^s}{c_{i(D)}^s} + \log \frac{c_{i(D)}^s}{c_{i(D)}^s} - \log \frac{c_{i(C)}^s}{c_{i(D)}^s}
\] (7-21)

When the sample electrolyte is I, one can assume that there would be less I in the membrane phase in Case D than in Case C (\(c_{i(D)}^s < c_{i(C)}^s\) and \(\log \frac{c_{i(D)}^s}{c_{i(C)}^s} > 0\)), which can be explained by the following assumption. In Case D, the ligand sites in the membrane would be partly (or mainly) occupied by J from the inner solution. When the sample electrolyte is J, there would be less J in the membrane phase in Case C than in Case D (\(c_{j(C)}^s < c_{j(D)}^s\) and \(\log \frac{c_{j(D)}^s}{c_{j(C)}^s} > 0\)).

Based on the above analysis, Eq. 7-21 becomes:

\[
\log K_{i,j}^{\text{pot}} (D) > \log K_{i,j}^{\text{pot}} (C) \quad (7-22)
\]

From Eq. 7-22, it is concluded that when the inner electrolyte is the interfering ion J (Case D), the selectivity of the membrane electrode would be reduced.

From the above, it is concluded that:

1. When different active molalities of the inner electrolyte are applied in the inner solution respectively, the greater the active molality of the inner electrolyte, the better the selectivity of membrane electrode.

2. When an interfering ion is used as the inner electrolyte instead of the primary ion, the selectivity of membrane electrode is reduced.
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7.1.3 Results and Discussions

In order to study the influence of the inner solution on the selectivity, a Mg$^{2+}$-selective membrane with the neutral ligand ETH 5506 was used to measure the selectivity of Mg$^{2+}$ over Ca$^{2+}$ (SSM and VIM). Different concentrations of certain electrolyte (Mg$^{2+}$) and different types of electrolytes (Mg$^{2+}$, Ca$^{2+}$) were utilized as the inner reference electrolyte (Table 7.1). For VIM measurements, two solutions were measured which had an identical physiological electrolyte composition but varying Ca$^{2+}$ concentrations (Figure 7.2).

Table 7.1 Influence of the inner electrolyte on the selectivity of Mg$^{2+}$-selective membrane (SSM and VIM, mean, n=6)

<table>
<thead>
<tr>
<th>Electrode 1 (Case A or Case C)</th>
<th>Electrode 2 (Case B)</th>
<th>Electrode 3 (Case D)</th>
<th>Electrode 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner solution</td>
<td></td>
<td></td>
<td>0.140 M Na</td>
</tr>
<tr>
<td>0.1 M Mg$^{2+}$</td>
<td>0.01 M Mg$^{2+}$</td>
<td>0.1 M Ca$^{2+}$</td>
<td>0.004 M K$^+$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0013 M Ca$^{2+}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0004 M Mg$^{2+}$</td>
</tr>
<tr>
<td>log $K_{Mg, Ca}^{pot}$ (SSM)</td>
<td>-1.82±0.04</td>
<td>-1.61±0.03</td>
<td>-1.78±0.04</td>
</tr>
<tr>
<td>log $K_{Mg, Ca}^{pot}$ (VIM)</td>
<td>-1.32±0.03</td>
<td>-1.14±0.02</td>
<td>-1.03±0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-0.70±0.05</td>
</tr>
</tbody>
</table>

7.1.3.1 Influence of the inner solution activity on the selectivity

Table 7.1 shows that the Mg$^{2+}$-selective electrode (ligand: ETH 5506) with a concentrated inner solution (0.1 M MgCl$_2$) showed an improved selectivity compared to the electrode with a diluted inner solution (0.01 M MgCl$_2$). The experimental result matches the prediction of the theory.

As was discussed in section 7.1.1, the membrane phase of Electrode 2 has more "free ligand sites" than Electrode 1 due to its less concentrated inner solution. When the sample solution is 0.1 M CaCl$_2$, the membrane phase can extract more Ca$^{2+}$ into the membrane phase by complexation with ETH 5506. This effect leads to a loss of selectivity (see Table 7.1).
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7.1.3.2 Influence of the inner solution composition on the selectivity

The selectivities of two identical Mg\(^{2+}\)-selective membranes containing different types of inner electrolyte (0.1 M MgCl\(_2\) and 0.1 M CaCl\(_2\) respectively) are compared in Table 7.1. As was predicted in section 7.1.2, the selectivity decreases when the solution of 0.1 M Ca\(^{2+}\) is used as inner solution instead of the solution of 0.1 M Mg\(^{2+}\).

In Electrode 3, the Ca\(^{2+}\) ions enter the membrane phase from the inner solution by forming complexes with ETH 5506. When the sample solution is 0.1 M MgCl\(_2\), the uptake of Mg\(^{2+}\) into membrane phase would be hindered because the ligand sites of ETH 5506 are partly occupied by Ca\(^{2+}\) from the inner solution. It can be seen from Figure 7.2 that Electrode 3 shows a reduced Mg\(^{2+}\) selectivity over Ca\(^{2+}\) than Electrode 1.

Moreover, a previous study showed that the stoichiometry of the Ca\(^{2+}\)-ligand (1:2) is higher than the Mg\(^{2+}\)-ligand (1:1) \([104]\). When Ca\(^{2+}\) but no Mg\(^{2+}\) is used as the inner electrolyte, more ligand sites would be occupied. This effect could also account for the decrease of the selectivity of Mg\(^{2+}\) over Ca\(^{2+}\).

7.1.3.3 A mixed solution as the inner electrolyte solution

When the inner solution is a physiological background electrolyte solution (Electrode 4), the electrode exhibits a much-reduced affinity for Mg\(^{2+}\) in the sample solution. Consequently, the Mg\(^{2+}\)-selectivity of the electrode membrane is decreased as is shown in Figure 7.2 and Table 7.1. The physiological background solution contains not only Ca\(^{2+}\), but also other interfering electrolytes such as K\(^{+}\) and Na\(^{+}\) and the level of Mg\(^{2+}\) is comparably low (c\(_{\text{Mg}^{2+}}\)=0.3~0.6 mM).

The inner solution in Electrode 4 contains 0.4 mM Mg\(^{2+}\) with a physiological background, including other interfering electrolytes, e.g. Na\(^{+}\), K\(^{+}\) and Ca\(^{2+}\). Such a mixed inner solution makes the situation more complicated. In the physiological background solution, c\(_{\text{Mg}^{2+}}\) is much lower than the other ions (e.g. c\(_{\text{Na}^{+}}\)=140 mM and c\(_{\text{Mg}^{2+}}\)=0.3~0.6 mM where the former is about 300 times more concentrated than the
latter). The Mg\textsuperscript{2+}-selective membrane using this mixture as the inner solution shows poorer selectivity of Mg\textsuperscript{2+} over Ca\textsuperscript{2+} than the electrode membrane using 0.1 M MgCl\textsubscript{2} as the inner solution.

![Figure 7.2](image)

**Figure 7.2.** Response curves of the electrode membrane with different inner electrolytes (see Table 7.1). The measurement was carried out in a flow through measuring cell.

Sample A: \( c_{\text{Ca}^2+} = 1.0 \text{ mM}; c_{\text{Mg}^2+} = 0.4 \text{ mM}; c_{\text{K}^+} = 4.0 \text{ mM}; c_{\text{Na}^+} = 140 \text{ mM} \)

Sample B: \( c_{\text{Ca}^2+} = 1.3 \text{ mM}; c_{\text{Mg}^2+} = 0.4 \text{ mM}; c_{\text{K}^+} = 4.0 \text{ mM}; c_{\text{Na}^+} = 140 \text{ mM} \)

**7.1.4. Conclusions**

This work shows the influence of the inner electrolyte on the selectivity of the electrode membrane. The theoretical derivation and the experimental results lead to the following conclusions:

1. A concentrated inner electrolyte solution (primary ion I) leads to an increase of selectivity.
2. When the inner electrolyte is the interfering ion J, the membrane shows a loss of selectivity against the interfering ion.
3. When the inner solution contains a mixture of the primary ion and interfering ions, the selectivity variation depends on the proportion of each electrolyte in the mixed inner solution.

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7.2 Mg\(^{2+}\)-selective membranes in physiological samples

For studying the behaviour of Mg\(^{2+}\)-selective membranes in physiological samples, a membrane electrode based on ETH 5506 was used to measure Mg\(^{2+}\) in a bovine serum sample and was compared to the commercially available AVL Mg\(^{2+}\)-selective membrane electrode. This work was carried out with Dr. Ghahramani from AVL. The standard solutions were obtained from AVL. AVL does not publish the composition of these standards. Figure 7.3 shows the response curves of the two electrodes.

Table 7.2. Emf values of the two membranes in calibrators and bovine serum (refer to Figure 7.3)

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Emf in calibrators before serum contact (mV)</th>
<th>Emf in calibrators after serum contact (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td>AVL</td>
<td>-47.16</td>
<td>-44.06</td>
</tr>
<tr>
<td>ETH</td>
<td>-34.70</td>
<td>-29.42</td>
</tr>
</tbody>
</table>

Figure 7.3. Response of Mg\(^{2+}\)-selective membranes in physiological samples in a flow through system. In stop-mode, each sample was measured for 60 seconds and the bovine serum was measured for 300 seconds.

"ETH" Mg\(^{2+}\)-selective membrane based on the ligand of ETH 5506;
"AVL" commercial Mg\(^{2+}\)-selective membrane from AVL;
A AVL calibrator of Standard A for AVL-988/4 Electrolyte Analyzer;
C AVL calibrator of Standard C for AVL-988/4 Electrolyte Analyzer;
C contains higher Mg\(^{2+}\) concentration than A.
It can be seen from Figure 7.3 and Table 7.2 that
1. both membranes showed stable response curves during the measurement in calibrators and bovine serum.
2. each membrane showed no significant change of behaviour after contact with bovine serum for 300 seconds.
3. the sensitivity of each membrane did not decrease after the contact with the bovine serum as shown by the calculation of emf difference between $\text{emf}_{(AVL-Std-C)}$ and $\text{emf}_{(AVL-Std-A)}$.
4. the membrane "ETH" showed a higher $\text{Mg}^{2+}$-sensitivity than the membrane "AVL" (Table 7.2).

In addition to the above measurement, another test was also carried out to compare the two membranes regarding their behaviour with additional calibrators for the physiological measurement of $\text{Mg}^{2+}$ in a flow through cell (Figure 7.4).

Figure 7.4. Behaviour of two electrode membranes in calibrators and bovine serum.
A (AVL Standard-A), B (AVL-Standard-B), C (AVL-Standard-C) and D (AVL-Standard-D) are the standard calibrators for AVL Electrolyte Analyzer and S is the bovine serum. The interval for each solution is 60 seconds.
$\text{emf}_{A}$-$\text{emf}_{A}$ $\text{Mg}^{2+}$-selectivity against $\text{Ca}^{2+}$;
$\text{emf}_{C}$-$\text{emf}_{A}$ $\text{Mg}^{2+}$-sensitivity;
$\text{emf}_{A}$-$\text{emf}_{B}$ $\text{Mg}^{2+}$-selectivity against $\text{Na}^{+}$.
Based on the comparison of the two membranes, the following conclusions can be drawn.

1. Both electrode membranes exhibit similar response curves during the whole measuring process.

2. The membrane “ETH” shows a more stable response when in contact with the serum sample and the better repeatability for its five contacts with the serum sample.

3. The membrane “ETH” shows better Mg$^{2+}$-selectivity against Ca$^{2+}$ ($\text{emf}_B$-$\text{emf}_A$) and better Mg$^{2+}$-sensivity ($\text{emf}_C$-$\text{emf}_A$).

4. During the calibration procedure, the response of the membrane “ETH” exhibits a “wash-out” effect so that the emf has a trend to be negative. This phenomenon may come from the fact that the membrane “ETH” has the better Mg$^{2+}$-selectivity against Ca$^{2+}$ and it takes a longer time for the membrane to reach a stable emf in the calibrators containing different levels of Ca$^{2+}$.

5. The membrane “AVL” has a better Mg$^{2+}$-selectivity against Na$^+$ ($\text{emf}_A$-$\text{emf}_D$) which could be due to the membrane composition which contains less KTpClPB than “ETH”.

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7. Clinical and biological applications of Mg\(^{2+}\)-selective electrodes

7.3 Symmetric potentiometric cell for physiological measurements

In physiological solutions (serum/plasma), the concentration of the extracellular free Mg\(^{2+}\) varies from 0.3 mM to 0.6 mM. The protein and lipid species in the sample may cause an asymmetric potential and emf drifts of the Mg\(^{2+}\)-selective membranes, which can be checked with the flow-through symmetric cells as shown in Figure 7.5.

![Figure 7.5. Configuration of the symmetric flow-through cell. The cell was manufactured in ETH workshop and CCS (Centre for Chemical Sensors/Biosensors). The reference electrodes were obtained from Hamilton Bonaduz AG.](image)

The organisation of the above flow through cell may then be represented as follows:

\[
\text{ref. electrode} \quad | \quad \text{sample} \quad | \quad \text{membrane} \quad | \quad \text{inner electrolyte} \quad | \quad \text{ref. electrode}
\]

The potential corresponds to the active molality of the analyte I at both sides of the membrane.

\[
E_M = \Delta E_0 + \frac{a_i(\text{sample})}{a_i(\text{inner})} \log \frac{a_i(\text{sample})}{a_i(\text{inner})}
\]

(7-23)
potential contributions between the two inner reference electrodes, e.g. liquid junction potentials.

Assuming that the same reference electrodes are used in the two half cells and the electrolyte solutions in two half cells are identical \( (a_{i(\text{sample})} = a_{i(\text{inner})}) \), the emf of the potentiometric cell should be zero.

\[
E_M = \Delta E_0 + s_i \log \frac{a_{i(\text{sample})}}{a_{i(\text{inner})}} = 0
\]  

(7-24)

\[
\Delta E_0 = 0
\]  

(7-25)

Eq. 7-24 and 7-25 mean that the potential contribution from the liquid junctions in the two reference electrodes and the electrolyte solutions can be disregarded. If the molal activity \( a_{i(\text{inner})} \) in the reference half cell is kept constant, the varying molal activity of analyte in the sample half cell can be estimated in a relevant range from the known concentration and the ionic strength of the aqueous reference solutions [143].

\[
a_{i(\text{sample})} = a_{i(\text{inner})} 10^{E_M/s_i}
\]  

(7-26)

In contact with physiological samples, e.g. human plasma or serum, the electrode membrane selectively responds to the specific analyte in the biological matrix. The extent of the sample-induced membrane asymmetry is mainly contributed by the additional Donnan potential arising from the protein content of the specimen [144]. An electrically symmetric potentiometric cell can be set up which is indifferent to short-time contacts (< 20 minutes) with the biological matrix provided that the membrane has the suitable composition and the cell set-up is appropriately modified. In this case, a reliable calibration is possible and based on Eq. 7-26 it is possible to estimate \( a_{i(\text{sample})} \) directly from the \( E_M \) provided the slope and \( a_{i(\text{inner})} \) are known [145].

To study the behaviour of electrodes in physiological samples, the Mg\(^{2+}\)-selective membrane containing ionophore ETH 5506 was characterized in a symmetric flow-through cell. The inner reference solution was a mixture of the most important electrolytes in the physiological range \( (c_{\text{Na}^+} = 140 \text{ mM}, c_{\text{K}^+} = 4.0 \text{ mM}, c_{\text{Ca}^{2+}} = 1.3 \text{ mM}, \)
For checking the membrane boundary potential bias in measuring the physiological sample, a biologically buffered sample of *Eurotrol* (ECW-0444) was measured with the system. The selectivity variations caused by the biological matrix were measured. The response curve is shown in Figure 7.6.

![Response curve](image)

**Figure 7.6.** The influence of the physiological sample (ECW-0444) on the response of the membrane in a symmetric flow through cell.

Selectivity coefficients (VIM) were measured in the two solutions (varying $c_{\text{Ca}^{2+}}$ within the physiological range) in a physiological background:

- **Solution 1**
  - $c_{\text{Ca}^{2+}}=1.3$ mM, $c_{\text{Na}^{+}}=140$ mM, $c_{\text{K}^{+}}=4.0$ mM, $c_{\text{Mg}^{2+}}=0.4$ mM,
  - *Eurotrol* $c_{\text{Ca}^{2+}}=2.5$ mM, other electrolyte in physiological range, biologically buffered.

- **Solution 2**
  - $c_{\text{Ca}^{2+}}=1.0$ mM, $c_{\text{Na}^{+}}=140$ mM, $c_{\text{K}^{+}}=4.0$ mM, $c_{\text{Mg}^{2+}}=0.4$ mM,
  - ECW-0444 $c_{\text{Ca}^{2+}}=2.5$ mM, other electrolyte in physiological range, biologically buffered.

After the contact with the biologically buffered *Eurotrol* sample (ECW-0444) for 10 minutes, the electrode membrane shows no significant change of its selectivity for Mg$^{2+}$ against Ca$^{2+}$ ($\log K_{\text{Mg,Ca}}^{\text{pot}}$ VIM), which implies that short-time contacts with the biologically buffered sample does not influence the selectivity behaviour of the membrane.

**Table 7.3.** Response of the Mg$^{2+}$-selective membrane in a symmetric flow-through cell

<table>
<thead>
<tr>
<th><em>Eurotrol</em> contact</th>
<th>$\text{Slope}^{(1)}$ (mV/dec) $T=25\pm 1.5^\circ\text{C}$</th>
<th>$\text{Slope}^{(2)}$ (mV/dec) $T=25\pm 1.5^\circ\text{C}$</th>
<th>$\log K_{\text{Mg,Ca}}^{\text{pot}}$ (VIM) $n=4$</th>
<th>$\log K_{\text{Mg,Ca}}^{\text{pot}}$ (SSM) $n=4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>24.55±0.32</td>
<td>29.51±0.09</td>
<td>-0.70±0.05</td>
<td>-1.78±0.07</td>
</tr>
<tr>
<td>After</td>
<td>24.86±0.41</td>
<td>29.64±0.12</td>
<td>-0.72±0.04</td>
<td>-1.82±0.05</td>
</tr>
</tbody>
</table>
The slope of the response function in physiological background solutions is decreased because the sensitivity of the membrane electrode is reduced to some extent by the interfering electrolytes (Ca$^{2+}$, K$^+$ and Na$^+$). Consequently, the poor selectivity of the membrane in a physiological situation is due to the decreased slope and the existence of interfering ions. It is worth pointing out that using an inner electrolyte with a physiological background in a symmetric flow through cell ($c_{Ca^{2+}}$=1.3 mM, $c_{Na^+}$=140 mM, $c_{K^+}$=4.0 mM, $c_{Mg^{2+}}$=0.4 mM) leads to a reduced selectivity compared to using pure Mg$^{2+}$ solution ($c_{Mg^{2+}}$=0.1 M) as the inner solution. (Table 7.1). The reason for this has been explained in Section 7.1.
For physiological measurements with ISEs, reliable repeatability should be achieved. The asymmetric potential induced by the biological matrix can be checked in a symmetric potentiometric cell. Figure 7.7.a and 7.7.b show the repeatability of the electrode membrane in a symmetric cell. In Figure 7.7.a, the sample solution contain electrolytes in the physiological range but no biological matrix and in Figure 7.7.b, the electrode membrane measures the sample solutions with- and without a biological matrix. It can be seen that the biological species in physiological samples do not contribute significantly to the emf drift in a symmetric cell.

![Graph showing repeatability of electrode membrane in a symmetric cell](image)

**Figure 7.7.a.** Repeatability of the electrode membrane in a symmetric flow through cell (without biologically buffered samples)

**Solution 1:** $c_{\text{Na}^+} = 140$ mM, $c_{\text{K}^+} = 4.0$ mM, $c_{\text{Ca}^{2+}} = 1.3$ mM, $c_{\text{Mg}^{2+}} = 0.3$ mM,

**Solution 2:** $c_{\text{Na}^+} = 140$ mM, $c_{\text{K}^+} = 4.0$ mM, $c_{\text{Ca}^{2+}} = 1.3$ mM, $c_{\text{Mg}^{2+}} = 0.4$ mM,

Interval for different samples: 5 minutes.
Figure 7.7.b. Repeatability of an electrode membrane in a symmetric cell with biologically buffered samples (Eurotrol standard solutions)

Conditioning solution: $c_{Na^+}=140$ mM, $c_{K^+}=4.0$ mM, $c_{Ca^{2+}}=1.3$ mM, $c_{Mg^{2+}}=0.4$ mM,

Biologically buffered Eurotrol standard solutions:

SH-H1: $c_{Na^+}=159.6$ mM, $c_{K^+}=0.1$ mM, $c_{Ca^{2+}}=0.1$ mM, $c_{Mg^{2+}}=0.0$ mM

SH-H2: $c_{Na^+}=130.0$ mM, $c_{K^+}=9.0$ mM, $c_{Ca^{2+}}=0.7$ mM, $c_{Mg^{2+}}=0.0$ mM

SH-H3: $c_{Na^+}=145.0$ mM, $c_{K^+}=9.0$ mM, $c_{Ca^{2+}}=2.0$ mM, $c_{Mg^{2+}}=0.0$ mM

SH-H4: $c_{Na^+}=154.7$ mM, $c_{K^+}=3.0$ mM, $c_{Ca^{2+}}=0.8$ mM, $c_{Mg^{2+}}=0.0$ mM

SH-H5: $c_{Na^+}=151.7$ mM, $c_{K^+}=4.5$ mM, $c_{Ca^{2+}}=1.2$ mM, $c_{Mg^{2+}}=0.0$ mM

The interval between different samples was 5 minutes.
8. Silicone interference to Mg\textsuperscript{2+}-ISEs

8.1 Introduction

In clinical analysis of Mg\textsuperscript{2+} concentration with ISEs, the measured results could at times be larger than the total magnesium concentration \[45\]. Further investigation of this interference found that the siliconization of the sample collection tubes, e.g. \textit{Vacutainer}, might be the source of such phenomena. Mg\textsuperscript{2+}-ISEs in “silicone-contact” sample solutions showed more positive emf value and longer response times than in a “silicone-free” sample. Figure 8.1 shows the “silicone interference” of the measured potential of the Mg\textsuperscript{2+}-selective electrodes.

![Figure 8.1. The “silicone interference” to Mg\textsuperscript{2+}-ISEs](image)

“Si-contact”: 0.1 M MgCl\textsubscript{2} solution with 4 hours storage in “\textit{Vacutainer}” tube;

“Si-free”: 0.1 M MgCl\textsubscript{2} solution without contact to “\textit{Vacutainer}” tube.

The measurement was carried out in a flow through system.

We found that the “silicone interference” depended on the type of cation in the sample solution (e.g. Mg\textsuperscript{2+}, Ca\textsuperscript{2+}, K\textsuperscript{+}). The emf shift of the “silicone interference” was larger in a 0.1 M NaCl solution than in both 0.1 M CaCl\textsubscript{2} and MgCl\textsubscript{2} solutions. Consequently, the “silicone-interference” affects the Mg\textsuperscript{2+}-selectivity against different cations (Ca\textsuperscript{2+}, Na\textsuperscript{+}) to different degrees (Table 8.1). Several hypotheses for explaining
such an effect have been proposed [45]. Based on the experiments, an explanation for the "silicone interference" is that apolar silicone substances from the Vacutainer diffused into the sample solution and could be adsorbed at the membrane-to-sample interface, which changed the extraction capability of the membrane. The lipophilic "silicone layer" would have a higher affinity to the less hydrophilic cations (Na⁺<Ca²⁺<Mg²⁺). Hence the residues of the emf shift by "silicone interference" were larger for the solution containing less hydrophilic cations (Table 8.1). However, such interference can be reduced or even excluded by adjusting the matrix of the membrane with polymers of different polarities.

Table 8.1. The "silicone interference" in different solutions (mean ± 2SD, n=6)

<table>
<thead>
<tr>
<th>0.1 M cation solution</th>
<th>Mg²⁺</th>
<th>Ca²⁺</th>
<th>Na⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface charge density</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>zÅ⁻², z=1, or 2</td>
<td>0.26</td>
<td>0.14</td>
<td>0.08</td>
</tr>
<tr>
<td>Δemf (mV)=</td>
<td>7.43±1.05</td>
<td>14.00±1.00</td>
<td>41.00±0.21</td>
</tr>
<tr>
<td>emf_{si-contact}−emf_{si-free}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>si-free</td>
<td>29.86±0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>si-contact</td>
<td>26.91±0.06</td>
<td></td>
</tr>
<tr>
<td>log K_{Mg,Ca}^{free}</td>
<td>si-free</td>
<td>-1.98±0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>si-contact</td>
<td>-1.81±0.03</td>
<td></td>
</tr>
<tr>
<td>log K_{Mg,Na}^{free}</td>
<td>si-free</td>
<td>-3.62±0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>si-contact</td>
<td>-2.57±0.02</td>
<td></td>
</tr>
</tbody>
</table>
8. Silicone interference to Mg\textsuperscript{2+}-ISEs

8.2 The “silicone interference” at the liquid junction of the reference electrode

In order to find the location of the “silicone interference”, the reference electrode was tested to check if the “silicone interference” affects the liquid junction potential (Figure 8.2).

![Figure 8.2. Schematic representation for checking the “silicone interference” at the reference electrode.](image)

Figure 8.2. Schematic representation for checking the “silicone interference” at the reference electrode.

In Figure 8.2, ref. 1 and ref. 2, are two identical calomel reference electrodes where the reference electrolyte is the saturated KCl and the bridge electrolyte is 3 M KCl. First, the emf difference ($\Delta E_1$) between ref. 1 and ref. 2 is measured in the “silicone-free” 0.1 M MgCl\textsubscript{2} solution. Then only ref. 1 is dipped in the “silicone-contact” 0.1 M MgCl\textsubscript{2} solution for about 4 hours. The emf difference ($\Delta E_2$) between ref. 1 and ref. 2 is measured again in the “silicone-free” solution. $\Delta E_1$ and $\Delta E_2$ gave identical values, which implies that the “silicone interference” does not arise at the liquid junction of the reference electrode but at the membrane surface.

Several polymers with high polarities were considered for use as membrane matrices so that apolar silicone in the sample cannot be easily adsorbed onto the membrane surface. If a relatively apolar substance like silicone oil is added to the membrane during the membrane preparation, the membrane exhibits high apolarity so that it might be possible that the sample-induced “silicone interference” could be disregarded.
8. Silicone interference to Mg$^{2+}$-ISEs

\[
\left(\begin{array}{c}
\text{Si-O} \\
\text{Si-O} \\
\text{Si-O} \\
\text{Si-O} \\
\end{array}\right)_{n}
\]

silicone oil

\[
\left(\begin{array}{c}
\text{Cl} \\
\text{Cl} \\
\end{array}\right)_{n}
\]

PVC (poly(vinyl chloride))

\[
\left(\begin{array}{c}
\text{Cl} \\
\text{OH} \\
\text{Cl} \\
\text{O} \\
\text{Cl} \\
\end{array}\right)_{n}
\]

PVC-OH (ETH 3528, copolymer of hydroxylated vinyl chloride/acetate vinyl chloride, OH-group 17.49 wt%)

\[
\left(\begin{array}{c}
\text{O(CH}_2\text{)}_4\text{O} \\
\text{N} \\
\text{N} \\
\text{O} \\
\end{array}\right)_{n}
\]

Tecoflex (polyurethane)

\[
\text{HO-Si-O-(Si-O-Si)}_{n}\text{OH}
\]

Silicone rubber

Figure 8.3. Alternative substances as polymer matrices
8. Silicone interference to Mg$^{2+}$-ISEs

8.3 Alternative polymeric membranes

8.3.1 Mixture of PVC and Hydroxylated PVC as the membrane matrix
The hydroxylated PVC in the experiment is ETH 3528 (co-polymer of hydroxylated vinyl chloride / acetate vinyl chloride, OH-group: 17.49 wt%). With the higher content of PVC-OH, the membrane was more fragile, the appearance less homogenous and the response time was longer. A membrane with a suitable ratio of PVC and PVC-OH (w$_{\text{PVC}}$:w$_{\text{PVC-OH}}$=30:1) showed an adequate response (slope=29.63 mV/decade, selectivity coefficient (SSM): log $K_{\text{Mg,Ca}}^{\text{pot}}$ = -1.8, log $K_{\text{Mg,K}}^{\text{pot}}$ = -3.1, log $K_{\text{Mg,Na}}^{\text{pot}}$ = -3.4). But unfortunately, the Δemf induced from the “silicone contact” sample was still several mVs (Table 8.3).

8.3.2 Addition of silicone oil
Using silicone oil as an apolar additive in the membrane, the addition of the suitable amount of silicone oil (w$_{\text{PVC}}$:w$_{\text{silicone oil}}$ = 4:1) to the membrane resulted in a homogenous PVC membrane and an adequate response (slope=30.1 mV/decade, log $K_{\text{Mg,Na}}^{\text{pot}}$ = -2.0, log $K_{\text{Mg,Na}}^{\text{pot}}$ = -3.2, log $K_{\text{Mg,Na}}^{\text{pot}}$ = -4.8). This electrode membrane showed a reduced “silicone-interference”.

8.3.3 Mixed membrane matrix of PVC and Polyurethane
The aliphatic polyurethane (Tecoflex) is known as the bio-compatible polymer that has sufficient apolarity to reduce the adsorption of the biological species in plasma, serum or whole blood samples [146-149]. The membrane using the mixture of PVC and polyurethane as matrix is expected to reduce the “silicone-interference”. It can be seen from Table 8.2 and Figure 8.4 that the membranes containing high amount of polyurethane are quite indifferent to the “silicone interference” in respect to the emf shift. However, the addition of polyurethane causes a loss of selectivity. A compromised composition of the membrane (MT3 in Table 8.2) is prepared with 10:1 weight ratio of PVC and polyurethane (w$_{\text{PVC}}$:w$_{\text{polyurethane}}$=10:1), together with the proper quantity of silicone oil. This membrane composition exhibits sufficient response of sensitivity and selectivity. The emf drift by the “silicone interference” is significantly reduced to 1.98 mV (Table 8.2 and Figure 8.4).
8. Silicone interference to Mg\(^{2+}\)-ISEs

Table 8.2. Selectivity of the membranes with different amount of polyurethane. Total amount of PVC and polyurethane is ~55 mg. The ionophore in use is ETH 5506 and the measurements are carried out in flow through cell with the inner electrolyte solution of 0.1 M MgCl\(_2\).

<table>
<thead>
<tr>
<th>Membrane</th>
<th>MT1</th>
<th>MT2</th>
<th>MT3</th>
</tr>
</thead>
<tbody>
<tr>
<td>W(<em>{\text{Tecoflex}}):W(</em>{\text{PVC}})</td>
<td>1:1</td>
<td>1:5</td>
<td>1:10</td>
</tr>
<tr>
<td>slope (mV/dec)</td>
<td>26.20</td>
<td>28.56</td>
<td>29.26</td>
</tr>
<tr>
<td>log (K_{\text{Mg,Ca}}^{\text{pot}})</td>
<td>-0.76</td>
<td>-1.24</td>
<td>-1.81</td>
</tr>
<tr>
<td>log (K_{\text{Mg,K}}^{\text{pot}})</td>
<td>-1.62</td>
<td>-2.02</td>
<td>-2.72</td>
</tr>
<tr>
<td>log (K_{\text{Mg,Na}}^{\text{pot}})</td>
<td>-1.88</td>
<td>-2.72</td>
<td>-3.92</td>
</tr>
<tr>
<td>(\Delta \text{emf (mV)}) (=\text{emf}<em>{\text{ctntr}}-\text{emf}</em>{\text{rsw}})</td>
<td>0.58</td>
<td>1.06</td>
<td>1.98</td>
</tr>
</tbody>
</table>

Figure 8.4. The "silicone-interference" of the membranes with different amounts of polyurethane. Solution 1 is a physiological like solution: \(c_{\text{NaCl}}\): 140 mM; \(c_{\text{KCl}}\): 4.0 mM; \(c_{\text{CaCl}_2}\): 1.3 mM and \(c_{\text{MgCl}_2}\): 0.4 mM "Solution 1*" is identical to "Solution 1" but pre-treated in "Vacutainer". MT1, MT2 and MT3 are the membranes listed in Table 8.2.
8.4 Conclusion

The "silicone interference" on the Mg\textsuperscript{2+}-ISEs in clinical analysis is critical for obtaining reliable results. Based on the assumption that a thin apolar silicone layer is adsorbed at the membrane-sample interface, alternative polymers were added to the membrane matrix. With the mixed matrix of PVC and polyurethane and the addition of silicone oil to the membrane, the electrode exhibits an adequate response with regard to the sensitivity (slope=29.26 mV/decade) and selectivity (log $K_{\text{Mg, Ca}}^{\text{pot}} = -1.81$, log $K_{\text{Mg, K}}^{\text{pot}} = -2.72$, log $K_{\text{Mg, Na}}^{\text{pot}} = -3.92$). The emf shift by the "silicone interference" can also be significantly reduced (Table 8.2).

Many other polymers were also investigated to achieve the goal of excluding the "silicone-interference". They were PVDC (polyvinylidene chloride), polystyrene, cellulose triacetate, and silicone rubber [150-153]. Unfortunately, these polymers did not meet the requirements for the clinical measurement of Mg\textsuperscript{2+}-ISEs. Until now, the only electrode membrane composition that could reduce this interference was the mixed matrix of PVC and polyurethane with the addition of silicone oil. This significantly reduced the interference.

Table 8.3. Behaviour of membranes with an alternative matrix

<table>
<thead>
<tr>
<th>Matrix (weight ratio)</th>
<th>PVC: PVC-OH</th>
<th>PVC: Si-oil</th>
<th>PVC: Tecoflex: Si-oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVC</td>
<td>30:1</td>
<td>3:1</td>
<td>10:1:2.5</td>
</tr>
<tr>
<td>Slope mV/decade</td>
<td>29.86±0.08</td>
<td>29.63±0.82</td>
<td>30.1±0.45</td>
</tr>
<tr>
<td>log $K_{\text{Mg, Ca}}^{\text{pot}}$</td>
<td>-1.98</td>
<td>-1.80</td>
<td>-1.95</td>
</tr>
<tr>
<td>log $K_{\text{Mg, K}}^{\text{pot}}$</td>
<td>-3.21</td>
<td>-3.08</td>
<td>-3.24</td>
</tr>
<tr>
<td>log $K_{\text{Mg, Na}}^{\text{pot}}$</td>
<td>-3.62</td>
<td>-3.42</td>
<td>-3.80</td>
</tr>
<tr>
<td>$(\Delta\text{emf}^*)$ mV</td>
<td>+3.09</td>
<td>+2.73</td>
<td>+2.01</td>
</tr>
</tbody>
</table>

$(\Delta\text{emf}^*) = \text{emf}_{\text{Si-conatcted}} - \text{emf}_{\text{Si-free}}$

The physiological background solution used for checking the "silicone interference" was:

- $c_{\text{NaCl}}$: 140 mM
- $c_{\text{KCl}}$: 4.0 mM
- $c_{\text{CaCl}_2}$: 1.3 mM
- $c_{\text{MgCl}_2}$: 0.4 mM
9. Experimental

9.1 Reagents

For these experiments, solutions were made with the bidistilled water and all salts were bought from Aldrich Chemie (Buchs, Switzerland), Fluka Chemie AG (Buchs, Switzerland), E. Merck AG (Darmstadt, Germany). The substances that were used in membrane preparations were either obtained from Fluka Chemie AG (Buchs, Switzerland) or were synthesized in the research group (cf. Table 9.1)

Table 9.1. The relevant chemicals

<table>
<thead>
<tr>
<th>Substance</th>
<th>mol. wt.</th>
<th>Product Quality</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETH(^T) 2001</td>
<td>C(<em>{25})H(</em>{38})N(_2)O(_2)</td>
<td>398.59</td>
<td>CCS</td>
</tr>
<tr>
<td>ETH(^T) 2002</td>
<td>C(<em>{22})H(</em>{34})N(_2)O(_2)</td>
<td>370.53</td>
<td>CCS</td>
</tr>
<tr>
<td>ETH(^T) 2003</td>
<td>C(<em>{26})H(</em>{38})N(_2)O(_2)</td>
<td>384.56</td>
<td>CCS</td>
</tr>
<tr>
<td>ETH 7025</td>
<td>C(<em>{40})H(</em>{66})N(_6)O(_6)</td>
<td>863.29</td>
<td>CCS</td>
</tr>
<tr>
<td>ETH 3832</td>
<td>C(<em>{54})H(</em>{60})N(_6)O(_6)</td>
<td>925.39</td>
<td>CCS</td>
</tr>
<tr>
<td>ETH(^T) 5504</td>
<td>C(<em>{60})H(</em>{60})N(_6)O(_6)</td>
<td>991.41</td>
<td>CCS</td>
</tr>
<tr>
<td>ETH 5506</td>
<td>C(<em>{63})H(</em>{68})N(_6)O(_6)</td>
<td>1033.49</td>
<td>CCS</td>
</tr>
<tr>
<td>ETH(^T) 2022</td>
<td>C(<em>{40})H(</em>{66})N(_6)O(_6)</td>
<td>728.97</td>
<td>CCS</td>
</tr>
<tr>
<td>K22B5</td>
<td>C(<em>{38})H(</em>{62})N(_2)O(_4)</td>
<td>700.91</td>
<td>CCS</td>
</tr>
<tr>
<td>ETH 8045</td>
<td>C(<em>{20})H(</em>{37})NO(_3)</td>
<td>411.58</td>
<td>CCS</td>
</tr>
<tr>
<td>o-NPOE</td>
<td>C(<em>{14})H(</em>{21})NO(_3)</td>
<td>215.33</td>
<td>Selectophore(\text{®})</td>
</tr>
<tr>
<td>DOS</td>
<td>C(<em>{26})H(</em>{38})O(_4)</td>
<td>476.69</td>
<td>Selectophore(\text{®})</td>
</tr>
<tr>
<td>TEHP</td>
<td>C(<em>{24})H(</em>{37})O(_4)P</td>
<td>744.04</td>
<td>Selectophore(\text{®})</td>
</tr>
<tr>
<td>Chloroparaffin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KT(_2)CIPB</td>
<td>C(<em>{54})H(</em>{10})BCL(_{3})K</td>
<td>496.12</td>
<td>Selectophore(\text{®})</td>
</tr>
<tr>
<td>PVC</td>
<td></td>
<td>&gt;50 000</td>
<td>Selectophore(\text{®})</td>
</tr>
<tr>
<td>PVC-OH</td>
<td></td>
<td></td>
<td>CCS</td>
</tr>
<tr>
<td>Polyurethane</td>
<td>(Tecoflex)</td>
<td></td>
<td>Thermedics</td>
</tr>
<tr>
<td>Silicone oil</td>
<td></td>
<td></td>
<td>Selectophore(\text{®})</td>
</tr>
<tr>
<td>Siloprene K1000</td>
<td></td>
<td></td>
<td>Selectophore(\text{®})</td>
</tr>
<tr>
<td>Siloprene Crosslinking Agent K-11</td>
<td></td>
<td></td>
<td>Selectoplane(\text{®})</td>
</tr>
<tr>
<td>Polystyrene</td>
<td></td>
<td></td>
<td>Selectophore(\text{®})</td>
</tr>
<tr>
<td>MgCl(_2).6H(_2)O</td>
<td></td>
<td>Analytically pure</td>
<td>Fluka</td>
</tr>
<tr>
<td>CaCl(_2).2H(_2)O</td>
<td></td>
<td>Analytically pure</td>
<td>Fluka</td>
</tr>
<tr>
<td>KCl</td>
<td></td>
<td>Analytically pure</td>
<td>Fluka</td>
</tr>
<tr>
<td>NaCl</td>
<td></td>
<td>Analytically pure</td>
<td>Fluka</td>
</tr>
<tr>
<td>HCl</td>
<td></td>
<td>Analytically pure</td>
<td>Fluka</td>
</tr>
<tr>
<td>LiCl</td>
<td></td>
<td>Analytically pure</td>
<td>Fluka</td>
</tr>
<tr>
<td>THF</td>
<td></td>
<td>Analytically pure</td>
<td>Fluka</td>
</tr>
</tbody>
</table>
9. Experimental

The "ETH" denotes the "ETH, Technopark"; The "CCS" means the Centre for Chemical Sensors/Biosensors and bioAnalytical Chemistry ETH.

- Standards for the thin-layer chromatography (C₁-C₈) were obtained from Ciba-Geigy AG, Switzerland.

- The Eurotrol quality-control standard solutions were obtained from Eurotrol B.V. Netherlands. The biologically buffered Eurotrol standard solutions were used for checking the influence of the biological matrix in the sample on the electrode response (Table 9.2).

Table 9.2. Eurotrol standard solutions (M)

<table>
<thead>
<tr>
<th>Eurotrol-I</th>
<th>ECW-0444</th>
<th>C_{Mg²⁺}</th>
<th>C_{Ca²⁺}</th>
<th>C_{Na⁺}</th>
<th>C_{K⁺}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.00250</td>
<td>unknown</td>
<td>Bio-buffered</td>
<td></td>
</tr>
</tbody>
</table>

| Eurotrol-II | SH-H1     | 0.00010  | 0.15960  | 0.0001  | No Mg²⁺ |
|             | SH-H2     | 0.00700  | 0.13000  | 0.0090  | Bio-buffered |
|             | SH-H3     | 0.00200  | 0.14500  | 0.0090  |         |
|             | SH-H4     | 0.00075  | 0.15475  | 0.0030  |         |
|             | SH-H5     | 0.00125  | 0.15175  | 0.0045  |         |

The detail compositions of Eurotrol standards are not available.
9. Experimental

9.2 Emf measurements

9.2.1 Potentiometer

The measurements were performed at ambient temperature. The 8-channel electrode monitor was equipped with FET operational amplifiers AD515 KH (input impedance 1013Ω/2pF; bias current<150 pA; Capacity neutralisation; Analog Devices, Norwood, MA), an active low pass filter in each channel for noise rejection and a latchable CMOS Multiplexer DG 529 (Siliconix GmbH, D-7024 Filderstadt 1 FRG) controlled by an Apple IIe Personal Computer (Apple Computer, Cupertino CA). A Solartron-Schumberger 7150 Digital Multimeter (resolution: 1mV; full scale ± 2V; Solartron Instrumentation Group, Farnborough, Hampshire, England; a division of Schlumberger Electronics Ltd, UK) was used with remote control through an IEEE 488 interface by the Apple IIe, programmed in Apple UCSD Pascal.

9.2.2 Membrane preparations

The PVC liquid membranes used in this work were composed of 1 wt% ionophore, 33 wt% poly(vinyl chloride) (PVC, high molecular weight; Fluka Selectophore®) and 64-65 wt% plasticizer. The lipophilic anionic site, potassium tetrakis (4-chlorophenyl) borate (KTpcClPB) was added with the varying molar ratios relative to the ionophore. The different membrane components whose total weight was 180 mg were dissolved in 2 ml of freshly distilled tetrahydrofuran (THF) and shaken at room temperature to obtain a homogeneous solution. The solution was then transferred to a glass o-ring of 24 mm diameter which was fixed onto a horizontal glass plate. After the slow evaporation of the THF solvent at room temperature over approximately 12 hours, a membrane of between 100~150 μm thickness was obtained.

9.2.3 Electrode preparation

From the membrane prepared as above, a membrane disc (ϕ=7mm) was cut out and fitted into a polyamide Philips IS-561 electrode bodies containing the Ag/AgCl inner reference electrodes. A solution of fixed concentration (usually 0.1 M MgCl₂) was used as the inner electrolyte. Before the measurements, the electrodes were conditioned in a solution of the same concentration as the inner solution (usually 0.1 M MgCl₂) for 24 hours. A free-flow free-diffusion calomel electrode containing a
9. Experimental

A bridge electrolyte of 3 M KCl was used as the reference electrode during the emf measurements. The emf measurements were carried out at ambient temperature with the following cell:

\[
\text{Hg, Hg}_2\text{Cl}_2 | \text{KCl (satd.)} \rightleftharpoons \text{KCl (3M)} \rightleftharpoons \text{sample solution} | \text{membrane} | \text{inner solution} | \text{AgCl,Ag.}
\]

The measured emf values were corrected with regard to the liquid junction potential according to the Henderson equation (cf. section 4.3.1). Single-ion activities were calculated using the Pitzer program [154]. Aqueous MgCl₂ solutions (concentrations ranged from 1 μM to 0.1M) were used to calibrate electrode. The emf values were read out until the potential drift was less than 0.5 mV/h. One single data acquisition could last between 10-30 min for concentrations greater than 0.1mM and 20-60 min for concentrations less than this. For comparing the selectivity of the ionophores, selectivity coefficients were measured using the separate solution method in 0.1M metal chloride solution (SSM, cf. section 3.3). To reduce the “memory effects” of the electrode membrane, the following solution sequence was normally chosen: MgCl₂ > CaCl₂ > NaCl > KCl > HCl. With each change of solution, the electrodes were rinsed with distilled water and with the new solution.
9. Experimental

9.3 Flow through system

The measurements in a flow-through system were carried out in stop-flow mode and the flow through cell is shown in Figure 9.1.

![Figure 9.1. Potentiometric flow-through cell](image)

The diameter of the flow through tunnel was 1 mm and the slot for the membrane-contacting sample was 4 mm long. During the measurement, the sample solution was delivered by a peristaltic pump at a speed of 1.5 mL min\(^{-1}\) till the front of the sample solution just passed the tip of the reference electrode in the cell and then the measurements were carried out in stop-mode. The emf values were read out until the potential drifts were less than 0.5 mV/hour. Between each change of solution, the flow-through cell was rinsed with the bidistilled water.
9. Experimental

9.4 Lipophilicity measurements

The lipophilicity (logP) of each ionophore was determined with the method reported by O. Dinten et al [155]. By means of lipophilicity, the partition coefficient $k$ of a compound between the membrane and the aqueous solution can be estimated. Knowing the lipophilicity, it is possible to calculate the loss of membrane component with time and to estimate the lifetime of a membrane. The more lipophilic the membrane components, the less the leaching from the membrane. Hence such membranes have longer lifetimes [156]. However, there is an upper limit to the lipophilicity of the membrane because as the lipophilicity increases, the mobility of ions in the membrane is decreased [84].

Reversed-phase thin-layer chromatography has been suggested as a suitable method for the experimental determination of lipophilicity values (log$P_{\text{TLC}}$) [155,157]. With this technique, lipophilicity value in the range of log$P_{\text{TLC}}$>8 can be obtained by comparing the extrapolated value for the 1-octanol/water (log$P_{\text{oct}}$) extraction system. In the thin layer chromatography (TLC), the retention of a component was characterized by the $R_f$ value, which was defined as the ratio of the moving distance of the component to the moving distance of the solvent front. The $P_{\text{TLC}}$ values were calculated from the results of the reversed-phase chromatography from the following relationship [155]:

$$P_{\text{TLC}} = \frac{V_m}{V_s} \left( \frac{1}{R_f} - 1 \right)$$  \hspace{1cm} (9-1)

where $V_m$ was the volume of the mobile phase and $V_s$ was a volume representative of the stationary phase. Bathe-Smith and Westall [158] defined $R_m$ as:

$$R_m = \log \left( \frac{1}{R_f} - 1 \right)$$  \hspace{1cm} (9-2)

and

$$\log P_{\text{TLC}} = R_m + \log r \text{ with } r = \frac{V_m}{V_s}$$  \hspace{1cm} (9-3)
In a calibration procedure, $R_m$ values for calibration standards were calculated from the experimental $R$, by using Eq. 9-2 and were correlated by linear regression with the assigned values of $\log P_{oct}$. From Eq. 9-3, $\log r$ can be from the intercept. $R$, values were experimentally determined, and the $R_m$ values were calculated by using Eq. 9-2. The $\log P_{TLC}$ was estimated by using the linear regression equation evaluated by the calibration standards. By this procedure, the $\log P_{TLC}$ of the neutral ionophores and plasticizers can be well identified with the basic quantities, $\log P_{oct}$.

In initial experiment, the $R_f$ values of the standards were measured and $R_m$ values were then calculated from Eq. 9-2. A calibration curve of $R_m$ against $\log P_{TLC}$ of the standards was obtained from linear regression analysis. From this calibration curve, the $\log P_{TLC}$ of the sample could be obtained.

The standard calibrators were given by Ellgehausen [159]: pesticides $C_1$-$C_8$ (Ciba-Geigy AG, Basel, Switzerland); $C_1$ (CGA 10832) $\log P_{TLC}$=6.34; $C_2$ (GS 19851) $\log P_{TLC}$=5.10; $C_3$ (GS 23992) $\log P_{TLC}$=4.73; $C_4$ (GS14260) $\log P_{TLC}$=3.70; $C_5$ (GS13529) $\log P_{TLC}$=3.46; $C_6$ (Duron) $\log P_{TLC}$=2.89; $C_7$ (GS29696) $\log P_{TLC}$=1.82; $C_8$ (Fenuron) $\log P_{TLC}$=0.88. Ten milligrams of the compounds was dissolved in 1 mL of methylene chloride (Fluka Chemie AG, Switzerland).

Solutions of neutral ionophores and plasticizers were investigated in concentrations of 10 mg/mL of ethanol (Fluka Chemie AG, Switzerland).

Mobile phase was an ethanol-water mixture with 7:3 ratio. Stationary phase was RP-C18-silica gel (Merck).

Three of eight calibration standards with different lipophilicities were applied on the thin silica gel layer. 8-12 ml samples were applied in the same way. The spots of the compounds were detected by ultraviolet spectroscopy (UV) at 254 nm and developed in the iodine vapour.
9. Experimental

9.5 Permittivity (dielectric constant) measurements

The permittivity (dielectric constant) \( \varepsilon \) of plasticizer, influences the membrane's selectivity, as was described in Eq. 4-43. The value of \( \varepsilon \) can be derived from the impedance measurement of the membrane compounds. Figure 9.2 shows the set-up and parallel circuit of the impedance measurement cell. The Faradiac impedance \( Z \) of the sample is composed of a real part \( Z' \) and an imaginary part \( Z'' \):

\[
Z = Z' + iZ'' = \frac{1}{G + iB}
\]  

(9-4)

where

\( G = 1/Z' \) conductance of the circuit

\( B = 1/Z'' \) susceptance of the circuit

\( G + iB \) admittance of the circuit

\( G \) and \( B \) can be measured by the experiments.

![Diagram of the set-up and circuit](image)

Figure 9.2 a) Set-up of the permittivity measurement; b) the circuit for the permittivity measurement. \( I_1 \) is the current via the capacitor "\( C \)"; \( I_2 \) is the current via the resistor "\( R \)".

The dielectric constant \( \varepsilon \) of the sample can also be described by:
\[ \varepsilon = \varepsilon' + i\varepsilon'' \]
\[ = \frac{b}{\omega} + ig / \omega \]
\[ = \frac{B\alpha}{\omega} + iG\alpha / \omega \]

where

\[ \varepsilon' = B\alpha \quad \text{real part of permittivity (dielectric constant)} \]
\[ \varepsilon'' = G\alpha \quad \text{imaginary part of permittivity (dielectric constant)} \]
\[ \alpha = \frac{d}{A} \quad \text{geometric factor which can be obtained from the standard reference, eg. propylen carbonate (\varepsilon = 72.2).} \]
\[ d \text{ and } A \text{ are the thickness and the area of the measuring cell.} \]
\[ \omega = 2\pi f \quad \text{frequency of alternative current.} \]

The impedance was measured by a Hewlett Packard 4192A LF Impedance Analyzer. The scanning frequency ranged from 51Hz to 13 MHz. All measurements included complex impedance and dielectric constants at a temperature of 20°C. Between two measurements, the cell was rinsed with 70% ethanol.

The instrument used for ac-impedance analysis in Chapter 6 was the AUTOLAB Frequency Response Analyzer (Eco Chemie B.V. Utrecht, Netherlands). The ac-impedance spectrum of the electrode membrane was measured in a symmetric electrochemical cell arrangement (Figure 6.3). The area of the membrane under study in the measuring cell exposed to the electrolyte was 0.78 cm² (one side). The membrane was placed in the corresponding chloride-containing solution on both sides. Because the Ag/AgCl electrodes have a comparably low resistance and high capacitance, they did not interfere with the impedance measurement of the membrane. The potential bias between the two Ag/AgCl electrodes did not exceed 0.5 mV.

9.6 Synthesis

The Mg²⁺-selective ionophores, ETH 5506, ETH⁺ 5504, ETH⁺ 2001 ETH⁺ 2002, ETH⁺ 2003 ETH⁺ 2022 and K22B5 [36] as well as the plasticizer ETH 8045 and hydroxylated PVC were synthesized in our laboratory [1,56,81].
10. Appendix

10.1 Mg$^{2+}$-selective ionophores

- **ETH 5506**: $X=\text{CH}_2$, $n=1$, $R=1$-adamantyl
- **ETH 5504**: $X=\text{CH}_2$, $n=0$, $R=1$-adamantyl
- **ETH 3832**: $X=\text{CH}_2$, $n=1$, $R=n$-heptyl

- **ETH$^T$ 2001**: $R_1=\text{I}$; $R_2=\text{CH}_3$; $R_3=\text{CH}_3$
- **ETH$^T$ 2002**: $R_1=\text{I}$; $R_2=\text{H}$; $R_3=\text{H}$
- **ETH$^T$ 2003**: $R_1=\text{I}$; $R_2=\text{H}$; $R_3=\text{CH}_3$

- **K22B5**: $R_1=\text{I}$; $R_2=\text{H}$; $R_3=\text{H}$
- **ETH$^T$ 2022**: $R_1=\text{I}$; $R_2=\text{CH}_3$; $R_3=\text{CH}_3$
### 10.2 Membrane components

#### Plasticizers

<table>
<thead>
<tr>
<th>No.</th>
<th>Code</th>
<th>Structure</th>
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</thead>
<tbody>
<tr>
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<td>0-NPOE</td>
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<tr>
<td>2</td>
<td>ETH 8045</td>
<td><img src="image" alt="ETH 8045" /></td>
</tr>
<tr>
<td>3</td>
<td>ETH 5373</td>
<td><img src="image" alt="ETH 5373" /></td>
</tr>
<tr>
<td>4</td>
<td>ETH 4314</td>
<td><img src="image" alt="ETH 4314" /></td>
</tr>
<tr>
<td>5</td>
<td>DOS</td>
<td><img src="image" alt="DOS" /></td>
</tr>
<tr>
<td>6</td>
<td>ETH 2041</td>
<td><img src="image" alt="ETH 2041" /></td>
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#### Polymers

<table>
<thead>
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</thead>
<tbody>
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<td>1</td>
<td>PVC</td>
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</tr>
<tr>
<td>2</td>
<td>PVC-OH</td>
<td><img src="image" alt="PVC-OH" /></td>
</tr>
<tr>
<td>3</td>
<td>Polyurethane</td>
<td><img src="image" alt="Polyurethane" /></td>
</tr>
<tr>
<td>4</td>
<td>Silicone rubber</td>
<td><img src="image" alt="Silicone rubber" /></td>
</tr>
</tbody>
</table>

#### Borate

<table>
<thead>
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<th>No.</th>
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<th>Structure</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>KTpClPB</td>
<td><img src="image" alt="KTpClPB" /></td>
</tr>
</tbody>
</table>
## 10.3 Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>CCS</td>
<td>Centre for Chemical Sensor/Biosensors and bioAnalytical Chemistry, Swiss Federal Institute of Technology.</td>
</tr>
<tr>
<td>ISE</td>
<td>Ion-Selective Electrode</td>
</tr>
<tr>
<td>ISFET</td>
<td>Ion-Selective Field Effect Transistor</td>
</tr>
<tr>
<td>SSM</td>
<td>Separate Solution Method</td>
</tr>
<tr>
<td>FIM</td>
<td>Fix Interfering Method</td>
</tr>
<tr>
<td>MPM</td>
<td>Matched Potential Method</td>
</tr>
<tr>
<td>VIM</td>
<td>Varying Interference Method</td>
</tr>
<tr>
<td>SAM</td>
<td>Specific Application Method</td>
</tr>
<tr>
<td>EMF</td>
<td>Electromotive Force</td>
</tr>
<tr>
<td>ETH 8045</td>
<td>[12-(4-ethylphenyl)dodecyl]-2-nitrophenyl ether</td>
</tr>
<tr>
<td>ETH 7025</td>
<td>N,N',N''-Tris[3-(heptylmethylamino)-3-oxopropionyl]8,8'-iminodioctylamine</td>
</tr>
<tr>
<td>ETH 5506</td>
<td>1,3,5-tris[10-(1-adamantyl)-7,9-dioxo-6,10-diazaundecyl]benzene</td>
</tr>
<tr>
<td>ETH T 5504</td>
<td>1,3,5-tris[9-(1-adamantyl)-6,8-dioxo-5,9-diazadecyl]benzene</td>
</tr>
<tr>
<td>ETH 4314</td>
<td>2-nitrophenyl-2-phenyldodecyl ether</td>
</tr>
<tr>
<td>ETH 3832</td>
<td>1,3,5-tris(10-methyl-7,9-dioxo-6,10-diazahexadecyl)benzene</td>
</tr>
<tr>
<td>ETH 3528</td>
<td>copolymer hydroxylated vinyl chloride/acetate vinyl chloride, OII- group 17 wt%</td>
</tr>
<tr>
<td>ETH T 2022</td>
<td>4,13-[bis(N-methyl-N-adamantylcarbamoyl) acetyl]-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane</td>
</tr>
<tr>
<td>ETH T 2001</td>
<td>N,N'-dimethyl-N,N'-diadamantylmalonamide</td>
</tr>
<tr>
<td>ETH T 2002</td>
<td>N,N'-diadamantylmalonamide</td>
</tr>
<tr>
<td>ETH T 2003</td>
<td>N-methyl-N,N'-diadamantylmalonamide</td>
</tr>
<tr>
<td>K22B5</td>
<td>4,13-[bis(N-adamantylearbamoyl) acetyl]-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane</td>
</tr>
<tr>
<td>o-NPOE</td>
<td>2-nitrophenyl octyl ether</td>
</tr>
<tr>
<td>DOS</td>
<td>Bis(2-ethylhexyl)sebacate</td>
</tr>
<tr>
<td>TEHP</td>
<td>Tris(2-ethylhexyl)phosphate</td>
</tr>
</tbody>
</table>
10.4 Biological setting points for total and ionized molalities of electrolytes [160-163]

<table>
<thead>
<tr>
<th>Primary ion</th>
<th>Reference interval, adults, plasma total ion concentration (mmol L(^{-1}) plasma)</th>
<th>Mean setting point of active molality (mmol kg(^{-1}) H(_2)O)</th>
<th>Activity coefficient (\gamma_k) for I=0.1609 mol kg(^{-1}), 37°C Debye-Hückel; Pitzer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Na(^+)</td>
<td>134-143</td>
<td>105</td>
<td>0.742 0.743</td>
</tr>
<tr>
<td>Potassium K(^+)</td>
<td>3.3-4.8</td>
<td>3.0</td>
<td>0.728 0.7263</td>
</tr>
<tr>
<td>Calcium Ca(^{2+})</td>
<td>2.03-2.57</td>
<td>1.00-2.16</td>
<td>0.325 0.3417</td>
</tr>
<tr>
<td>Magnesium Mg(^{2+})</td>
<td>0.66-0.95</td>
<td>0.46-0.665</td>
<td>0.336 0.3507</td>
</tr>
<tr>
<td>Hydrogen activity H(_3)O(^+) (pH)</td>
<td>pH 7.35-7.45</td>
<td>10(^{-7.50})</td>
<td>0.770</td>
</tr>
<tr>
<td>Chloride Cl(^-)</td>
<td>99-111</td>
<td>83.9</td>
<td>0.741 0.7446</td>
</tr>
</tbody>
</table>
10. Appendix

10.5 Required selectivity coefficients (SSM, SSM\text{cons})

Required selectivity coefficients for the detection of the main inorganic cations in physiological solutions, assuming an allowable error of 1.0% at the lower level of the physiological range of the detecting ion. Measurements were carried out under two conditions. Firstly, using the lower level of the physiological range of the primary ion and the upper level of the physiological range of the interfering ion (Eq. 4-48). Secondly, using the lower level of physiological range of primary ion but variation of the interfering ion around its mean value (Eq. 4-59).

\[
\begin{align*}
\alpha_{\text{Mg}^{2+}} & : 0.19 \text{ mM} \\
\alpha_{\text{Ca}^{2+}} & : 0.38 \text{ mM} \\
\alpha_{\text{K}^+} & : 2.95 \text{ mM} \\
\alpha_{\text{Na}^+} & : 102.91 \text{ mM}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Primary</th>
<th>Conventional SSM $\log(K_{ij}^{\text{no calab}})$</th>
<th>Consistent SSM $\log(K_{ij}^{\text{no calab}})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>\text{Mg}^{2+}</td>
<td>\text{Ca}^{2+}</td>
<td>-2.43</td>
</tr>
<tr>
<td></td>
<td>\text{K}^+</td>
<td>-0.88</td>
</tr>
<tr>
<td></td>
<td>\text{Na}^+</td>
<td>-3.84</td>
</tr>
<tr>
<td>\text{Ca}^{2+}</td>
<td>\text{Mg}^{2+}</td>
<td>-1.83</td>
</tr>
<tr>
<td></td>
<td>\text{K}^+</td>
<td>-0.55</td>
</tr>
<tr>
<td></td>
<td>\text{Na}^+</td>
<td>-3.52</td>
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<tr>
<td>\text{K}^+</td>
<td>\text{Mg}^{2+}</td>
<td>-2.80</td>
</tr>
<tr>
<td></td>
<td>\text{Ca}^{2+}</td>
<td>-2.93</td>
</tr>
<tr>
<td></td>
<td>\text{Na}^+</td>
<td>-3.64</td>
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<tr>
<td>\text{Na}^+</td>
<td>\text{Mg}^{2+}</td>
<td>-1.19</td>
</tr>
<tr>
<td></td>
<td>\text{Ca}^{2+}</td>
<td>-1.32</td>
</tr>
<tr>
<td></td>
<td>\text{K}^+</td>
<td>-0.54</td>
</tr>
</tbody>
</table>
10.6 Results of titration on new Mg\textsuperscript{2+} ionophores with KTpClPBlionophore in electrode membranes

For studying the newly synthesized Mg\textsuperscript{2+} ionophores (ETH\textsuperscript{T} 2001, ETH\textsuperscript{T} 2002, ETH\textsuperscript{T} 2003, K22B5 and ETH\textsuperscript{T} 2022), a series of membranes were prepared with variation of the ratio of KTpClPBlionophore. The selectivity coefficients of each membrane were measured using SSM (n=6). The selectivity coefficients are plotted for each ionophore in section 5.3.

### Ionophore: ETH\textsuperscript{T} 2001

<table>
<thead>
<tr>
<th>KTpClPBlionophore (mol%)</th>
<th>log $K_{\text{Mg, Ca}}^\text{pot}$</th>
<th>log $K_{\text{Mg, K}}^\text{pot}$</th>
<th>log $K_{\text{Mg, Na}}^\text{pot}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>-0.4±0.06</td>
<td>-0.6±0.05</td>
<td>-1.4±0.07</td>
</tr>
<tr>
<td>40</td>
<td>-0.7±0.08</td>
<td>-0.9±0.06</td>
<td>-1.7±0.06</td>
</tr>
<tr>
<td>60</td>
<td>-0.3±0.05</td>
<td>0.3±0.03</td>
<td>-0.8±0.09</td>
</tr>
<tr>
<td>80</td>
<td>-0.2±0.06</td>
<td>1.5±0.04</td>
<td>-0.2±0.03</td>
</tr>
<tr>
<td>120</td>
<td>-0.2±0.05</td>
<td>3.8±0.11</td>
<td>1.1±0.10</td>
</tr>
<tr>
<td>160</td>
<td>-0.2±0.09</td>
<td>4.4±0.13</td>
<td>1.4±0.11</td>
</tr>
</tbody>
</table>

### Ionophore: ETH\textsuperscript{T} 2002

<table>
<thead>
<tr>
<th>KTpClPBlionophore (mol%)</th>
<th>log $K_{\text{Mg, Ca}}^\text{pot}$</th>
<th>log $K_{\text{Mg, K}}^\text{pot}$</th>
<th>log $K_{\text{Mg, Na}}^\text{pot}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
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<td>40</td>
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<td>-1.6±0.08</td>
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### Ionophore: ETH\textsuperscript{T} 2003

<table>
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<tr>
<th>KTpClPBlionophore (mol%)</th>
<th>log $K_{\text{Mg, Ca}}^\text{pot}$</th>
<th>log $K_{\text{Mg, K}}^\text{pot}$</th>
<th>log $K_{\text{Mg, Na}}^\text{pot}$</th>
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<td>-3.1±0.11</td>
</tr>
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<td>2.3±0.04</td>
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<td>160</td>
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### Ionophore: K22B5

<table>
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<th>KTpClPB/ionophore (mol%)</th>
<th>log $K_{\text{Mg, Ca}}^{\text{pot}}$</th>
<th>log $K_{\text{Mg, K}}^{\text{pot}}$</th>
<th>log $K_{\text{Mg, Na}}^{\text{pot}}$</th>
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<tbody>
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### Ionophore: ETH\textsuperscript{T} 2022

<table>
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<th>KTpClPB/ionophore (mol%)</th>
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<th>log $K_{\text{Mg, K}}^{\text{pot}}$</th>
<th>log $K_{\text{Mg, Na}}^{\text{pot}}$</th>
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11. References


(7) Frant, M. S. Analyst 1994, 119, 2293.


11. References


11. References

(74) Bakker, E. Electroanal. 1997, 9, 7.
11. References


11. References


11. References


11. References


12. Curriculum Vitae

Born on the 27th of July 1965 in Zhengzhou, Henan Province, People’s Republic of China

1972-1977 Primary School of Attachment in Zhengzhou University, Zhengzhou, China
1977-1982 Henan Provincial Experimental Middle and High School, Zhengzhou, China
1982-1986 Undergraduate Student at the Department of Chemistry of Zhengzhou University, Zhengzhou, China
1986-1989 Graduate Student for Master of Sciences at the Department of Chemistry of Zhengzhou University, Zhengzhou, China
1989-1994 Teaching and Research Assistant at the Department of Chemical Engineering, Zhengzhou Institute of Technology, Zhengzhou, China
1994-1999 Doctoral work at the Centre for Chemical Sensors/Biosensors and bioAnalytical Chemistry (CCS) of Swiss Federal Institute of Technology (ETH) Zürich, Switzerland; Supervisors: Prof. Dr. U.E. Spichiger and Prof. Dr. G. Folkers
1995-1997 Language Course for German at the Vocational School of the Canton Zürich, Zürich, Switzerland
1997-1999 Assistant to the Practical Courses in Analytical Chemistry at the University of Applied Sciences, Wädenswil, Switzerland.