เมมเบรนอิเล็กโทรดและออปโทดที่สามารถจดจำไอออนแบบแอลโลสเตอริก

นางสาววัลลภา วงศ์แสน

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MEMBRANE ELECTRODES AND OPTODES PERFORMING ALLOSTERIC RECOGNITION OF IONS

Miss Wanlapa Wongsan

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
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วัลลภา วงศ์แสน : เมมเบรนอิเล็กโทรดและออปโทดที่สามารถจดจำไอออนแบบแอลโลสเตอริก. (MEMBRANE ELECTRODES AND OPTODES PERFORMING ALLOSTERIC RECOGNITION OF IONS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ.ดร.ธวัชชัย ตันฑุลานิ, 64 หน้า.

ได้ออกแบบและสร้างพอลิไวนิลคลอไรด์ (พีวีซี) เมมเบรนอิเล็กโทรดแบบใหม่ ที่สามารถจดจำไอออนแบบแอลโลสเตอริกระหว่างไอออนฟลูออไรด์และโซเดียม โดยภายในแผ่นเมมเบรนบรรจุฟีนิลโบโรนิคแอซิดเพื่อเป็นสารตั้งต้นในการเกิดแอนไอออนิคไซต์ โดยมีฟลูออไรด์เป็นตัวกระตุ้นเพื่อทำให้เกิดแอนไอออนิคไซต์ และมีโซเดียมไอโอโนฟอร์ (NaX) ซึ่งจำเพาะต่อโซเดียมในตัวกลางพอลิเมอร์พีวีซีที่มี DOS เป็นพลาสติไซเซอร์ โดยในขั้นต้นเตรียมออปโทดเมมเบรน เพื่อศึกษาอันตรกิริยาระหว่างฟีนิลโบโรนิคแอซิดกับฟลูออไรด์ ด้วยเทคนิคยูวีวิสิเบิลสเปกโทรสโกปีพบว่า ฟีนิลโบโรนิคแอซิดสามารถเกิดสารเชิงซ้อนกับฟลูออไรด์ได้ และสามารถตอบสนองต่อฟลูออไรด์ที่ความเข้มข้น 10^{-8} M ได้ จากนั้นใช้เทคนิคโพเทนชิโอเมตรีเพื่อศึกษาการตอบสนองของเมมเบรนต่อไอออนโซเดียม พบว่าพีวีซีเมมเบรนที่มี DOS เป็นพลาสติไซเซอร์และมี *p-tert*-butylcalix[4]arene-tetraacetic acid tetraethyl ester (NaX) และ 4-[(4-*tert*-butyl-2-methyl phenoxy)methyl]phenylboronic acid (50% mol เมื่อเทียบกับ NaX) เป็นองค์ประกอบ เมื่อแช่เมมเบรนในสารละลายโซเดียมฟลูออไรด์ความเข้มข้น 10^{-2} M ให้สมบัติ Na-ISE ที่ดี โดยให้ค่าความชันเท่ากับ 57.48 mV/decade และมีขีดจำกัดการตรวจวัดโซเดียมเท่ากับ 7.42×10^{-7} M การตอบสนองของเมมเบรนอิเล็กโทรดต่อโซเดียมขึ้นกับค่าความต้านทานของเมมเบรนซึ่งเป็นผลจากการแช่แผ่นเมมเบรนในสารละลายฟลูออไรด์ จากการทดลองพบว่ากราฟระหว่างค่าความต้านทาน (R_m) กับความเข้มข้นให้ช่วงความเป็นเส้นตรงในการวิเคราะห์ปริมาณฟลูออไรด์คือ 10^{-8}–10^{-1} M การแช่เมมเบรนในสารละลาย Bu_4NF ที่ความเข้มข้นต่างๆ ให้ความสัมพันธ์ของกราฟที่ไม่เป็นเส้นตรง ดังนั้นแอลโลสเตอริกเมมเบรนอิเล็กโทรดที่เตรียมจึงมีความจำเพาะในการเลือกจับกับ Na^+ เมื่อใช้ F^- เป็นตัวกระตุ้น จากผลการทดลองคาดว่าสามารถนำไปใช้ตรวจวัดความเข้มข้นของฟลูออไรด์ที่ความเข้มข้นต่ำๆ ในตัวอย่างที่มีน้ำเป็นตัวทำละลายได้

ภาควิชา เคมี ลายมือชื่อนิสิต..

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก ....

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WANLAPA WONGSAN : MEMBRANE ELECTRODES AND OPTODES PERFORMING ALLOSTERIC RECOGNITION OF IONS. ADVISOR: PROF. THAWATCHAI TUNTULANI, Ph.D., 64 pp.

New polyvinyl chloride (PVC) membrane electrodes which show allosteric ion recognition between F^- and Na^+ were proposed and fabricated. The membranes incorporating phenylboronic acid as a precursor for anionic sites, F^- as an effector to generate anionic site and NaX ionophore as a receptor for Na^+ ions in DOS-plasticized PVC were fabricated. The study was initially relied on optode experiments. The optode membrane was prepared to study the interaction of phenylboronic acid and F^- in the membrane phase using UV-Vis spectroscopy. The result showed that phenylboronic could form complex with F^-, and the membrane responded to F^- at concentration as low as 10^{-8} M. Moreover, potentiometry was used to study the response of the membrane towards Na^+. DOS-plasticized PVC membranes containing *p*-*tert*-butylcalix[4]arene-tetraacetic acid tetraethyl ester (NaX) and 4-[(4-*tert*-butyl-2-methylphenoxy)methyl]phenylboronic acid (50% mol compared to NaX) were found to give the best characteristic of Na-ISEs upon conditioning the membranes in 10^{-2} M NaF: a response slope of 57.48 mV per decade and detection limit of 7.42×10^{-7} M. Potentiometric responses of Na-ISEs were found to depend largely on membrane resistances (R_m) varied with concentrations of fluoride ions used in membrane conditioning steps. Electrochemical impedance spectra showed a linear relationship between R_m and concentration range of NaF (1×10^{-1}–1×10^{-8} M). Conditioning optimal membranes with various concentrations of Bu_4NF gave a non-linear relationship. The proposed allosteric membrane electrode, thus, showed permselectivity toward Na^+ using F^- as an effector. The results can be potentially used for detection of low concentration of F^- in aqueous solution.

Department : Chemistry Student's Signature
Field of Study : Chemistry Advisor's Signature
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LIST OF SCHEMS

LIST OF ABBREVIATIONS

DOS	Bis(2-ethylhexyl)sebacate
EMF	Electromotive force
g	Gram
pK_a	Acid dissociation constant
Hz	Hertz
ISE	Ion selective electrode
EIS	Electrochemical impedance spectroscopy
L	Ionophore
M	Molar
mL	Millilitre
$mmol\ kg^{-1}$	Millimole per kilogram
PVC	Poly(vinyl chloride)
α	Normalized absorbance, degree of deprotonation
THF	Tetrahydrofuran
wt %	Percent by weight
µL	Microliter
R_s	Solution resistance
R_m	Membrane resistance
C_{dl}	Double layer capacitance
A_D	Absorbance of the nonprotonated form of the chromoinophore
A_P	Absorbance of the fully protonated form of the chromoionophore
F	Farad

CHAPTER I

INTRODUCTION

In biochemistry, allosteric refers to the regulation of an enzyme or a protein by binding an effector molecule at the protein's allosteric site. Effectors can either enhance or decrease the protein's activity. Along with the development of supramolecular host-guest chemistry, the design of allosteric receptors is significance because the guest (similar to an effector in biochemistry) can either enhance or decrease the binding or catalytic efficiency of the sensor-molecule [1]. Most recently, several examples of allosteric supramolecular receptors for metal ions and various organic guest molecules have been reported [2]. Many groups have reported the heteroditopic ion receptors displaying allosteric behavior of enhanced binding of anions in the presence of alkali metal ions [3-6]. In most cases, a metal ion is the allosteric effector which can improve the sensitivity and selectivity of an allosteric sensor.

In mimicking the transport of ions in biological membranes, chemists have used artificial membrane to extract or transport metal ions into the organic phases in order to detect such ions. Ion selective PVC membrane electrodes (ISEs) and optodes have been explored extensively and used in detections of metal ions and anions in environmental and medical interests [7]. In the case of metal ion selective electrodes, the anionic additive is required in order to improve the permselectivity of the membrane and reduce its electrical resistance. General anionic additives employed in PVC membrane electrodes and optodes are derivatives of borate salts such as potassium tetrakis[4–chlorophenyl]borate (KT*p*ClPB).

In this thesis, we propose a new idea for allosteric recognition of ions using a lypophillic phenylboronic acid derivative, 4-[(4-*tert*-butyl-2-methylphenoxy) methyl] phenylboronic acid, as a precursor of an anionic site in sodium ion selective membrane electrodes (Na-ISEs) and F^- as an effector to generate anionic sites and thus fulfill the property of anionic sites in membrane electrodes [8]. It was hypothesized that with the appropriate membrane ingredients, the presence of F^-

would help improving the performance of Na^+-selective electrode. The negatively charged complex upon selectively binding F^- acts as ionic additive and can reduce the electrical resistance of the sensing membrane.

Ion selective electrodes and electrochemical impedance spectroscopy were applied to study an allosteric recognition behavior of the proposed membrane because this is a non-destructive technique. EIS will be used to determine the electrical properties of the polymeric membranes such as the electrical resistance and capacitance under working conditions [9]. Therefore, electrochemical impedance spectroscopy was employed to measure the resistance of fabricated Na-ISE membranes. This implied the ability of membranes to transfer Na^+, which varied along the concentrations of F^- in the membrane treatment process.

In this research, a new polymeric membrane sensor which shows allosteric ion recognition between F^- and Na^+ was fabricated and examination of its behavior using potentiometry and EIS techniques. The fabricated of allosteric Na-ISE using F^- as an effector can then possibly be applied for F^- determination in aqueous solution.

1.1 Research Objectives

1. To prepare and study membrane optodes and membrane electrodes performing an allosteric recognition of F^- and Na^+ ions.

2. To examine the resistance of fabricated allosteric membranes using electrochemical impedance spectroscopy.

1.2 Scope of this research

The polymeric membrane incorporating 4-[(4-*tert*-butyl-2-methylphenoxy) methyl]phenylboronic acid for fluoride ion recognition, *p*-*tert*-butylcalix[4]arene-tetraacetic acid tetraethyl ester (NaX) as a receptor for sodium ion in a plasticized poly(vinyl chloride) (PVC) matrix was prepared. The proposed membrane was used to study allosteric ion recognition between F^- and Na^+. The proof of F^- uptake by phenylboronic acid derivative into the membrane was carried out by optodes using

UV-Vis spectroscopy measurements. The performance of Na-ISEs using F^- as an effector was investigated using potentiometry. The membrane resistance of optimal membranes conditioning with various concentrations of NaF was then measured using electrochemical impedance spectroscopy.

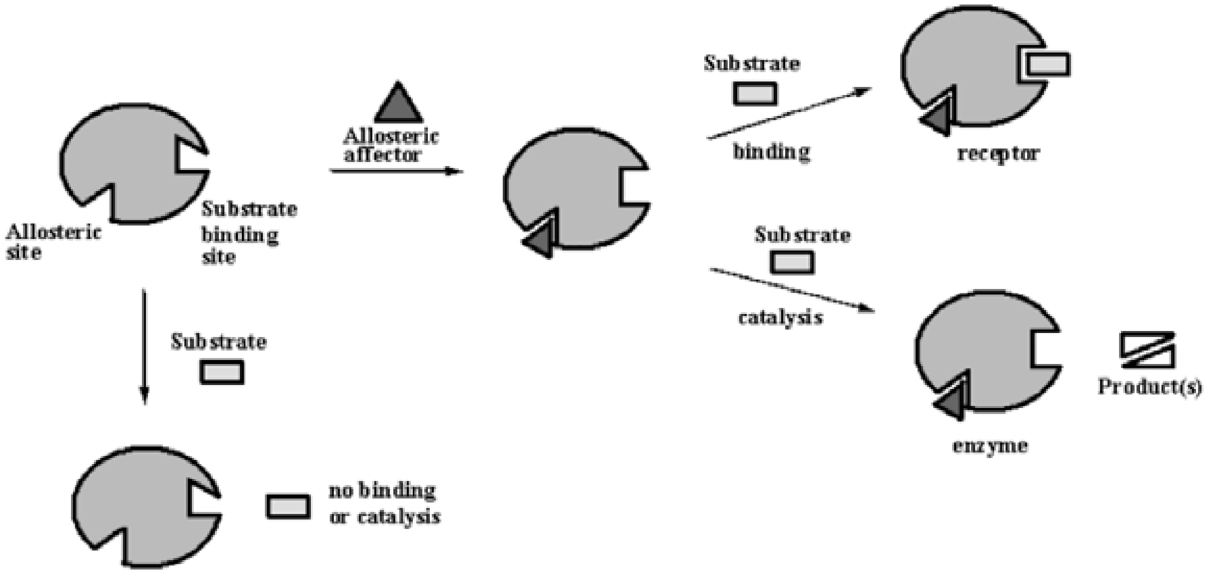
1.3 Benefits of this research

We expect to obtain DOS-plasticized PVC membrane electrodes which showed allosteric recognition of Na^+ using F^- as an effector. Our proposed membranes could be used to detect a low concentration of F^- using EIS.

CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Allosteric supramolecular receptors [10]



Scheme 2.1 Monomeric allosteric protein: activation of substrate binding (If protein is a receptor) or catalytic conversion (If protein is an enzyme) by an allosteric effector [10].

The concept of allosteric proteins was developed in the early 1960s by Monod and Koshland. Allosteric is derived from the Greek root *allo*, meaning “the other”. The binding of a regulatory molecule or ion to a specific allosteric site of the protein, structurally distinct from the active site, brings about an alteration of the conformation of the protein that indirectly modifies the properties of the biologically active site. This indirect mode of action via a distinct regulatory site is today the most commonly accepted meaning of the word “allosteric”.

Allosteric modulation of activity is fundamental for cellular function and is a common feature of biological receptors and enzymes, in particular those involved in metabolic pathways. The effector can either enhance (positive allosterism) or decrease (negative allosterism) the binding or catalytic efficiency of the protein. Ultimately, activity is switched ON (see Scheme 2.1) or OFF. The simplest mode of allosteric regulation, represented by a monomeric protein having distinct subunits for the binding of substrate and effector, is illustrated in Scheme 2.1.

In the case of an enzyme, the effector can influence either the affinity (the binding constant) for a substrate or the catalytic efficiency (rate of conversion of active site bound substrate), or both. In practice monomeric allosteric proteins are very rare. Most allosteric proteins are oligomeric. They consist of several subunits, often two or four subunits. The regulatory process is usually more complicated since conformational changes induced by an allosteric effector binding to one subunit can be transmitted to the other subunits.

The biomimetic design of allosteric systems is of great significance, because they are readily applicable to the efficient regulation of capture and release of analytes, catalytic reactions, and information transduction to the remote site that are frequently seen in biological systems. From the viewpoint of constructing a molecular sensory system, the methodology is very useful to amplify, integrate, and convert the weak chemical or physical signals into other signals. Allosteric complexation could be also used to transcribe “digital” behaviors in the world of molecules, because, ultimately, their behaviors may be switched “on” or “off” only at the specific threshold conditions regulated by, for example, the effector concentration. Allosteric systems provide a means of obtaining chemical feedback that is a necessary step toward achieving total control over molecular-scale chemical processes. An additional characteristic feature of allosterism is nonlinear binding which, by definition, requires that the initial binding of a guest has a different effect from subsequent host-guest interactions and avoids an information randomization.

Basically, the allosteric systems are classified into four different categories: (a) negative heterotropic, (b) positive heterotropic, (c) negative homotropic, and (d) positive homotropic. The simplest mode of allosteric action takes the form of heterotropic allosterism, where the binding of one chemical species influences the binding of a second chemical species. Homotropic allosterism, which is more important for the efficient regulation of equilibria and catalyses, however, is considerably more difficult to achieve in artificial systems, because the initial binding of a guest species must have a different effect from that of the subsequent interactions between the same host and guest molecules.

2.1.1 Homotropic allosteric systems

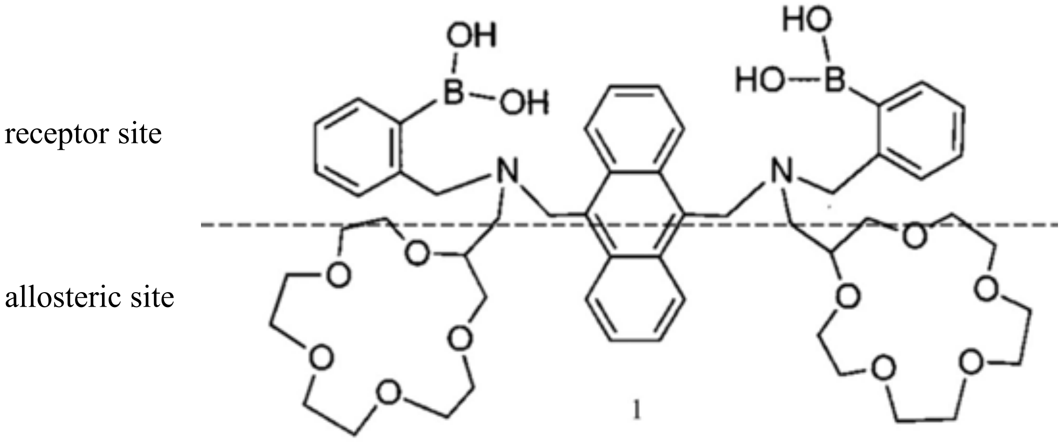
In artificial systems, homotropic allosterism is more difficult to reproduce, and examples are accordingly rare, because the initial binding of a guest species must have a different effect from that of the subsequent interactions between the same host and guest. Especially in the positive homotropic one, the guest binding information in a subunit should be passed to other all subunits in unison.

2.1.2 Heterotropic allosteric systems

In the design of a heterotropic allosteric system, a guest binding at one site must cause a negative or positive effect on a different guest binding at the remote second site. To design a negative heterotropic system, the first guest binding must induce a major conformational change so as to make the second guest binding less favorable; whereas, to design a positive heterotropic system, the first guest binding must make the remote site more suitable for the second guest binding.

2.1.2.1 Negative heterotropic allosteric systems

The glucose receptor **1** incorporates two crown units and an anthracene fluorescent reporter groups. On binding of saccharides, fluorescence increases as a consequence of an increased acidity of boronic acid and a therefore strengthened Lewis-acid base interaction with the tertiary amine, which becomes a less efficient quencher of the fluorophore. Alkaline and alkaline earth metal ions, for example Na^+ and in particular Ba^{2+}, disfavor binding of D-glucose to the ditopic cleft of receptor, as monitored by fluorescence decrease and circular dichroism [11].



allosteric effector : Na^+, K^+,Sr^{2+}, Ba^{2+}

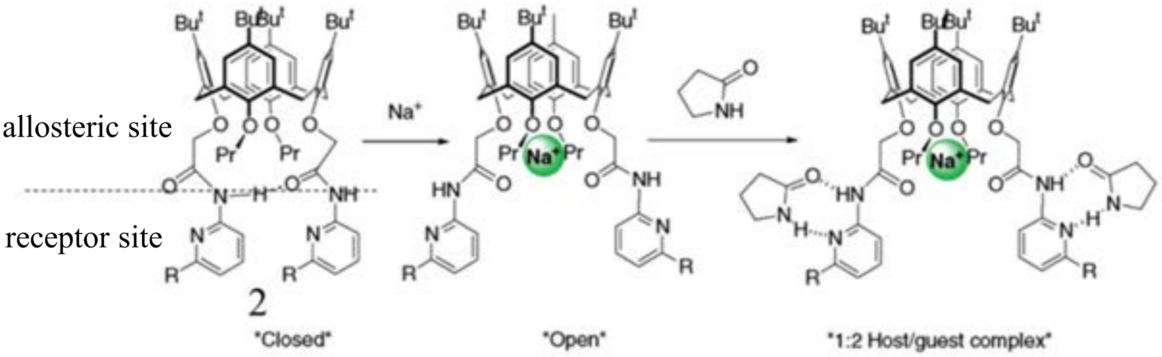
substrate : D-glucose

Figure 2.1 Structure of 1 corresponding to negative heterotropic allosteric systems.

The concept of negative heterotropic allosterism is very important in dynamic molecular or ion recognition systems, for example, to release guests only in the presence of some certain threshold concentration of effectors, to regenerate guest-selective electrodes, to control the membrane transport rate of a specific guest by an effector, and so forth.

2.1.2.2 Positive heterotropic allosteric systems

It is already known that in calix[4]arene esters and amides the four carbonyl groups are turned outward to reduce electrostatic repulsion among carbonyl oxygens, whereas bound Na^+ mechanically changes from the exo-annulus carbonyls to the endo-annulus carbonyls for the carbonyl oxygens to trap a Na^+ ion acting as an effector. Thus considered that the metal-induced structural change can be used to generate an “open” form from a “closed” form in an allosteric manner. In chloroform:acetonitrile (9:1 v/v), compound 2 exists as a “closed” form because of the formation of intramolecular hydrogen bonds and cannot bind its complementary guests (e.g., lactams). On the other hand, Na^+ bound to the ionophoric cavity cleaves the intramolecular hydrogen bonds, and the exposed receptor sites can bind the guests through intermolecular hydrogen bonds [12].



R = H

allosteric effector : Na^+

substrate :

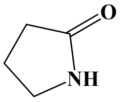


Figure 2.2 Molecular structure of compound 2.

2.2 Components of the polymeric ion-selective membrane

The polymeric membrane is a water immiscible liquid of high viscosity containing the components listed below.

Polymer matrix

The polymeric matrix provides mechanical stability to the membrane. In ideal cases it has no chemical interaction with the sensed ions and it is inert. The glass transition temperature of the polymers employed in polymeric membranes should be below room temperature [13]. Many types of polymer as silicon rubber [14], some methacrylates [15] and polyurethanes [16,17] have been used, while poly(vinyl chloride) (PVC) is commonly used in polymeric ISE-membranes.

Plasticizer

Initially, in order to provide mobility of membrane constituents and decrease its viscosity within the membrane phase, plasticizers were applied to polymer matrix. Plasticizer acts as a membrane solvent, affecting membrane selectivity through both extraction of ions into organic phase and influencing their complexation with the ionophore [18-20]. The plasticizer must be compatible with the polymer and other membrane components have to be soluble in it. Typical PVC plasticizers used for ISE membranes are 2-nitrophenyl octyl ether (o-NPOE, polar) and bis(2-ethyl hexyl)sebacate (DOS, non-polar).

Lipophilic ionic sites

Ionic site provides electro-neutrality of the membrane so that no significant amount of counter ions can be co-extracted into the membrane together with primary ion. Therefore, the membrane is permeable only for ions of the same charge sign as primary ion (Donnan exclusion) and demonstrates theoretical Nernstian response. Moreover, lipophilic ionic sites reduce electrical resistance of the membrane [21].

Ionophore

The ionophore, or ion carrier, or ligand, has the main influence on the selectivity of the ISE membrane. It can form reversible and relatively strong and selective complexes with target ions and does not complex other ions. Additionally, the ionophore structure must contain numerous lipophilic groups to keep a small leaching rate from the membrane to the sample phase [22].

Chromoionophore

Chromoionophore is lipophilized derivatives of azobenzene, nile blue, fluorescein and stilbene. They possess high molar absorptivity value (greater than 5×10^4 cm^{-1} M^{-1}), good chemical and photostability and particularly interacts with proton lead to a change in the optical properties of the polymer [23].

2.3 Important concepts of ion selective electrodes (ISEs)

Response mechanism

The electrochemical measuring cell (Figure 2.3) composes of two galvanic half-cells, ion-selective membrane as working electrode and Ag/AgCl as reference electrode. The working electrode potential of an electrochemical cell was measured by reference electrode. It should have a constant electrochemical potential on condition that no current flows through it.

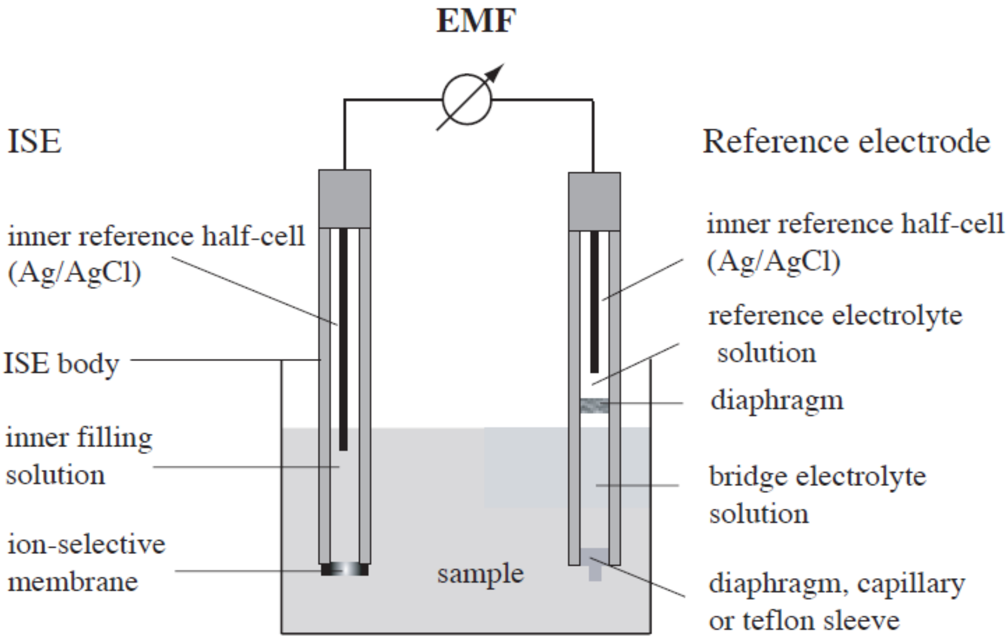


Figure 2.3 Schematic diagram of an ISE measuring cell [24].

A voltmeter or multi-channel measuring station was used to measure the total potential difference (*electromotive force*, EMF) increasing between working electrode and reference electrode. The EMF measured under zero-current conditions between two electrodes is a sum of local potential differences originating at each electrode/solution interface.

Activity (a_x)

Activity is the effective concentration of a free ion in a solution. It is dependent on the total ionic strength of a solution. In dilute solutions with low ionic strength, the ions are relatively far apart and free to move so that the activity and concentration are practically identical. In the case of ISE measurements, the activity of the measured ion determines the proportion of ions passing through the ion-selective membrane and thus the magnitude of the voltage developed by the electrode. The activity of an ion in solution for any known concentration may be calculated from the formula [25]:

$$a_x = \gamma_x \times C \qquad (2.1)$$

Where γ_x is the activity coefficient and C is the molar concentration

i) Ionic strength (μ)

The ionic strength of a solution is a measure of the total effect of all the ions exist in the solution and is given by the equation:

$$\mu = 0.5 \times \Sigma C_i Z_i^2 \qquad (2.2)$$

Where C_i is the species molar concentration and Z_i is the charges of ions

ii) *Activity coefficient* (*γ_x*)

The activity coefficient is dependent on the ionic strength of the solution. It becomes consecutively lower as the ionic strength increases, owing to inter-ionic interactions. The activity coefficient for any ion in solution can be estimated using the Debye-Hückel equation that permits the calculation of activity coefficients of ions from their charge and their average size.

$$- \quad og\ \gamma_x = \frac{. \qquad x\sqrt{\mu}}{. \qquad x\sqrt{\mu}} \qquad (2.3)$$

Where: Z_x = the ionic charge, μ = the ionic strength of the solution

$_x$ = effective diameter of the hydrated ion x in nanometers (10^{-9}m)

In solutions of very low ionic strength, when μ is less than . , the denominator of the equation becomes 1 and the equation can be simplified to:

$$- \quad og\ \gamma_x = \ . \qquad x\sqrt{\mu} \qquad (2.4)$$

Nernst equation

This is the basic equation, which involves the electrode potential to the activity of the measured ions in the test solution [26].

$$= \qquad - \frac{.}{n} \log \qquad (2.5)$$

Where, E = the total potential (in mV) developed between the sensing and reference electrodes

E^0 = a constant which is characteristic of the particular ISE/reference pair

n = the charge on the ion (with sign)

log (a) = the logarithm of the activity of the measured ion

An electrode is said to have a Nernstian response over a given concentration range if a plot of the potential difference versus the logarithm of the ionic activity of a given species in the test solution, is linear with a slope factor that is given by the Nernst equation.

Slope

The slope of an electrode is the gradient of the line formed by plotting the electrode response in millivolts against the logarithm of the activity (or concentration) of the measured ion. The theoretical Nernstian slope at 25°C is 59.16 mV/decade change in activity for monovalent ions and 29.58 mV/decade for divalent ions [26].

Response time

The duration of time that is essential to gain a stable electrode potential when the electrode is removed from one solution and placed in another of different concentration. There are many factors that affect response time such as the electrode type, the magnitude and direction of the concentration change, temperature, and the presence of interfering ions. For ISE specifications it is described as the time to complete 90% of the change to the new value and is mainly quoted as less than ten seconds [27].

Detection limits

In practice, the ideally specific electrode behavior as expressed by the Nernst equation can be obtained only for a certain range of ion activity. Every ISE has a lower and upper detection limit where the response starts to deviate from the theoretical slope and electrode loses specificity towards primary ion. According to the IUPAC recommendation, the detection limit (DL) is defined by cross-section of the two linear calibration curves (Figure 2.4).

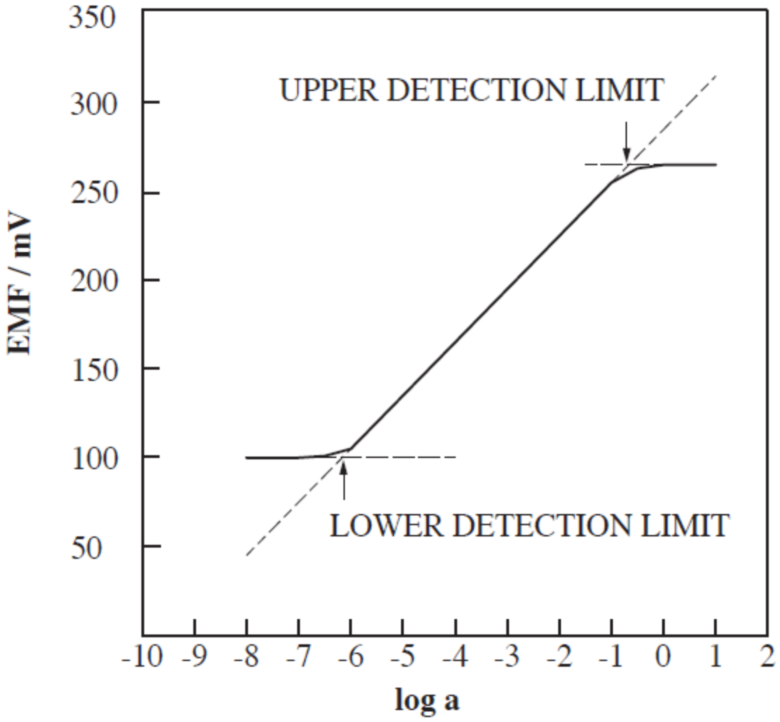
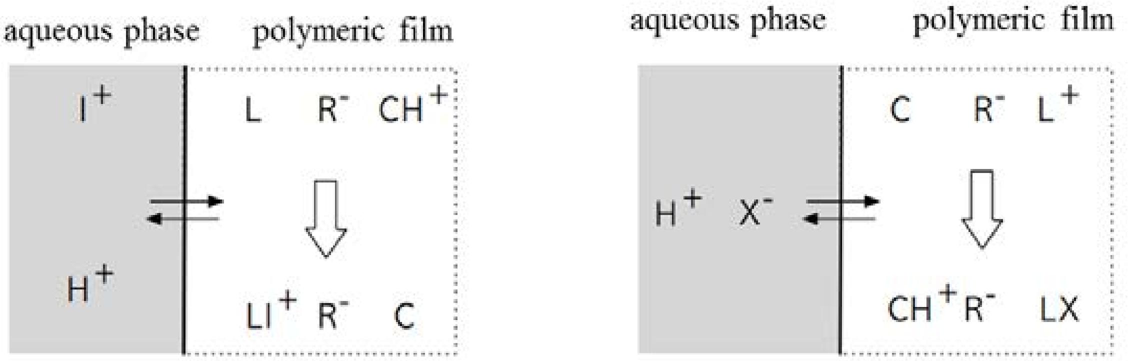


Figure 2.4 Definition of the upper and lower detection limits of an ion-selective electrode according to IUPAC recommendations [28].

2.4 Ion-selective optical sensor [29]

Optical chemical sensors expressed in this thesis are generally called "bulk optodes," which comprise of a plasticized polymeric phase containing chromoionophores and ionophores. Ionophores dissolved in the optode membrane simplify an extraction of interesting ions into the optode membrane and a concomitant deprotonation or protonation of the chromoionophore takes place to maintain charge neutrality in the optode membrane.



ion-exchange mechanism for cation detection

co-extraction mechanism for anion detection

Figure 2.5 Response mechanism of ion-selective optical sensors.

The amount of proton that left the optode membrane is equal to the amount of cation extracted into the optode membrane. Hence, the degree of deprotonation (or protonation) of chromoionophore is a quantitative measure for the degree of extraction of interesting ion as can be seen in the following equilibrium:

$$CH^{+}R^{-} + L + I^{+}_{(aq)} \rightleftharpoons C + LI^{+}R^{-} + H^{+}_{(aq)} \qquad (2.6)$$

where C is the chromoionophore, R^- is the anionic site, L is the ionophore for ion I^+, and species in the organic phase are denoted without any subscript. While optodes for cations utilize the ion exchange mechanism, in the case of anion-selective optical sensors, anions are co-extracted into the film along with a proton because the anion ionophore is typically a charged carrier type species (Figure 2.5). Therefore, the equilibrium is usually as follows:

$$\mathrm{C + L^{+}R^{-} + H^{+}_{(aq)} + X^{-}_{(aq)} \rightleftharpoons CH^{+}R^{-} + LX^{-}} \qquad (2.7)$$

where L^+ is the ionophore for anion X^-. Since the ion-selective optical sensor involves proton response, pH should be fixed during the measurement by using an appropriate buffer as a sample phase. The above mechanisms are displayed to explain how the ion-selective optical sensor works. There are many differences of these rules in the literature based on the charges of chromoionophores and ionophores as well as on whether or not the ionophores themselves also present optical properties.

2.5 Electrochemical impedance spectroscopy (EIS) [30]

Electrochemical impedance spectroscopy is a useful method of qualifying of numerous electrical properties such as conductivity, capacitance, and inductance of materials and their interfaces with electronically conducting electrodes. The concept of electrical resistance is based on the capability of circuit elements to hinder the flow of electrical current. The Ohm's law describes resistance, R, in terms of voltage, E, and current, I, ratio:

$$= \frac{}{\mathrm{I}} \qquad (2.8)$$

However, this ratio is restricted by the ideal resistor: Its resistance is independent of frequency of voltage signals and alternating current (AC) - alternating current are assumed to be in phase with each other.

The excitation or input signal expressed as a function of time is:

$$\mathrm{e = Esin}\ (\omega \mathrm{t}) \qquad (2.9)$$

where E is the amplitude of the AC potential with the radial frequency $\omega = \ \pi f$ and f is a frequency expressed in Hertz (Hz).

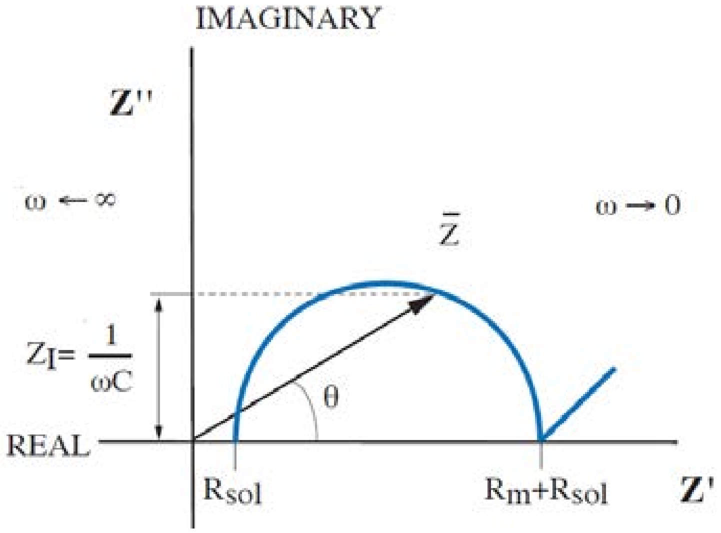
In a linear system the response or output signal, i, with the amplitude I is shifted in phase:

$$\mathrm{i = Isin}(\omega \mathrm{t} \quad \theta \qquad (2.10)$$

An expression analogous to the Ohm's law allows us to calculate the impedance of the system as:

$$Z(\omega) = \frac{\sin \omega t}{I\sin \omega t \quad \theta} = Z^{0} \frac{\sin \omega t}{\sin \omega t \quad \theta} \tag{2.11}$$

The impedance vector in Nyquist impedance plot is described by two components, real, Z' and imaginary, Z'' as shown in Figure 2.6.



$$Z(\omega) = Z' + jZ''$$

$$Z' = |Z| \cos \theta$$

$$Z'' = |Z| \sin \theta$$

Figure 2.6 Impedance spectra presentation: Nyquist plot and impedance vector in the complex plane.

Simplified randles cell

EIS data is generally examined by fitting it to an equivalent electrical circuit model. The simplified randles cell is one of most common cell models. It consists of a solution resistance (R_S), a double layer capacitor (C_{dl}) and a membrane resistance (R_m or charge transfer). The membrane resistance is in parallel with the double layer capacitance. The equivalent circuit for a simplified randles cell is shown in Figure 2.7.

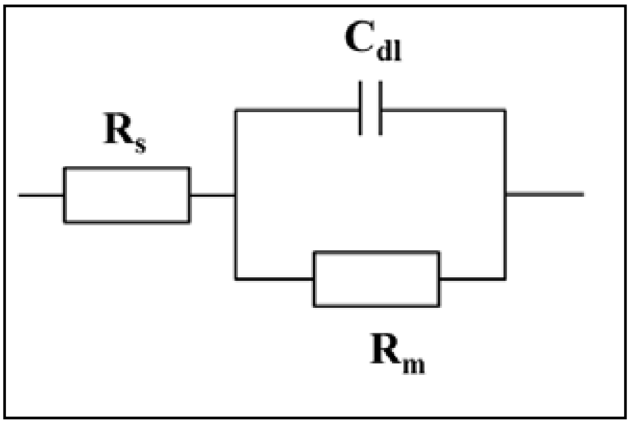


Figure 2.7 Simplified randles cell schematic diagram.

The Nyquist plot for a simplified randles cell is regularly a semicircle. The solution resistance can found by reading the real axis value at the high frequency intercept. The real axis value at the other (low frequency) intercept is the sum of the solution resistance and the membrane resistance. The diameter of the semicircle is therefore equal to the membrane resistance as shown in Figure 2.8.

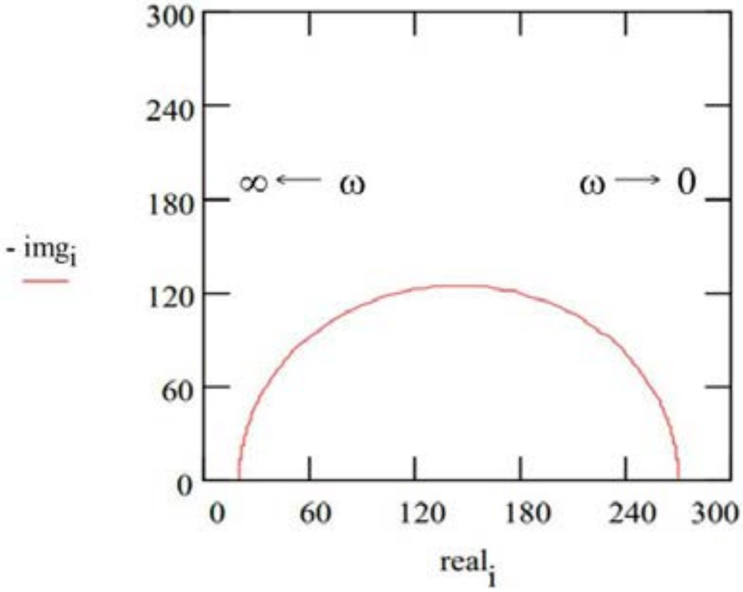


Figure 2.8 Nyquist plot for a typical simplified randles cell.

2.6 Fluoride measurements

Fluoride measurements are obtained continuous attention from sensor research communities. According to the high toxicity of fluoride, the concentration of fluoride in natural water should be monitored. The levels of fluoride in drinking water is importance because higher levels can cause effects the range from dental fluorosis to serious bone disorder [31,32] whereas low levels lead to tooth decay [33]. Many methods that mentioned about detection of fluoride in aqueous solution have been reported such as UV-Vis absorption and fluorescence spectroscopy [34-39], inductively couple plasma/mass spectrometry (ICP/MS) [40], RP-HPLC separation with UV-Vis detector [41], flow injection analysis (FIA) [42,43], co-precipitation [44], colorimetric naked eye detection [45-47]. Although the most beneficial fluoride ion selective sensor is based on a LaF_3 crystal (solid-state ion-selective electrode), a method which is accurate but flimsy and time consuming [48,49]. From previous research, there are several techniques to determine fluoride ions at low concentration levels. Unfortunately, there are some disadvantages among these methods such as complicated techniques, expensive instruments and sample pretreatment requirements.

Therefore, electrochemical technique was chosen to determine fluoride in aqueous solution due to its has several advantages as simplicity, high sensitivity, low cost, good selectivity, non-destructive analysis compared to other techniques [50].

2.7 Literature review

The development of an inexpensive fluoride selective devices is of interest particularly using liquid polymer membrane to design potentiometric or optical sensors [51,52]. Recently, a number of new ionophores have been reported for detection of fluoride. The usual approaches for detection of fluoride ion have used the specific Lewis acid-base interaction like the strong affinity of a boron atom toward the fluoride ion. Thus, many researchers demonstrated the use of phenylboronic acid derivatives as a receptor for determine fluoride ion concentration.

Jańczyk *et al.* [53] synthesized orgonoboron compounds pinacol ester of 2, 4, 6-trifluorophenylboronic acid (PE-PBA) and employed this compound as ionophores for fluoride ions in PVC membrane electrodes. It was found that the membrane electrodes containing the PE-PBA exhibited a Nernstian slopes as -58.5 mV/decade from 10^{-3} to 10^{-1} M of the responses toward fluoride ions at pH = 3.5. The detection limit of 7×10^{-4} M was obtained by the presence of acetate ion from the background buffer solution.

Cha *et al.* [54] reported the effect of boronic acid on the fluoride-selective chemosignaling behavior of a merocyanine dye. The dye containing *N*-methylquinolinium and phenylboronic acid (PBA) exhibited fluoride- selective behavior over other anions. Selective displacement of merocyanine dye with a fluoride ion influenced dissociation of the dye complex into the free dye and PBA-F^- adducts result in a great chromogenic signal for the fluoride ion. UV-Vis spectroscopy was used to analyze this signal. This dye was used to determine F^- in the concentration range between 2.0×10^{-5} and 1.0×10^{-3} M. The detection limit of 9×10^{-6} M was obtained.

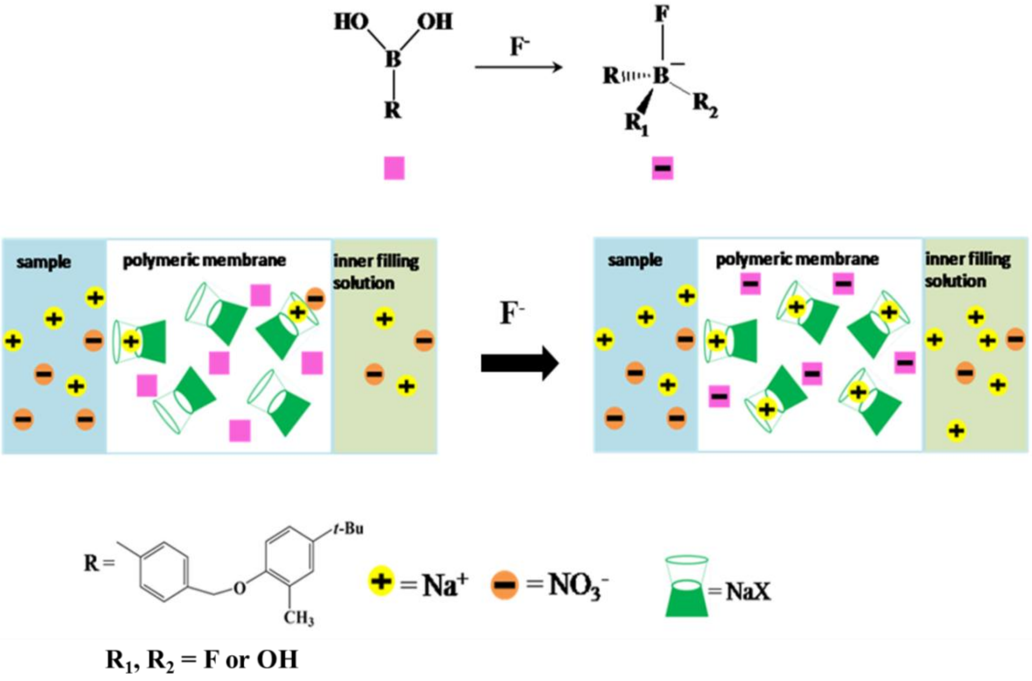
Xu *et al.* [55] reported the synthesis of the first fluoride receptors which is the asymmetric bidentate receptor containing a boronic acid and naphthoimidazolium moiety. ^{19}F NMR and fluorescence spectroscopy were employed to study the F^- binding ability of the compound in aqueous solution. The change of ratiometric fluorescence on addition of fluoride was observed. This compound is selective with F^- and does not response to other anions as Cl^-, Br^-, $CH_3CO_2^-$, HSO_4^- and $H_2PO_4^-$.

Swamy *et al.* [56] synthesized a new fluorescein derivative bearing a boronic acid group as a binding site to construct a fluorescent chemosensor for F^-. This sensor displays a selective fluorescent change with fluoride ion among the halide ions. The enhancement of fluorescent upon the addition of fluoride can be explained by blocking of the potoinduced eletron transfer (PET) mechanism which occurred by the interaction between fluoride and the boronic acid. The presence of the boronic acid-F^- adduct was confirmed by ^{11}B NMR experiments.

Badugu *et al.* [57] proposed the use of BAQBA-*N*-(2-boronobenzyl)-6-amino-quino-linium bromide as a novel water-soluble fluoride sensitive probe. This probe containing boronic acid fluorophore shows both an absorption and emission wavelength ratiometric response to fluoride at visible wavelengths in the concentration range 1-300 mM. The responses of the probes is based on the ability of the boronic acid group to interact with fluoride lead to the changing from the neutral form of the boronic acid group[R-B(OH)$_2$] to the anionic trifluoro form [R-B-F$_3$].

2.8 Hypothesis of this work

From the aforementioned reviews, the most importance components of ISE are ionophores and ionic additives. We use the principle of ISE to demonstrate our concept in allosteric recognition of ions in PVC membranes. In this case, phenylboronic acid derivative was used as a precursor of the anionic site in the membrane electrodes. Upon reacting with F$^-$, the phenylboronic acid-F$^-$ adduct is generated as illustrated in Scheme 2.2.



Scheme 2.2. Schematic representation of the allosteric ion recognition and transportation in polymeric membrane electrodes.

The reaction of phenylboronic acids derivative with fluoride ions leads to the establishment of series of the equilibria forming a single fluoride adduct $PhB(OH)_2F^-$, and from the OH^-/F^- exchange based on the protonation of hydroxide groups, providing species of 1:2 and 1:3 stoichiometry ($PhB(OH)F_2^-$ and $PhBF_3^-$) [58], as shown in Figure 2.9. All 3 species resulted in changes of the hybridization of the boron center from sp^2 to sp^3 and gave the negative charged boron complexes and thus fulfill the property of anionic sites in membrane electrodes.

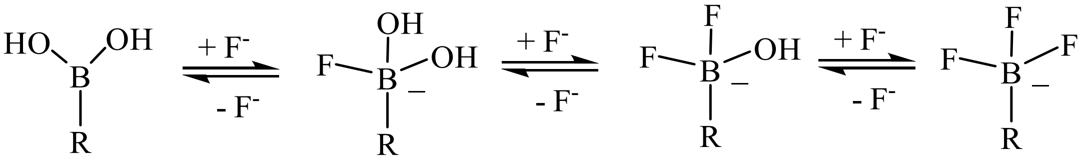


Figure 2.9 Equilibrium involved in the interaction between the phenylboronic acid derivative and fluoride.

The adduct becomes the anionic additive in the membrane electrode containing NaX as an ionophore for Na^+. Thus, F^- turns on the proper Na-ISE characteristics such as response slopes, detection limits and membrane resistances. An allosteric recognition of F^- and Na^+ in polymeric membrane was proved by ISEs and EIS.

CHAPTER III

EXPERIMENTAL

3.1 Instruments

A Varian Cary 50 Probe UV-Vis spectrophotometer was used to record all spectra, and absorbance measurements were conducted by the use of a homemade holder to hold a membrane glass slide. All pH values were determined with an Orion 2-Star Benchtop pH meter (Thermo Fisher Scientific). All EMF values were recorded with a 16-channel electrode monitor (Lawson Labs Inc., Malvern, PA 19355, USA), and a reference electrode Ag/AgCl (Metrohm 6.0726.100) containing 1 M LiOAc as salt bridge electrolyte was used. The impedance measurements were carried out using a potentiostat/galvanostat instrument (Autolab PG STAT 30, Eco Chemie B.V., Utrech, Netherlands) monitored by Frequency Response Analyser.

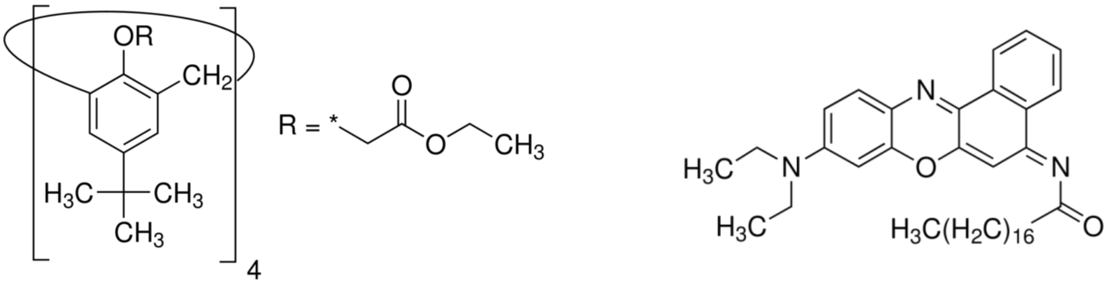
3.2 Chemicals

All chemicals were used without further purification and summarized in Table 3.1.

Table 3.1 Chemicals list and suppliers

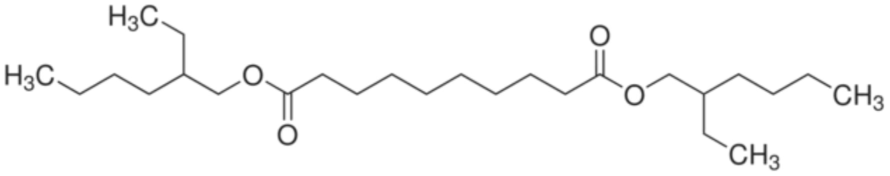
Chemicals	**Supplier**
p-tert-Butylcalix[4]arene-tetraacetic acid tetraethyl ester (NaX)	Fluka
4-[(4-*tert*-Butyl-2-methylphenoxy)methyl]phenylboronic acid	Sigma-Aldrich
9-(Diethylamino)-5-octadecanoylimino-5H-benzo[a]phenoxazine (Chromoionophore I, ETH 5294)	Fluka
High molecular weight poly(vinyl chloride) (PVC)	Fluka
Bis(2-ethylhexyl)sebacate (DOS)	Fluka
Stabilizer-free tetrahydrofuran (THF)	Fluka
Glycine	Sigma-Aldrich
Sodium nitrate ($NaNO_3$)	Carlo Erba
Sodium fluoride (NaF)	Merk
Sodium chloride (NaCl)	Merk
Sodium bromide (NaBr)	Fluka
Sodium sulfate (Na_2SO_4)	Sigma-Aldrich
Sodium dihydrogenphosphate (NaH_2PO_4)	BHD
Sodium cyanide (NaCN)	BHD
Sodium hydroxide (NaOH)	Merk
Sodium iodide (NaI)	Unilab
Sodium nitrite ($NaNO_2$)	Fluka
Hydrochloric (37 wt %)	Merk
Nitric acid (65 wt %)	Merck

Buffers and standard solutions were prepared in ultrahigh-purity Milli Q water (Bedford, Ma, USA) using Nanopure Millipore water purification system. Structures of components of polymeric membrane are shown in Figure 3.1.

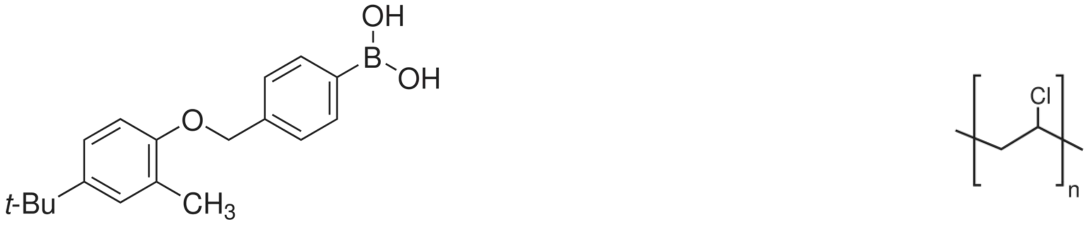


p-tert-butylcalix[4]arene-tetraacetic acid tetraethyl ester (NaX)

chromoionophore I



Bis(2-ethylhexyl)sebacate



4-[(4-*tert*-butyl-2-methylphenoxy)methyl]phenylboronic acid

PVC

Figure 3.1 Molecular structures of the components of polymeric membranes.

3.3 Optode studies

3.3.1 Preparation of optode membranes

The fluoride sensing optode was prepared by dissolving 20 mmol kg^{-1} of NaX, 10 mmol kg^{-1} of phenylboronic acid derivative, 2.5 mmol kg^{-1} of ETH 5294, poly(vinyl chioride) and a plasticizer DOS (1:2 by mass) in the total amount of 90 mg. The ratio of NaX and phenylboronic acid derivative used to prepare optodes is the same as the ratio used in ion selective electrode. All components were dissolved in 2.0 mL of THF. The cocktail was spread by pipetting 50 μL of the solution onto a square glass slide (22-mm No.1). After coating, the optode was left to dry at room temperature for at least 2 hours before use. The transparent and pinkish purple films were obtained indicating the presence of the deprotonated form of ETH 5294. The concentration of optode compositions are shown in Table 3.2.

Table 3.2 The composition of optode membranes

Compositions	**mmol kg^{-1}**	**mg**	**wt %**
NaX	20	1.81	2.00
phenylboronic acid derivative	10	0.28	0.31
ETH 5294	2.5	0.14	0.16
PVC		29.29	32.44
DOS		58.78	65.09

The composition of optode membranes was maintained to possess the mole ratio of ionophore (L) > ionic additive (R^-,R^+) > chromoionophore (C) [7].

3.3.2 Optimization of fluoride uptake by optode

3.3.2.1 Procedure of fluoride determination

The prepared optodes were placed in the optimized pH of 1 mM glycine/HCl buffer solution containing fluoride ions in the concentration range of 1.0×10^{-10} M to 1.0×10^{-1} M. Absorption spectra were recorded by the UV-Visible spectrophotometer in the wavelength range of 400-800 nm. The parameters including buffer solution pH and reaction time were optimized. The effect of interfering ions on the selectivity studies was investigated. All experiments were performed in triplicate.

3.3.2.2 Effect of the pH of the buffer solution

In order to evaluate the appropriate pH of the buffer solution, 1 mM glycine solutions of different pH values were prepared and used. The solution pH was adjusted by 1 M HCl. The previously prepared optodes were immersed in 1 mM glycine/HCl buffer solutions pH 1.5, 2.5 and 3.5 for 30 min before UV-Vis measurements. Absorption spectra were recorded by the UV-Visible spectrophotometer in the wavelength range of 400-800 nm. The effect of buffer solution pH was investigated by monitoring the presence of protonated peak at 665 nm at different pH values.

3.3.2.3 Effect of response time

The effect of response time was examined by placing the optodes in 10^{-2} M NaF at the optimum pH. The reaction time used was 5, 10, 20, 30, 40, 50 or 60 min. The effect of reaction time on the fluoride uptake by phenylboronic acid derivative in the optode membrane was studied by monitoring the highest absorbance intensity of protonated peak at 665 nm. Absorption spectra were recorded by the UV-Visible spectrophotometer in the wavelength range of 400-800 nm.

3.3.2.4 Effect of interfering ions

The effect of interfering ions was individually investigated by immersed the prepared optodes in 10^{-2} M solutions of $H_2PO_4^-$, SO_4^{2-}, NO_2^-, Cl^-, Br^-, I^-, CN^- and OH^- at optimum pH. Absorption spectra were recorded by the UV-Visible spectrophotometer in the wavelength range of 400-800 nm.

3.4 Ion selective electrode studies

3.4.1 Preparation of electrode membranes

The membrane solution for Na-selective electrode was prepared by dissolving 20 mmol kg^{-1} of NaX, phenylboronic acid derivative (0, 25, 50, 75 and 100 %mol relative to NaX ionophore and the ratio of PVC:DOS plasticizer (1:2 w/w) in the total amount of 220 mg. All components were dissolved in 2.5 mL of THF. The membrane cocktail was cast into a glass ring fixed on a glass support (i.d. = 30 mm). After evaporation of the solvent at room temperature, a transparent membrane with membrane thickness of ca. 200 μM was obtained, and a 7.5 mm-diameter disk was cut to put on an electrode body. Electrode membranes used in this thesis were prepared with different compositions as listed in Table 3.3

Table 3.3 Various compositions of electrode membranes

membrane number	**ionophore (mmol kg^{-1}, wt %)**	**boronic acid (mmol kg^{-1}, wt %)**	**PVC (wt %)**	**DOS (wt %)**
1	20, 1.98	0, 0	32.64	65.39
2	20, 1.98	5, 0.17	32.58	65.27
3	20, 1.98	10, 0.30	32.56	65.16
4	20, 1.98	15, 0.45	32.54	65.03
5	20, 1.98	20, 0.59	32.38	65.06

3.4.2 The EMF measurements

Potential responses of the membrane electrodes were measured by using a 16-channel electrode monitor (Lawson Labs Inc., Malvern, PA 19355, USA). The external reference electrode was a double junction type Ag/AgCl glass electrode (type 6.0726.100, Metrohm AG, CH-9010 Herisau, Switzerland) with 1 M LiOAc as a salt bridge electrolyte. 10^{-2} M NaCl was used as inner filling solution in the working electrode. The galvanic cell employed in this work consisted of: Ag, AgCl/3M KCl//1 M LiOAc//sample solution/membrane/inner filling solution/AgCl, Ag. Dynamic response curves and calibration plots were achieved through the adding step of a standard $NaNO_3$ solution from 1.0×10^{-7} to 1.0×10^{-2} M into 25 mL of Milli Q water. The solutions were magnetically stirred during the EMF measurements. The activities of ion are correlated to the EMF responses which are calculated from the Debye-Hückel method [59].

3.4.3 Optimization of the membrane electrode by potentiometry

3.4.3.1 Effect of the amount of phenylboronic acid derivative

The compositions of membrane were optimized by the fixed amount of NaX at 20 mmol kg^{-1}, the ratio of PVC:DOS plasticizer at 1:2 by weight, and varying the amount of phenylboronic acid derivative (0, 25, 50, 75and 100% mol relative to NaX ionophore) as shown in Table 3.3. The membrane without phenylboronic acid derivative was conditioned in 10^{-2} M $NaNO_3$ whereas the other membranes which contained the different amount of phenylboronic acid derivative were conditioned in 10^{-2} M NaF overnight before potentiometric experiments.

3.4.3.2 Effect of the concentration of conditioning solutions

The effect of the conditioned membrane in different concentration of NaF on the sensing ability of the membrane electrode was evaluated. Experiments were performed by conditioned the membrane in 10^{-8}, 10^{-5}, and 10^{-2} M of NaF overnight before EMF measurements.

3.5 Electrochemical impedance spectroscopy studies

3.5.1 The impedance measurements

A conventional three-electrode cell was used in the EIS studies: Na - ISE as the working electrode, a Ag/AgCl reference electrode and a platinum counter electrode. All experiments were conducted at room temperature. The membranes were conditioned in the different concentrations of NaF overnight using 10^{-2} M NaCl as the inner and outer electrolyte solution. Impedance measurements were carried out using a potentiostat/galvanostat instrument (Autolab PG STAT 30, Eco Chemie B.V., Utrecht, Netherlands) monitored by Frequency Response Analyser. The spectra were recorded within a frequency range 10 – 0.1 KHz with an AC voltage amplitude of 0.6 V. The impedance data were analyzed using the fitting program available in the Autolab software.

3.5.2 Optimization of the membrane electrode by impedance

In this study, the membranes were prepared with the same procedure and composition as described in section 3.4.1 (membrane electrode preparation) in order to optimize the membrane electrode by EIS as well. The membrane without phenylboronic acid drivative was conditioned in 10^{-2} M $NaNO_3$ whereas the other membranes which contained the different amount of phenylboronic acid derivative were conditioned in 10^{-2} M NaF overnight before impedance measurements. The influence of phenylboronic acid derivative on the electrical property of the membrane was evaluated.

3.5.3 Procedure of fluoride determination

The optimized membranes were conditioned in the different concentrations (1.0×10^{-10} – 1.0×10^{-1} M) of NaF overnight before impedance measurements. The impedance spectra of the membranes were recorded by electrochemical impedance spectroscopy within a frequency range 10 - 0.1 KHz with an AC voltage amplitude of 0.6 V.

CHAPTER IV

RESULTS AND DISCUSSION

In this work, we proposed a new idea for allosteric recognition of ions using a lypophillic phenylboronic acid derivative, 4-[(4-*tert*-butyl-2-methylphenoxy)methyl] phenylboronic acid, as a precursor of an anionic site in sodium ion selective membrane electrodes (Na-ISE) and F^- as an effector to generate anionic sites. The performance of the membrane was investigated using UV-Vis spectroscopy, potentiometry (ion selective electrodes). In addition, electrochemical impedance spectroscopy was employed to measure the resistance of fabricated Na-ISE membranes. This implied the ability of membranes to transfer Na^+, which varied along the concentrations of F^- in the membrane treatment process. A new strategy for F^- detection by the EIS method was presented.

4.1 The proof of F^- uptake by the phenylboronic acid derivative in the membrane by optodes

Optode is the optical sensor analogous to the electrochemical ISE. Their sensing mechanisms derived from the same principle. However, in the case of optodes, changing of signals is easier to observe from the indicator color incorporating in the membrane. Therefore, preliminary studies of F^- uptake into the membrane containing phenylboronic acid derivative was carried out using optode membranes. We prepared PVC membrane optodes with bis(2-ethylhexyl)sebacate (DOS) plasticizer. The polymeric membrane contained the ionophore NaX, phenylboronic acid derivative and chromoionophore (ETH 5294). The characteristic absorption bands at 540 and 665 nm corresponds to the deprotonated and protonated form of ETH 5294, respectively, as shown in Figure 4.1. Experiments were carried out in aqueous buffered solution. The boron atom of phenylboronic acid derivative acted as a selective receptor for F^- whereas ETH 5294 was selective to H^+. The uptake of F^- into the membrane occurred by the co-extraction mechanism [7] of H^+ and F^- ions to the membrane

phase following by a binding of H^+ to ETH 5294 and F^- to neutral phenylboronic acid derivative to form a phenylborate complex to maintain charge neutrality in the membrane phase. In this way, as each anion-proton pair is extracted into the organic phase, the counter ions (RX^- and CH^+) are automatically formed. This coextraction process was summarized in eq. (4.1) and (4.2), where L, R, C and CH^+ and RF^- represent NaX, phenylboronic acid, ETH 5294, protonated ETH 5294 and the phenylborate complex, respectively [60].

$$L_{(org)} + R_{(org)} + C_{(org)} + H^+_{(aq)} + F^-_{(aq)} \rightleftharpoons L_{(org)} + RF^-_{(org)} + CH^+_{(org)} \qquad (4.1)$$

$$L_{(org)} + RF^-_{(org)} + CH^+_{(org)} + Na^+_{(aq)} \rightleftharpoons LNa^+_{(org)} + RF^-_{(org)} + C_{(org)} \qquad (4.2)$$

In eq. (4.1), if a chromoionophore with a high pK_a value is employed, the equilibrium shifts to the right and sensitivity towards anion F^- will be improved. Hence, ETH 5294 ($pK_a = 11.41 \pm 0.03$ in DOS/PVC membrane [61]) was used to prepare the optode.

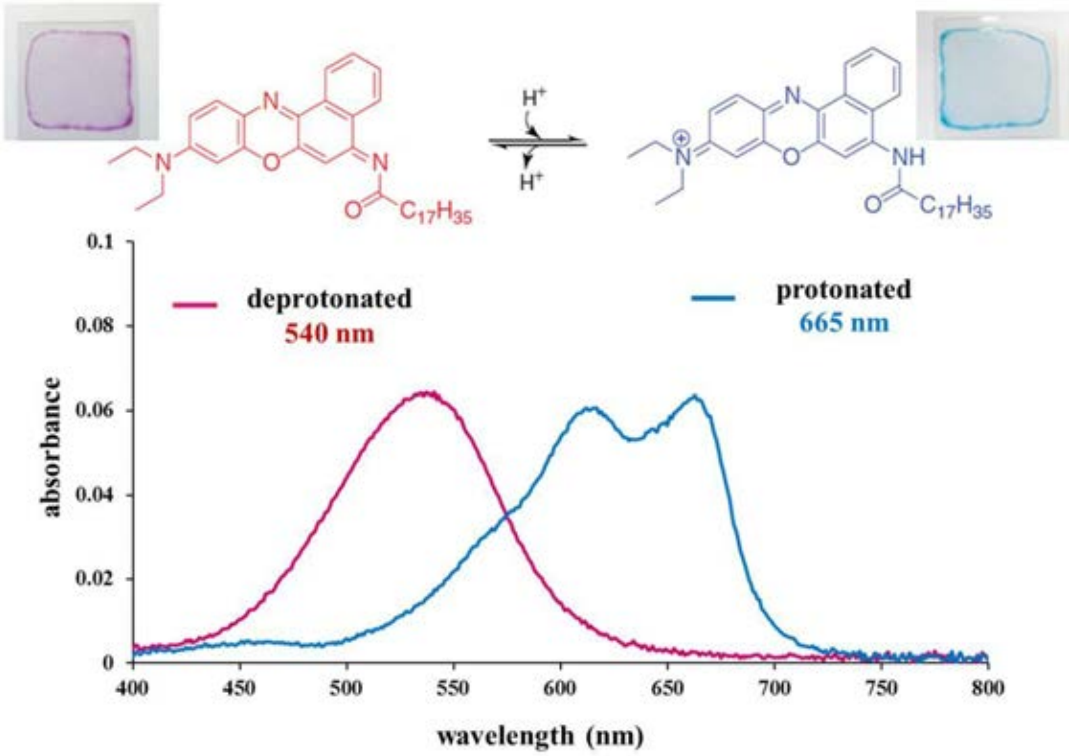


Figure 4.1 Absorption spectra of fluoride-selective optode membrane in fully protonated form (665 nm) and fully deprotonated form (540 nm) of ETH 5294 [62].

Optical response for fluoride uptaking was assessed by determining the degree of protonation (1-α) of the ETH 5294. The degree of protonation was correlated to the concentration of fluoride in the optical film as shown in eq. (4.3).

$$\frac{[\mathrm{CH}^+]}{C_T} = 1 - \alpha = \frac{A - A_D}{A_P - A_D} \tag{4.3}$$

$[CH^+]$ is the concentration of protonated chromoionophore relative to its total concentration, C_T. A is the absorbance at a given equilibrium. A_D and A_P are the absorbances with fully deprotonated (1-α = 0) and protonated (1-α = 1) chromoionophore at the same wavelength, respectively [63].

4.1.1 Effect of sample solution pH

Optical fluoride sensors consist of a plasticized polymeric phase doped with NaX, phenylboronic acid derivative and chromoionophore were prepared. The dyes, called chromoionophores are usually lipophilized pH indicators, does not complex any metal cations other than H^+ [64]. In this study, the optode response is based on the coextraction of F^- and H^+ from the aqueous phase into the membrane phase. Therefore, the pH of the sample solution has to be kept constant by buffering and the buffer solution in the experiment should not interfere the measurement of F^-. Hence, pH of the sample solution is the first parameter to be optimized.

In case of anion detection, buffer solution must be slightly acidic to avoid measuring a false response to hydroxide ion [65]. The effect of sample solution pH on the response of the optode was studied by placing the optode in 1 mM glycine/HCl at pH 1.5, 2.5 and 3.5. As shown in Figure 4.2, the absorption spectra were recorded at 665 nm after equilibration for 30 min. It was found that an appropriate buffer solution was pH 3.5 because at this pH the optode would not be protonated while at pH 1.5 and 2.5 the optode generated protonated peak at 665 nm. Therefore, pH 3.5 was chosen as the working pH of the sample solution.

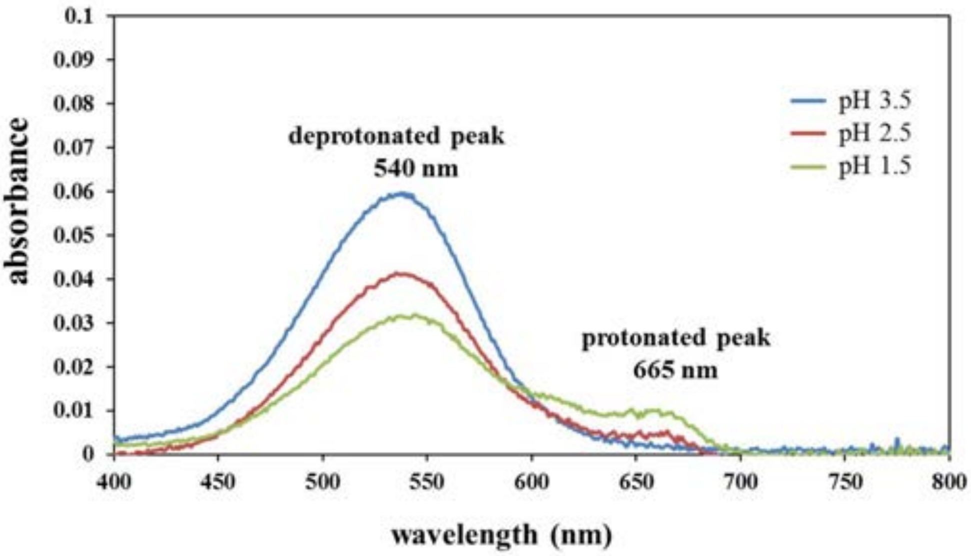


Figure 4.2 The effect of pH on the response of optode membrane at 665 nm in 1 mM glycine/HCl buffer solution.

4.1.2 Effect of response time

The response time of the optode membrane depends on many factors such as membrane composition, membrane thickness, activity of the measuring ion and pH of the measurement [66].

The response time of the optode was controlled by the time required for the analyte to diffuse from bulk solution to the membrane containing an ionophore. The response time of the fabricated optode was defined as the time required to reach equilibrium of signal absorbance. The response of the proposed optode was based on coextraction mechanism. Phenylboronic acid derivative as ionophore (L) induced the extraction of F^- into the membrane at the same time H^+ form aqueous phase was extracted by chromoionophore to maintain the electroneutrality of the system.

In this study, the absorption spectra of the optode membrane were recorded after bathing the membranes in 10^{-2} M NaF of glycine/HCl buffer solution pH 3.5. The results were shown in Figure 4.3. It took only 10 min to reach the maximum absorption intensity of the protonated peak at 665 nm. Therefore, the response time of 10 min was chosen for the bathing process.

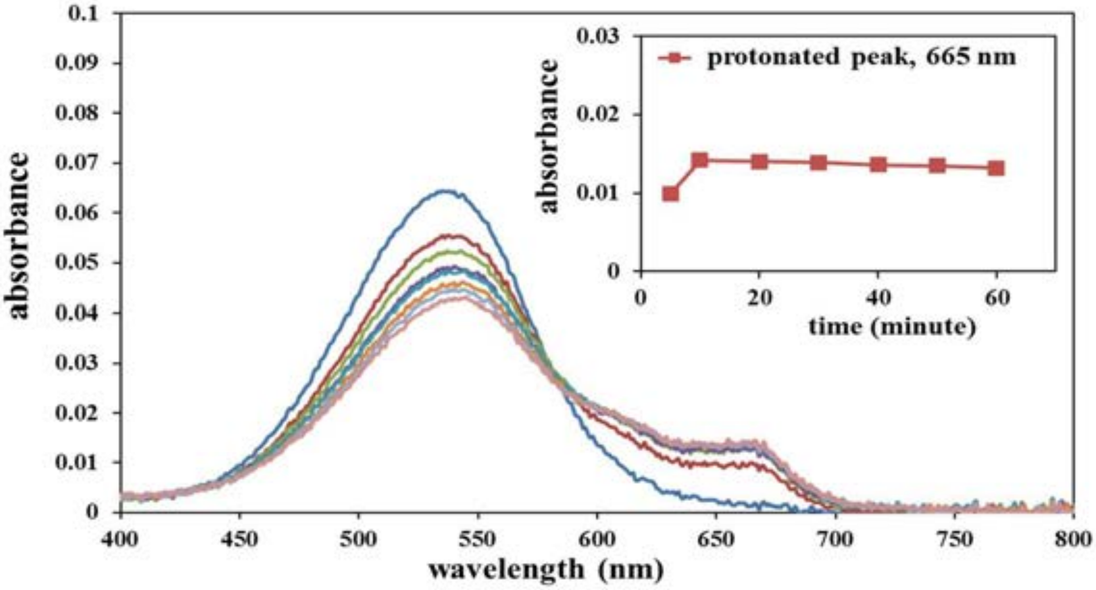


Figure 4.3 The response of optode membrane at 665 nm as a function of time in 10^{-2} M NaF glycine/HCl pH 3.5.

4.1.3 Effect of interfering ions

Anions used in this study were chosen from the lipophilic anions. Experiments were performed in 10^{-2} M of each interfering ions under the optimum conditions. The results were shown in Figure 4.4. The absorbance of the chromoionophore at 665 nm was monitored, only F^- binding yields and observable optical change which corresponds to the phenylboronic acid-F^- adduct.

The prepared membrane is not responsive to other anions such as $H_2PO_4^-$, SO_4^{2-}, NO_2^-, Cl^-, Br^- and I^-. It is well know that OH^- and CN^- can react with phenyl boronic acid to give the negative charged adducts similar to F^-. However, exposure of our prepared membrane to OH^- and CN^- resulted in white spots in the membranes probably due to membrane decomposition. Moreover, when placing the optode in OH^- or CN^- , the leaching of film components was observed.

The results indicated that the purpose optodes have high selectivity towards F^-.

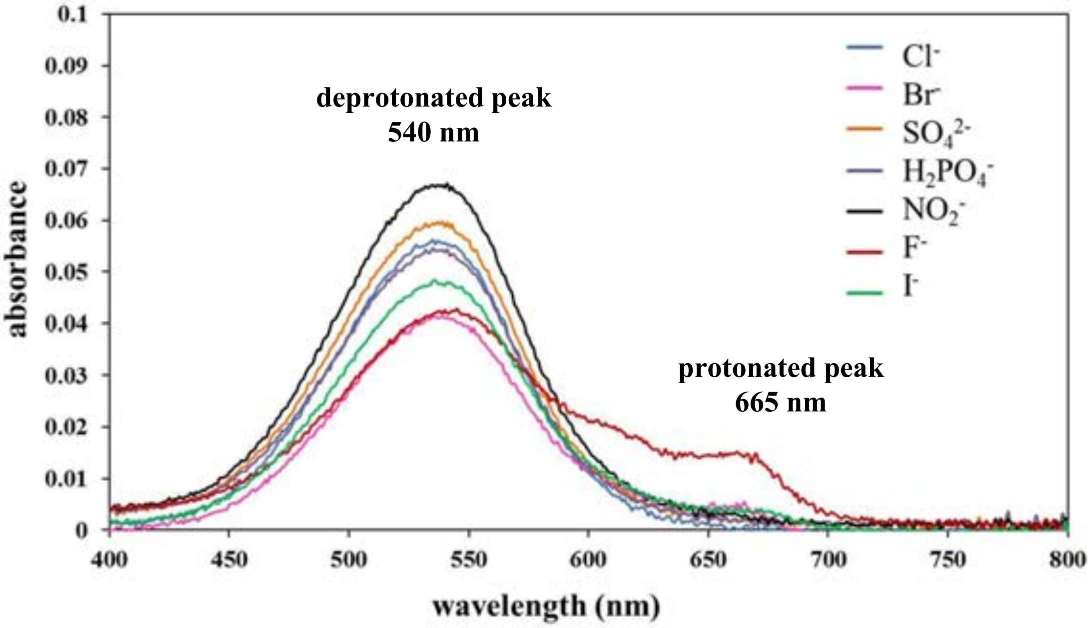


Figure 4.4 UV-Vis spectra of the membrane optode contacting various anions at a concentration of 10^{-2} M in 1 mM glycine/HCl pH 3.5 buffer solution.

4.1.4 Response behavior

In order to study of F^- uptake by the phenylboronic acid derivative in the membrane by optode, the fluoride sensing optode contained NaX , phenylboronic acid derivative as fluoride receptor and ETH 5294 as proton selective chromoionophore was prepared.

The blank membrane yielded an absorption band at 540 nm. F^- extraction into the polymeric membrane gave the optical response at 665 nm. We performed the measurement by varying concentration of NaF from 10^{-10} M to 10^{-1} M. Figure 4.5 shows absorption spectra of the membranes to various concentrations of NaF. It was found that this particular membrane responded to the concentration of F^- as low as 1×10^{-8} M. With increasing F^- concentration, the extent of protonation of ETH 5294 by proton coextraction became greater, yielding an increase in absorbance of the protonated form at 665 nm. The degree of protonation of the optodes measured in 10^{-8}-10^{-1} M F^- in glycine/HCl pH 3.5 (obtained from eq. 4.3) was shown in the inset of Figure 4.5. From the optode experiments, the membrane equilibrated to (1- α) = 0.56 at 10^{-2} M concentration of NaF. Further experiments for ion selective electrodes are carried out based on this concentration of NaF.

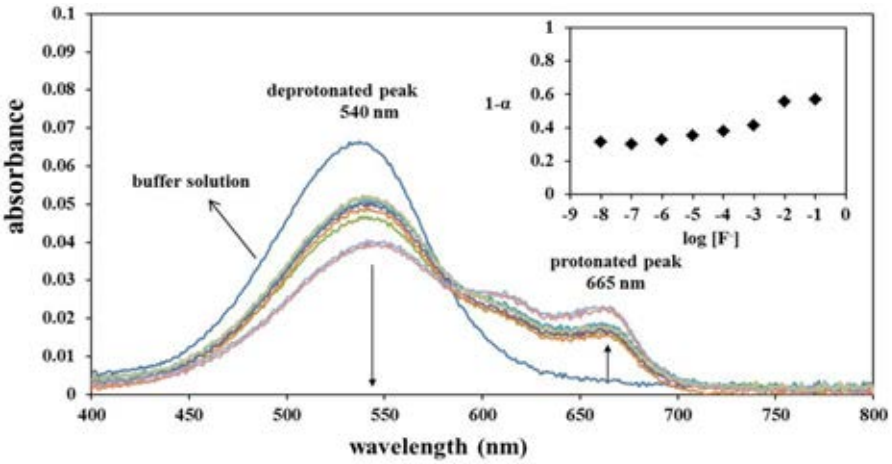


Figure 4.5 Absorption spectra of the membrane optode measured in 1 mM glycine/HCl buffer solution, pH 3.5 at varying F^- concentrations of 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} and 10^{-1} M. Inset shows the plot of degree of protonation (1-α) versus logarithm of fluoride ion concentration (M).

4.2 Fabrication of allosteric Na-ISEs using F^- as an effector

4.2.1 Optimization of the membrane electrode by potentiometry

4.2.1.1 Effect of the amount of phenylboronic acid derivative

Na-selective membranes were doped with different amount of phenylboronic acid derivative and their influence on potentiometric response was investigated. Amounts of phenylboronic acid derivative used in membranes were 25%, 50%, 75% and 100% mol compared to the ionophore NaX (20 mmol kg^{-1}). The response slopes of ISEs toward Na^+ (added as $NaNO_3$) are shown in Figure 4.6.

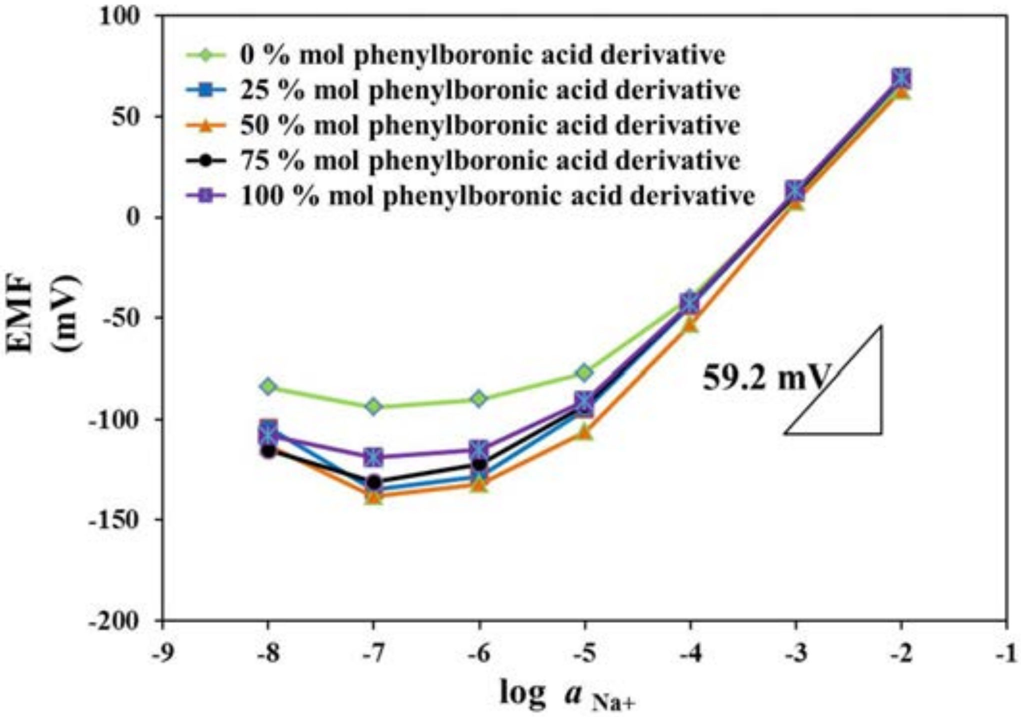


Figure 4.6 Response slopes of Na-ISEs prepared using various amounts of phenylboronic acid derivative.

Without phenylboronic acid derivative, the response slope of the membrane was found to be 48.68 mV/decade. In the presence of phenylboronic acid derivative, the response slopes of the membranes improved to be closer to Nernstian slope (59.2 mV/decade).

It was found that the presence of F^- ions could help improving the performance of Na^+- ISEs by coordinating to the Lewis acidic boron center of 4-[(4-*tert*-butyl-2-methylphenoxy)methyl]phenylboronic acid as an allosteric site for F^-. The resulting negatively charged complex would then act as an anionic site in the membrane phase and facilitate the Na^+ transfer. Time trace line observation of five membrane electrodes are presented in Figure 4.7.

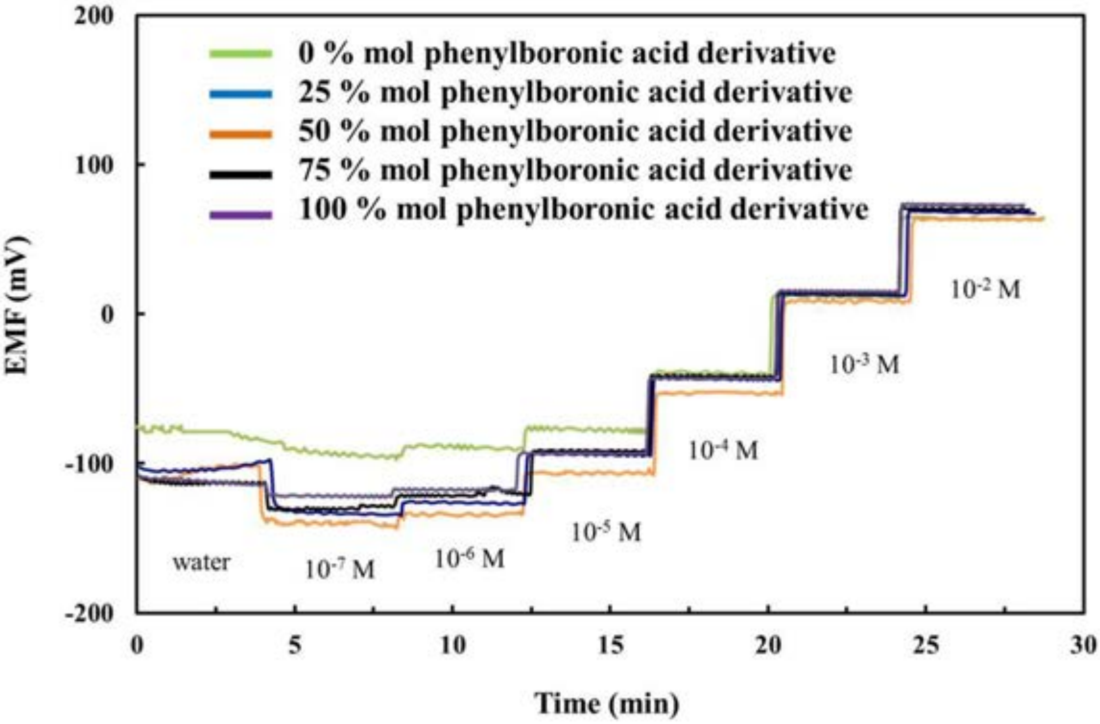


Figure 4.7 Time trace line observation of sodium-selective electrode.

The results in Table 4.1 show that the best Na-ISE was obtained from the membrane composed of 50% mol phenylboronic acid derivative, NaX 20 mmol kg^{-1} incorporated in the DOS plasticized PVC membrane (1:2; PVC:DOS), with a Nernstian slope of 57.48 ± 0.37 mV/decade, with a working concentration range of 1.0×10^{-5} - 1.0×10^{-2} M and a limit of detection of 7.42×10^{-7} M. Other ingredients also turn on Na-ISEs, but did not give the Nernstian response.

Table 4.1 Characteristics of Na-ISEs prepared using various amounts of phenylboronic acid derivative

membrane number	ionophore (mmol kg^{-1},wt %)	boronic acid (mmol kg^{-1},wt %)	slope[a]	detection limit (M)
1	20, 1.98	0, 0	48.68±0.48	6.06×10^{-6}
2	20, 1.98	5, 0.17	55.19±0.60	4.72×10^{-7}
3	20, 1.98	10, 0.30	57.48±0.37	7.42×10^{-7}
4	20, 1.98	15, 0.45	55.51±0.51	6.14×10^{-7}
5	20, 1.98	20, 0.59	54.75±0.41	9.97×10^{-7}

Note : All polymeric membranes were prepared using a 1:2 PVC to DOS plasticizer mass ratio, the slope values results from a linear rang $10^{-5} – 10^{-2}$ M. No buffer solution was employed in ISE measurements. Membrane No.1 was conditioned in 10^{-2} M $NaNO_3$. Membranes No.2-5 were conditioned in 10^{-2} M NaF.

[a] Standard deviations in triplicate experiments

4.2.1.2 Effect of the concentration of conditioning solutions

In this part, the effect of the concentration of bathing solutions was studied. It was found that the conditioning of the membranes has an influence on their resistance and sensitivity of the membranes [67].

The experiments were performed by conditioning the optimized membrane in 10^{-8}, 10^{-5} and 10^{-2} M NaF overnight before potentiometric experiments. Time trace lines and response slopes are shown in Figure 4.8, the membrane conditioned in 10^{-2} M NaF exhibited a near Nernstain slope of 57.26 mV/decade. The membrane has a relatively fast response time less than 10 second. Other membranes also turn on Na-ISEs, but did not give the Nernstian response. It could be seen that the slope of the membrane were increased when a concentration of the conditioning solutions also increased.

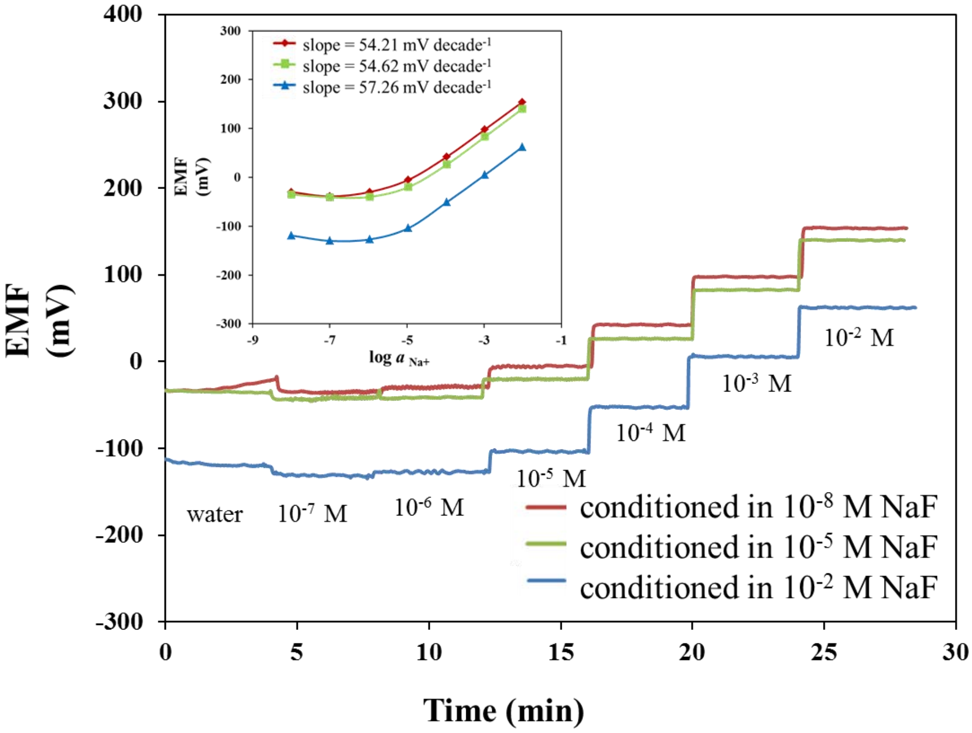


Figure 4.8 Effect of the concentration of bathing solutions on the membrane response in PVC/DOS.

4.3 Membrane efficiency in transportation of Na^+ using electrochemical impedance spectroscopy

4.3.1 Electrochemical impedance spectroscopy studies

In the membrane phase, a fraction of the neutral ligand is charged because the primary ion is complexed at the membrane:solution interface. 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 lipophilic anionic sites to a membrane is supposed to reduce the bulk membrane resistance significantly. In order to study the contribution of each membrane component (selective ligand, lipophilic ionic sites, plasticizer) to the membrane resistance, five membranes were prepared with different compositions.

The addition of an anionic additive to the membrane of a cation-selective electrode was not only to reduce the anionic interference observed in the presence of lipophilic anions but also the electrical resistance of the membrane is lowered [68,69]. The influence of adding boronic acid to DOS/PVC membranes on their resistance of sodium ion selective membranes based on NaX neutral carriers has been examined using electrochemical impedance spectroscopy.

Bulk membrane resistance is an important electrical property of an ISE. It is related to the ion transfer from the bulk solution to the membrane phase [70]. In practice, a low bulk membrane resistance indicates a high activity and mobility of ions within the membrane phase. The bulk membrane resistance of an ISE is mainly related to the membrane's composition (the type of ionophore and additives), its thickness, viscosity and the temperature. Impedance spectra can be used to determine the bulk membrane resistance, R_m, under defined conditions, and to compare R_m for different membrane compositions and different contacting electrolytes [71].

4.3.2 Optimization of the membrane electrode by EIS

Electrochemical impedance spectroscopy is used to measure the electrical resistance of the membrane upon ion transferring. We performed the EIS measurements of the membranes containing 0, 25, 50, 75 and 100% mol of phenyl boronic acid derivative compared to the NaX ionophore (20 mmol kg^{-1}). The obtained results are presented in Table 4.2.

Table 4.2 Membrane resistances and double layer capacitance of Na-ISEs prepared using various amounts of phenylboronic acid derivative

membrane number	**ionophore (mmol kg^{-1},wt %)**	**boronic acid (mmol kg^{-1},wt %)**	**R_m (Mohm)**	**C_{dl} (nF)**
1	20, 1.98	0, 0	3.57	0.292
2	20, 1.98	5, 0.17	2.10	0.401
3	20, 1.98	10, 0.30	1.25	0.550
4	20, 1.98	15, 0.45	2.34	0.377
5	20, 1.98	20, 0.59	2.43	0.364

Note : All polymeric membranes were prepared using a 1:2 PVC to DOS plasticizer mass ratio, the slope values results from a linear rang $10^{-5} – 10^{-2}$ M. No buffer solution was employed in ISE measurements. Membrane No.1 was conditioned in 10^{-2} M $NaNO_3$. Membranes No.2-5 were conditioned in 10^{-2} M NaF.

The semicircle corresponds to the bulk membrane resistance. It was seen that in case of fast transport of charge species through the membrane interface, only one arc which is referred to the membrane bulk resistance was observed on Nyquist plot (Z' vs. Z") [72]. Hence, the diameter of the semicircle is equal to the membrane bulk resistance in parallel to double layer capacitance of the membrane [73].

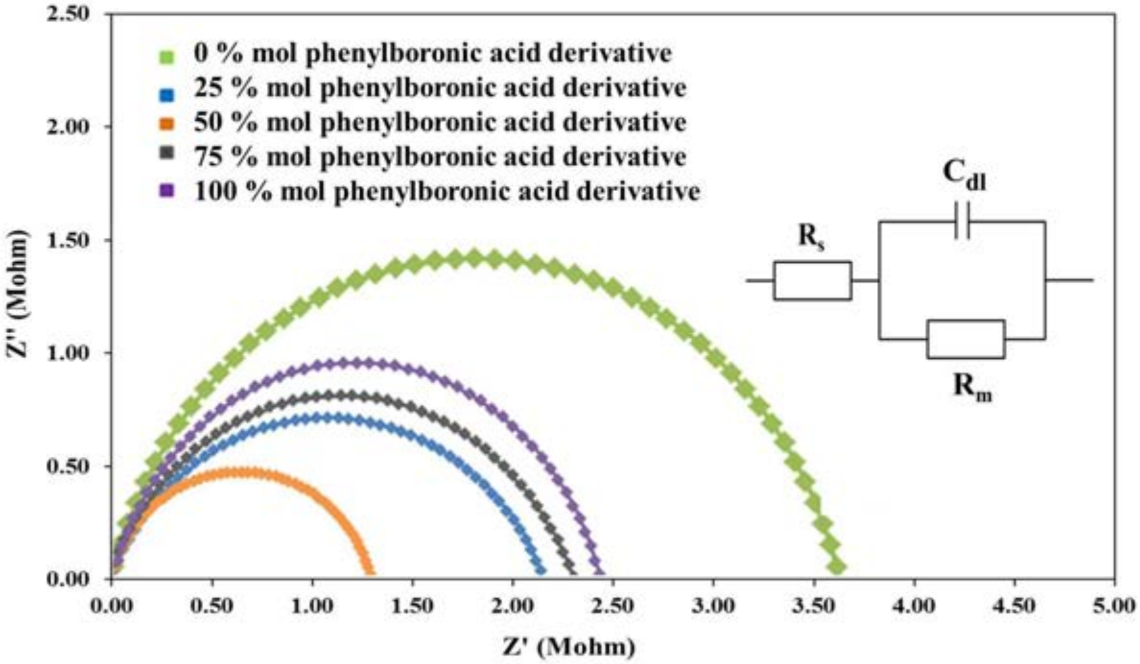


Fig 4.9 Relative impedance spectra for Na^+ - selective DOS/PVC membranes containing NaX ionophore (20 mmol kg^{-1}) and given amounts of phenylboronic acid derivative. Experimental conditions: 0.01 M NaCl as internal and external electrolyte solution, a.c. amplitude 0.6 V, frequency range 10 – 0.1 KHz.

The EI spectroscopy data were fitted to the equivalent circuit shown in the inset of Figure 4.9. It is composed of a parallel RC-element (R_m, membrane resistance; C_{dl}, double layer capacitance; R_s, solution resistance). The R_m (diameter of the semicircle in the Nyquist plot) is an important characteristic related to the ion transfer behavior within the membrane phase [74].

Our electrical approach is based on the monitoring of the R_m. Membrane No.1 (without phenylboronic acid derivative) was conditioned in 10^{-2} M $NaNO_3$ whereas membrane No.2-5 (containing 5, 10, 15 and 20 mmol kg^{-1} of phenylboronic acid derivative, respectively) were conditioned in 10^{-2} M NaF overnight to generate anionic additive. The membrane resistance of the five membranes were compared.

Membrane No.1 (without phenylboronic acid derivative) gave a high charge transfer resistance (R_m = 3.57 Mohm) due to no allosteric behavior, while the R_m of the membrane No.2-5 is too low as showed in Table 4.2. When phenylboronic acid derivative bind F^- ions, the negatively charged complex upon selectivity binding F^- acts as anionic additive then catalyzed the interfacial charge transfer process result in a reduction of the electrical resistance of the sensing membrane.

The results indicated that the membrane containing 50% phenylboronic acid derivative showed the least resistance of the membrane towards the transporation of Na^+ (as NaCl) into the membrane phase.

This behavior allowed us to setup the experiment conditions for sensitive and selective quantitative determination of F^- in aqueous solution using EIS. Therefore, the membrane containing 50% mol of phenylboronic acid derivative was chosen to perform preconditions in different concentration of F^-.

4.3.3 Determination of fluoride in aqueous solution

The aforementioned results suggested that amounts of F^- effector used in the conditioning steps of the allosteric membranes affected their potentiometric responses. This result signified the importance of lipophilic anionic sites on the membrane phase, which compensated the charge number of the ion–ligand complexes within the membrane as shown in Scheme 2.2. Kucza and coworkers have performed numerical simulations of steady-state potentials and impedance spectra of ion-selective membranes electrodes with ionic sites (different concentrations of primary ion in the bathing solution) [74]. Grabrielli et al. demonstrated by both experimental and calculation that K-ISEs containing various concentrations of an anionic site, BPh_4^-, yielded different membrane resistances (R_m) [74,76]. Grabrielli expressed the relationship between membrane parameters and the membrane resistance as shown in eq. 4.4:

$$R_m = \frac{RTd}{F^2(D_{ML}C_{ML} + D_M C_M)} \tag{4.4}$$

where d, D_{ML}, C_{ML}, D_M and C_M represent membrane thickness, diffusion coefficient and concentration of metal-ionophore complex (ML) and metal ion (M), respectively. R, T and F are gas constant, temperature and Faraday constant, respectively. If d and T are the same for every fabricated membrane and for potentiometric measurements and the ratio of ionophore/anionic site is greater than 1, the ML complex controls the conductivity [74,76]. Therefore, the membrane resistance R_m varies along the concentration of the ML complex which equals to the concentration of the anionic site in the membrane phase (C_Y) as shown in eq. 4.5.

$$R_m \alpha \frac{1}{C_Y} \tag{4.5}$$

Impedance spectra can be used to determine the bulk membrane resistance, R_m, under defined conditions, and to compare R_m for different membrane compositions (anionic sites) with the same contacting electrolytes. According to eq. 4.5, increasing concentration of the anionic site resulted in reduction of the membrane resistance. We then used the optimal membrane component (membrane 3) to perform preconditions of membranes in various concentrations of F^- (from 10^{-1} to 10^{-10} M) and measured EIS spectra of each membrane using 10^{-2} M NaCl as the inner and outer electrolyte solution. The results are displayed as semicircle Nyquist plots shown in

Figure 4.10. Membrane resistances and double layer capacitance of membrane electrode with phenylboronic acid derivative and NaX from EIS are presented in Table 4.3.

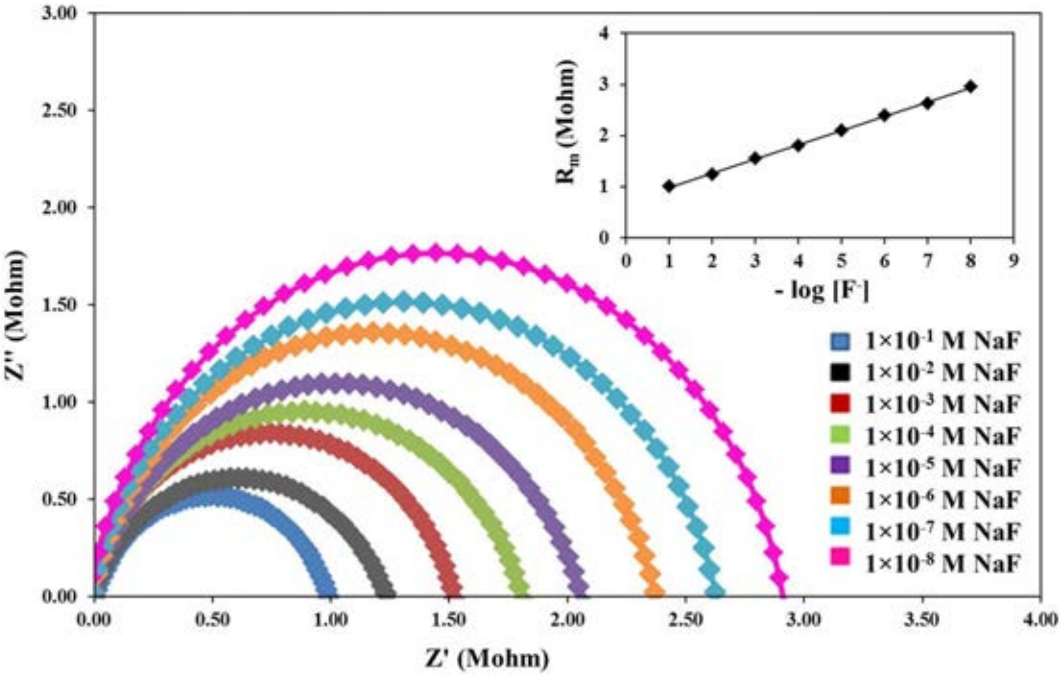


Figure 4.10 Relative impedance spectra and inset calibration curve of NaF in PVC membrane electrode with phenylboronic acid derivative and NaX.

The values of the interfacial parameters (R_{sol}, R_m and C_{dl}) could be obtained and calculated. R_m values of the membranes containing both phenylboronic acid derivative and NaX showed a linear relationship with the concentration range of F^- between 1×10^{-1} – 1×10^{-8} M as illustrated in the inset of Figure 4.10. The membrane pretreated with F^- lower than 10^{-8} M gave unfitted data, and semicircle Nyquist plots could not be obtained. This result agrees well with results obtained from optode membranes which showed the response to F^- concentration as low as 1×10^{-8} M. In addition, we also conditioned ISE membranes with various concentrations of Bu_4NF. Nyquist plots (data not shown) of these membranes did not show a linear relationship between the concentration range of Bu_4NF and the R_m values. This suggests the permselectivity of the fabricated allosteric membranes toward Na^+ using F^- as effector.

Table 4.3 Membrane resistances and double layer capacitance of membrane electrode with phenylboronic acid derivative and NaX from EIS

concentration (M)	**R_m (Mohm)**	**C_{dl}(nF)**
1.0×10^{-1}	1.01	0.622
1.0×10^{-2}	1.24	0.552
1.0×10^{-3}	1.55	0.479
1.0×10^{-4}	1.81	0.436
1.0×10^{-5}	2.10	0.402
1.0×10^{-6}	2.39	0.375
1.0×10^{-7}	2.62	0.351
1.0×10^{-8}	2.95	0.338

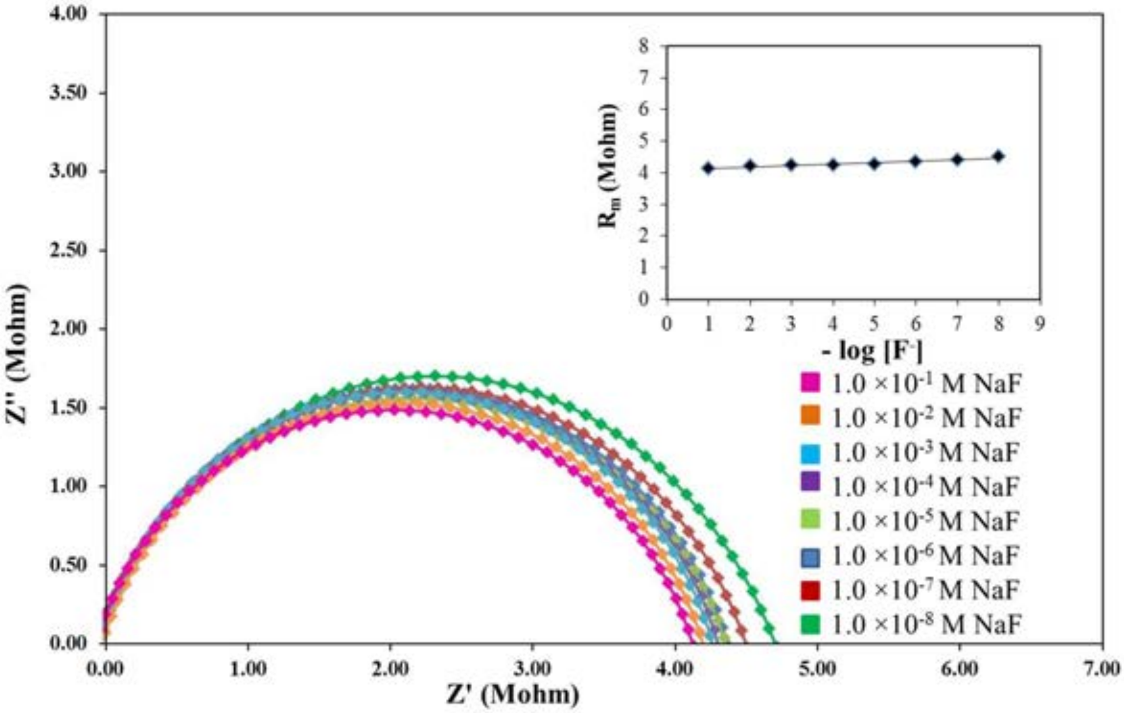


Figure 4.11 Relative impedance spectra and inset calibration curve of NaF in PVC membrane electrode with only phenylboronic acid derivative.

Membranes contained only 10 mmol kg^{-1} of phenylboronic acid derivative without NaX ionophore were also prepared. The membrane resistance and double layer capacitance of membrane electrode with only phenylboronic acid derivative from impedance spectroscopy are presented in Table 4.4.

The R_m values of the membrane containing only phenylboronic acid derivative gave small changes of R_m values in a range of 4.14 – 4.51 Mohm, when conditioned with 10^{-8} - 10^{-1} M of NaF, as shown in Figure 4.11. This result signifies the importance of the ionophore in ion transportation in the membrane. Without NaX, Na^+ transfer in the membrane was not efficient.

Table 4.4 Membrane resistances and double layer capacitance of membrane electrode with only phenylboronic acid derivative from EIS

concentration (M)	**R_m (Mohm)**	**C_{dl}(nF)**
1.0×10^{-1}	4.14	0.284
1.0×10^{-2}	4.22	0.280
1.0×10^{-3}	4.24	0.280
1.0×10^{-4}	4.25	0.279
1.0×10^{-5}	4.28	0.277
1.0×10^{-6}	4.35	0.274
1.0×10^{-7}	4.40	0.270
1.0×10^{-8}	4.51	0.262

Mazloum-Ardakani *et al.* showed that the dissymmetrical concentration of the internal solution has affected the characteristics of the membrane electrode [77]. We also varied the concentration of the inner solution (NaCl) of our electrode system. However, the dissymmetrical concentration of the inner and outer solution gave unfitted semicircle Nyquist plots. We also performed the EIS experiments using 10^{-2} M KCl and *t*-Bu_4NCl as inner and outer electrolyte solutions. The membrane resistance and double layer capacitance of membrane precondition in various concentrations of NaF using 10^{-2} M KCl as inner and outer electrolyte solution are presented in Table 4.5.

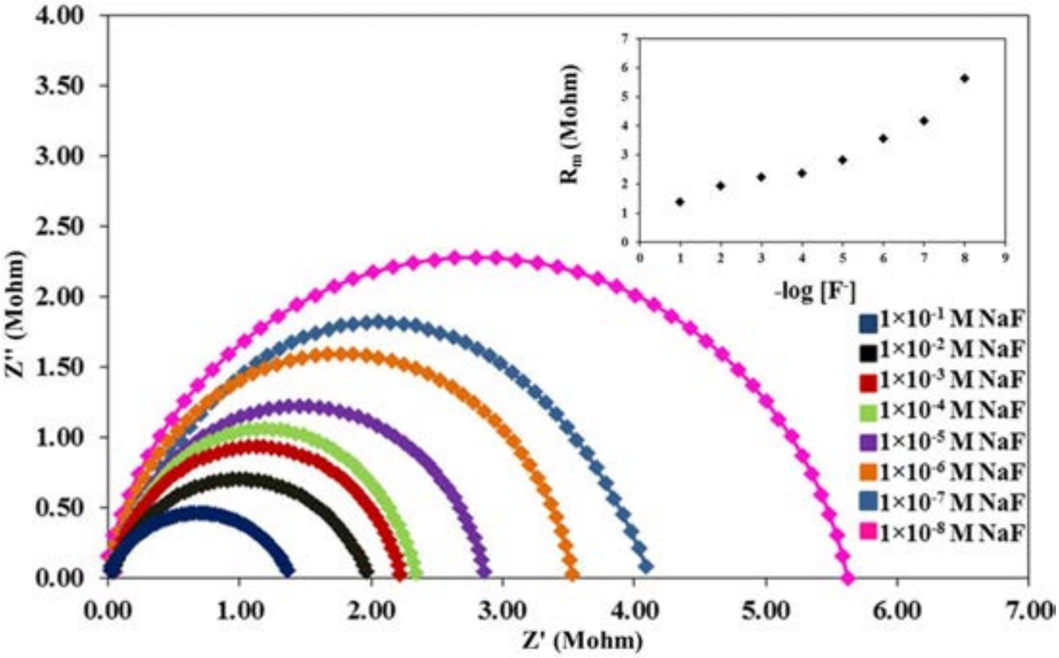


Figure 4.12 EIS of the membrane precondition in various concentrations of NaF using 10^{-2} M KCl as inner and outer electrolyte solution.

Table 4.5 Membrane resistances and double layer capacitance of the membrane precondition in NaF using 10^{-2} M KCl as inner and outer electrolyte solution

concentration (M)	**R_m (Mohm)**	**C_{dl}(nF)**
1.0×10^{-1}	1.38	0.532
1.0×10^{-2}	1.93	0.457
1.0×10^{-3}	2.24	0.395
1.0×10^{-4}	2.36	0.378
1.0×10^{-5}	2.82	0.356
1.0×10^{-6}	3.56	0.293
1.0×10^{-7}	4.16	0.285
1.0×10^{-8}	5.62	0.142

In the case of *t*-Bu_4NCl, the Nyquist plots cannot be obtained. Figure 4.12 shows the Nyquist plots of the membranes using 10^{-2} M KCl as inner and outer electrolyte solutions. It is obviously seen that the EIS responses did not give a linear relationship. This result signifies that the fabricated membrane can selectively respond to Na^+ rather than K^+.

There are many of the new applications of ISEs. It is significant to identify process occurring in the membrane. From the EIS experiments, it was found that EIS is a beneficial technique. Impedance measurements display the transport of a charged species across the membrane. The information that may occur at the electrode-electrolyte interface such as reaction mechanisms and electrode kinetics were obtained. We can apply this technique to study interfacial process in order to understand of ISE responses.

CHAPTER V

CONCLUSION

A new polymeric membrane sensor which showed allosteric ion recognition of F^- and Na^+ was prepared successfully. Ion selective membrane electrode was constructed by using a derivative of phenylboronic acid as a precursor of an ionic additive and a calix[4]arene (NaX) based receptor as an ionophore for Na^+. Firstly, the proof of F^- uptake by phenylboronic acid derivative in the membrane electrode was carried out by optode membrane. The measuring of F^- uptake via co-extraction mechanism of F^-/H^+ to form a complex with ETH 5294 and phenylboronic acid derivative was studied by UV-Vis spectrophotometry. The optode membrane responsed to F^- by increasing intensity of protonated peak at 665 nm after equibration in glycine/HCl buffer (pH 3.5) solution containing different concentrations of F^- and degree of protonation (1-α) of the optode measured in $10^{-8} – 10^{-1}$ M F^- was equal to 0.56 at 10^{-2} M NaF. The optode membrane showed high selectivity toward F^- over other anions such as $H_2PO_4^-$, SO_4^{2-}, NO_2^-, Cl^-, Br^- and I^-.

Allosteric Na-ISEs were fabricated using F^- as an effector to generate ionic additive. The performance of the proposed membrane was studied by potentiometry. The results show that the best Na-ISE was obtained from the membrane composed of 50% mol phenylboronic acid derivative compared to NaX (20 mmol kg^{-1}). The fabricated allosteric ISEs were demonstrated to detect Na^+ with good ISE characteristic with a response slope of 57.48 mV/decade and detection limit of 7.42×10^{-7} M upon preconditioning the membrane with 10^{-2} M NaF.

Potentiometric responses of Na-ISEs were found to depend on membrane resistances (R_m) varied with concentrations of fluoride ions used in membrane conditioning steps. EIS spectra showed a linear relationship of the concentration range of F^- (1×10^{-1} – 1×10^{-8} M) versus the membrane resistances. Results showed that the proposed allosteric membrane electrodes possessed permselectivity toward Na^+ using F^- as an effector. This method can be potentially used for detection of low concentration of F^- in aqueous solution which usually gives low detection limit in a micromolar range as suffered from high hydration energy of F^- in water.

Future work

The allosteric polymeric membrane electrode can be potentially useful to other ions and molecules that can react with the phenylboronic acid derivative such as cyanide and saccharides. Therefore, detection of cyanide and saccharide can be developed using the same strategy described in this thesis.

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