บัลค์ออปโทดเซ็นเซอร์สำหรับการตรวจวัดไอออนตะกั่ว

นางสาวฉันทนา บัวล้อม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตมหาบัณฑิต
สาขาวิชาเคมี   ภาควิชาเคมี
คณะวิทยาศาสตร์   จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2552

BULK OPTODE SENSORS FOR DETECTION OF LEAD ION

Miss Chantana Bualom

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2009
Copyright of Chulalongkorn University

Thesis Title   BULK OPTODE SENSORS FOR DETECTION OF LEAD ION

By   Miss Chantana Bualom

Field of Study   Chemistry

Thesis Advisor   Assistant Professor Wanlapa Aeungmaitrepirom, Ph.D.
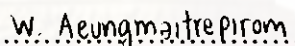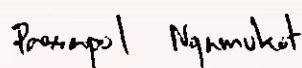
Thesis Co-Advisor Passapol Ngamukot, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree
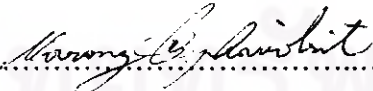
.... Dean of the Faculty of Science

(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

.... Chairman

(Assistant Professor Warinthorn Chavasiri, Ph.D.)

.... Thesis Advisor

(Assistant Professor Wanlapa Aeungmaitrepirom, Ph.D.)

.... Thesis Co-Advisor

(Passapol Ngamukot, Ph.D.)

.... Examiner

(Assistant Professor Narong Praphairaksit, Ph.D.)

.... External Examiner

(Wittaya Ngeontae, Ph.D.)

ฉันทนา บัวล้อม : บัลค์ออปโทดเซ็นเซอร์สำหรับการตรวจวัดไอออนตะกั่ว. (BULK OPTODE SENSORS FOR DETECTION OF LEAD ION), อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. วัลภา เอื้องไมตรีภิรมย์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร. ภัสสร์พล งามอุโฆษ, 74 หน้า.

ออปโทดเมมเบรนที่มีสภาพไวสร้างจากไอโอโนฟอร์ที่มีความจำเพาะสูงกับไอออนตะกั่ว โครโมไอโอโนฟอร์ (ETH 5294) และ แคทไอออนเอกซ์เชนจ์เจอร์ (KTpClPB) ในพีวีซี ออปโทด เมมเบรนใช้สำหรับตรวจวัดไอออนตะกั่วโดยเทคนิคการวัดการดูดกลืนแสงในระบบแบทช์และ ระบบไหล ศึกษาปัจจัยที่มีผล เช่น พีเอชของสารละลาย ชนิดของสารละลายบัฟเฟอร์ ความเข้มข้นของสารละลายปรับสภาวะและสารละลายตัวชะ เวลาในการปรับสภาวะและการชะ ไอออนตะกั่วออกจากเมมเบรน และเวลาการตอบสนอง เซนเซอร์นี้ตอบสนองต่อไอออนตะกั่วโดย เปลี่ยนจากสีฟ้าเป็นสีชมพูม่วงในสารละลายบัฟเฟอร์ทริส พีเอช 7.0 ที่มีไอออนตะกั่วความเข้มข้น ต่าง ๆ มีช่วงการตอบสนอง 3.16×10^{-6} - 5.0×10^{-5} โมลต่อลิตร และขีดจำกัดการตรวจวัดเท่ากับ 2.49×10^{-6} โมลต่อลิตรในระบบแบทช์ โดยใช้เวลาในการตอบสนอง 30 นาที การตอบสนองใน ระบบไหลอยู่ในช่วงความเข้มข้น 1.26×10^{-6} - 3.16×10^{-5} โมลต่อลิตร ขีดจำกัดการตรวจวัดเท่ากับ 8.97×10^{-9} โมลต่อลิตร โดยใช้เวลาในการตอบสนอง 15 นาที นอกจากนี้ พบว่า ออปโทด เมมเบรนมีความจำเพาะสูงต่อไอออนตะกั่วมากกว่าไอออนโซเดียม โพแทสเซียม แมกนีเซียม แคดเมียม ปรอท และเงิน การประยุกต์ออปโทดในการตรวจวัดไอออนตะกั่วในตัวอย่างน้ำต่าง ๆ ประสบความสำเร็จ และจากการเปรียบเทียบกับการตรวจวัดด้วยอินดัคทีพลีคัพเพิลพลาสมา ออพติคอลอิมิสชันสเปกโทรเมตรี พบว่า ผลการทดลองทั้งสองวิธีไม่แตกต่างกันอย่างมีนัยสำคัญ ที่ระดับความเชื่อมั่น 95 เปอร์เซนต์

ภาควิชา	เคมี	ลายมือชื่อนิสิต	ฉันทนา บัวล้อม
สาขาวิชา	เคมี	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก	
ปีการศึกษา	2552	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม	

5072248623 : MAJOR CHEMISRTY
KEYWORDS : BULK OPTODE / LEAD ION / ETH 5294 / SPECTROPHOTOMETRY

CHANTANA BUALOM: BULK OPTODE SENSORS FOR DETECTION OF LEAD ION. THESIS ADVISOR: ASST. PROF. WANLAPA AEUNGMAITREPIROM, Ph.D., THESIS CO-ADVISOR: PASSAPOL NGAMUKOT, Ph.D., 74 pp.

A sensitive optode membrane based on highly lead-selective ionophore, chromoionophore (ETH 5294) and cation exchanger (KTpClPB) in plasticized PVC was fabricated. The optode membranes were used for determination of Pb^{2+} by absorption spectrophotometry in batch and flow-through systems. The influence parameters such as pH solution, type of buffer, concentration of conditioning and regenerating solutions, time of condition and regeneration, and response time were studied. This sensor responded to Pb^{2+} by changing color from blue to pink purple in TRIS buffer containing different concentrations of Pb^{2+} at pH 7.0. The optode provided the response range of 3.16×10^{-8} to 5.00×10^{-5} mol L^{-1} Pb^{2+} with detection limit of 2.49×10^{-8} mol L^{-1} in batch system within the response time of 30 min. The dynamic range of 1.26×10^{-8} to 3.16×10^{-5} mol L^{-1} Pb^{2+} with detection limit of 8.97×10^{-9} mol L^{-1} were obtained in flow-through system within the response of 15 min. Moreover, the proposed optode membrane showed good selectivity toward Pb^{2+} over Na^+, K^+, Mg^{2+}, Cd^{2+}, Hg^{2+} and Ag^+. It was successfully applied to determine Pb^{2+} in real water samples and the results were compared with inductively coupled plasma optical emission spectrometry (ICP-OES). No significant different value was found between both methods at 95% of confidence level.

Department : ___Chemistry___   Student's Signature _Chantana Bualom_

Field of Study : ___Chemistry___   Advisor's Signature _W. Aeungmaitrepirom_

Academic Year : ___2009___   Co-Advisor's Signature _P. Ngamukot_

ACKNOWLEDGEMENTS

CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Figure   **Page**

Figure   **Page**

LIST OF SYMBOLS AND ABBREVIATIONS

AAS	Atomic absorption spectroscopy
A	Absorbance of the chromoionophore for a giving equilibrium
A_{prot}	Absorbance values of fully protonated of the chromoionophore
A_{deprot}	Absorbance values of fully deprotonated of the chromoionophore
aq	Aqueous sample phase
C	Chromoionophore
[C]	Concentration of deprotonated chromoionphore,
$[CH^+]$	Concentration of protonated chromoionphore
$[C]_{tot}$	Total concentration of chromoionphore.
°C	Degree celsius
DL	Detection limit
D_M	Distribution coefficient of the analyt M between phase
D	Diffusion coefficient
EDTA	Ethylenediaminetetraacetic acid
$g\ cm^{-1}$	Gram per centimeter
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
ISEs	Ion selective electrodes
J	Diffusion flux
K	Kelvin
K_{ML}	Complexation constant in membrane phase
L	Ionophore
M	Meter
mem	Membrane phase
mg	Milligram
$mol\ kg^{-1}$	Mole per kilogram
mL	Millilitre
$mol\ L^{-1}$	Mole per litre

mol m^{-3}	Mole per cubic meter
m^2 s^{-1}	Square meter per second
mol m^{-2}s^{-1}	Mole per square meter second
mL min^{-1}	Mililiter per minute
nm	Nanometre
PVC	Poly(vinyl chloride)
R$^-$	Cation exchanger or lipophillic anionic sites
RFA	Rapid flow-through analysis
SSM	Separated sample solution method
t	Time
THF	Tetrahydrofuran
TRIS	Tris(hydroxymethyl) aminomethane
UV-VIS	Ultraviolet visible spectroscopy
w/w	Weight by weigth
WHO	World health organization
α	Degree of protonation chromoionphore

CHAPTER I

INTRODUCTION

1.1 Statement of the Problem

Many worldwide countries are increasing awareness of environmental problems and decreasing environmental pollutions. In particular, the contamination of heavy metals is one of the serious problems because their low concentrations can cause harmful effect to plants, animals and human. Among heavy metals, lead is a common toxic pollutant in the environment as a result of its use in storage batteries, cable sheath, gasoline antiknock products and paint pigments [1]. The widespread uses cause environmental and health problems. The common technique for determination of lead is atomic absorption spectrometry (AAS) because it offers high sensitivity and high accuracy. However, it has some drawbacks because of matrix interference and also the detection limit of instrument is not compatible with the presence of lead in environmental samples. Moreover, World health organization (WHO) guidelines have permitted the maximum allowable lead concentration in drinking water to be 0.01 mg L^{-1} [2]. Therefore, the highly sensitive, selective and rapid method for determination of trace level of lead is desirable.

The use of chemical optical sensors (optode) for determination of metal ions can be considered as an alternative detection device to other types of sensor, which offer advantages such as simple preparation and procedure, relatively fast response, wide response range, reasonable selectivity and high sensitivity [3-5]. Recently, reviews covering the principles and mechanisms of bulk optode technique have been described [6-7]. The developments of bulk optode technique for determination of lead and other metal ions have been published [8-16]. However, the disadvantage of optical chemical sensors for determination of Pb^{2+} is their relatively long response times at low concentration levels. Meanwhile, the very low detection limit is still demanding.

1.2 Research objectives

The objectives of this work were focused on the development of selective determination of Pb^{2+} using bulk optode sensor and the improvement of response time.

1.3 Scope of the research

Initially, the optode membranes containing a lead-selective ionophore, a proton-selective chromoionophore and a cation exchanger (or lipophilic anionic sites) in plasticized poly(vinyl chloride) (PVC) matrix were prepared. Then, the influences of several parameters on response of optode membrane were carried out. In batch system, the parameters such as pH, types of buffer solution, response time, concentration of regenerating and conditioning solutions, times of condition and regeneration were investigated. The effects of flow rate and response time were investigated in flow-through system. Afterwards, the analytical performance of proposed method was evaluated in parameters of dynamic range and detection limit by using spiked known lead standard in the test solution. Finally, the optimum conditions were applied to real water samples with spiked method.

1.4 Benefits of the research

In this work, we expected to see further improvement of the response time for determination of Pb^{2+} at low concentration using lead-selective optode membrane coupling with flow-through cell. Moreover, the proposed method can be applied to determine Pb^{2+} in real water samples.

CHAPTER II

THEORY AND LITERATURE REVIEWS

2.1 Chemistry of lead [17]

Metal ions in aqueous solution require reaching a state of maximum stability through chemical reactions. Acid-base, precipitation, complexation and oxidation-reduction reactions all provide means through which metal ions in water are transformed to more stable forms. The solubility constant, complexation equilibrium constant, redox potential and pH affect to chemistry of Pb^{2+} in aqueous solution.

In this section, lead is represented in a detail of chemical property, principle and mechanism of bulk optode technique, determination of lead and optode membrane for selected metal ions.

Lead is the last member of the carbon family but in keeping with the general tendency for metallic character to increase with atomic number in a family. It shows typical metallic property which has an atomic number of 82, atomic mass of 207.19 and specific gravity of 11.34 g cm^{-1}. It has weak electropositive characteristic and strong electron accepter property. Lead forms compounds which has the oxidation state (IV) and (II). The former state is found in the organometallic compounds that have considerable environmental significance. Overall, lead compounds show great toxicity and cause environmental problems. Pb^{2+} forms comparatively covalent bonds with appropriate donor group in complex, generally favoring sulfur and nitrogen over oxygen donor.
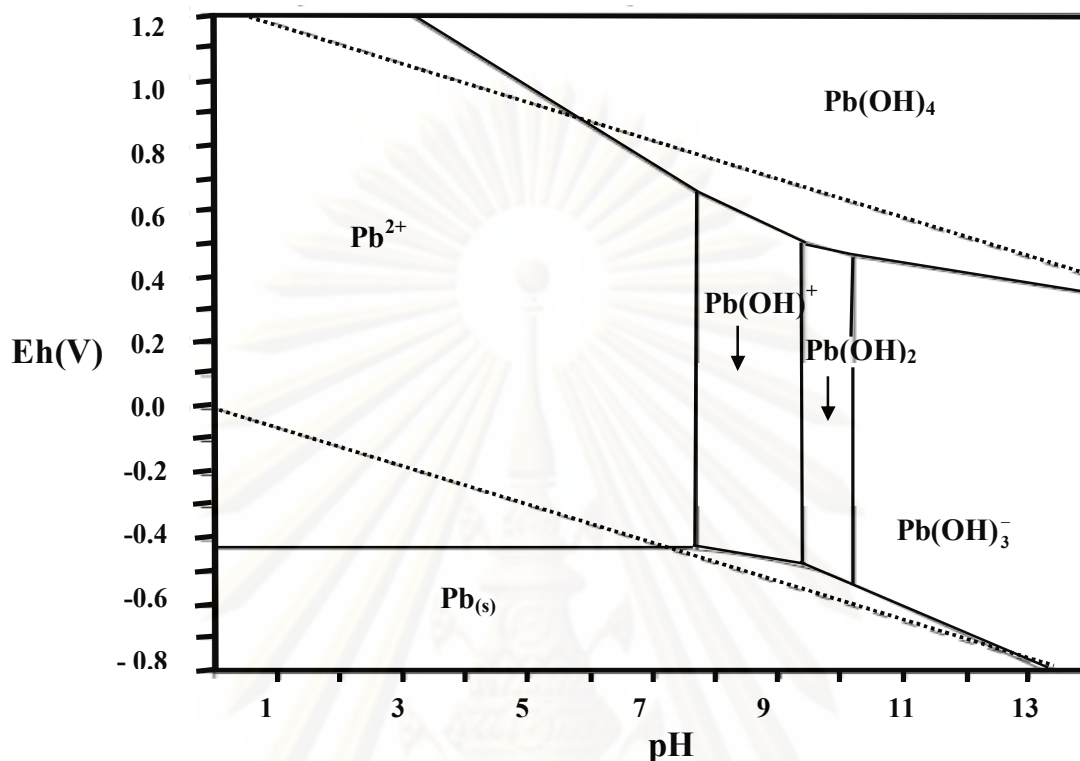
Figure 2.1 Eh-pH diagram of system Pb-O-H at 298 K, 10^{-5} Pa, modified from atlas of Eh-pH diagrams of Takeno, N. [18].

     The Eh-pH diagram for the lead system in water is shown in Figure 2.1. Forms of lead depend on pH and Eh. Pb^{2+} is the predominant species at relatively high hydrogen-ion activity and low electron activity (an acidic oxidizing medium). In basic reducing medium, with a very low hydrogen-ion activity and relatively high electron activity, lead performs hydrolysis reaction and displays multiple hydrolysis reactions giving $Pb(OH)^+$, $Pb(OH)_2$ and $Pb(OH)_3^-$. In oxidizing medium, $Pb(OH)_4$ is the primary species present. Pb(s) is stable in very high reducing medium.

     Currently, the toxicity of lead in the environment has caused extensive concern. Lead and its compounds play an important role in many industries that cause the pollutant to release environment. Various application and uses of lead are shown in Table 2.1. It can serve as origins of ingested lead in humans which enters our body system though air, water and food resulting lead poisoning. The levels of lead accumulate in air depend on location and weather. It is derived from soil and rocks, fallout, dust and vehicular

exhausts flow into water. Lead is a cumulative poison and can act through long-term ingestion of relatively small quantities which can be retained in the body for long periods, especially in bones. The acute lead poisoning in humans causes severe dysfunction in the kidneys, reproductive system, liver, brain and central nervous system and eventually causes death. Mild lead poisoning causes anemia, headache, sore muscles and irritable [19].

Table 2.1 Some applications and uses of lead [17]

	Application and uses
As metal	
Batteries	Lead-acid storage batteries are most widely used.
Water pipes	Some many still exist in old construction
Solder	As an alloy
Structural use	Used for roofing, glass mounting, etc.
Radiation shielding	As an alloy, also contains antimony and arsenic
Shot	Some bearing compositions
As inorganic lead	
Glass	Increases index of refraction; gives sparkle to crystal
Paints	White pigment and base, also colored pigments
Stabilizers in plastics	Heat stabilizer in PVC (often as organic acid salt)
As organic lead	
Gasoline additives	Generally phased out in undeveloped nations

According to WHO's guidelines for drinking water, maximum acceptable concentration of toxic metals are low (Table 2.2). Therefore, the determination of toxic metal at low level concentration is important for human health.

Table 2.2 WHO's guidelines for drinking-water quality, 2006 [2]

Element	Maximum acceptable concentration (mg L^{-1})
Arsenic (As)	0.01
Barium (Ba)	0.7
Cadmium (Cd)	0.003
Chromium (Cr)	0.05
Copper (Cu)	2.0
Lead (Pb)	0.01
Manganese (Mn)	0.4
Mercury (Hg)	0.006
Nickel (Ni)	0.07
Selenium (Se)	0.01

2.2 Principle and mechanism of bulk optode technique

An optode or optrode is an optical sensor device that optically measures a specific substance usually with the aid of a chemical transducer [20].

The principles and mechanisms of bulk optode technique based on plasticized PVC are described in term of selectivity, response time, dynamic measuring range, sensitivity and lifetime [6-7]. Optode membrane incorporates all components necessary for the recognition and extraction of the analyte. The optode membrane would extract the sensed ionic components into a plasticized polymeric membrane by mass transfer where the change of the optical signal of an incorporated component is detected by means of absorption or fluorescence. A simplified schematic representation of optode membrane based on the cation-exchange mechanism is illustrated in Figure 2.2.
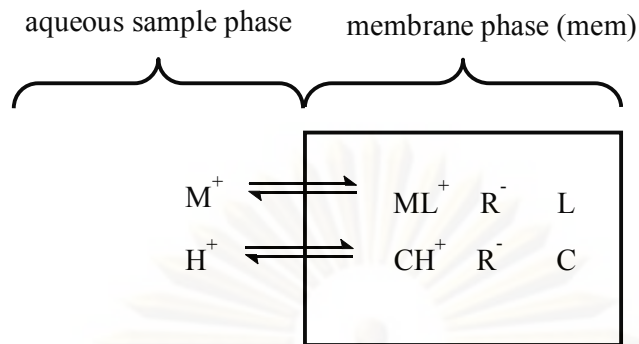
Figure 2.2 The neutral ionophore (L) based optode membrane with neutral chromoionophore (C) and negatively charged sites (R⁻). Square indicates species in the membrane phase.

All the mathematical description of the optode response is based on the following seven assumptions [6]:

(i) Solid support: the sensing optode membrane is attached on one side to a solid support and contacts the sample solution on the other side. The suitable properties of solid support are chemically inert and impermeable for all species.

(ii) Equilibrium: all optodes are considered as reversible equilibrium system. Further, it is assumed that no membrane components are present in the sample and that the analyte in the membrane phase is completely complexed by ionophore.

(iii) Impurities: impurities in the membrane phase are not considered as a part of the equilibrium system.

(iv) Interface: the size of the interface is assumed to be small as compared with the membrane thickness.

(v) Aggregation, stoichiometry: it is assumed no occur aggregations of the active components in the membrane phase, and that the stoichiometry of the complexes formed does not change in the considered dynamic analyte measuring range.

(vi) State of reference: in most optode membranes the concentration of the components is expressed in mol L⁻¹ or mol kg⁻¹. Optode membranes have been developed for analytes present in different phases, so that for the sample the appropriate state of reference should be chosen.

(vii) Activity coefficients: the composition of the bulk membrane is not changed dramatically over the whole analyte measuring range. The activity coefficients of the membrane components are assumed to approach unity or to be constant.

2.3 Extraction mechanism

In the extraction process many different equilibrium are concerned. The extensive experience from liquid-liquid extraction system may explain and discuss of the mechanism.

The analyte extraction can be described by a three-step scheme: diffusion of the analyte through the boundary membrane in the sample given by Equation (1), phase transfer and complexation-decomplexation by ionophore (L) followed by Equation (2) and diffusion of the species within the membrane [6].

(i) distribution of the analyt M between the aqueous sample (aq) and the membrane phase (mem)

$$M\ (aq) \xrightleftharpoons{\quad D_M \quad} M\ (mem) \tag{2.1}$$
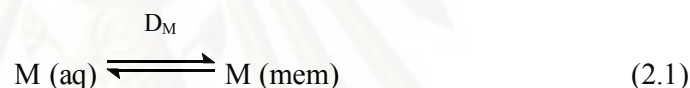
(ii) complexation in the membrane phase

$$L\ (mem) + M\ (mem) \xrightleftharpoons{\quad K_{ML} \quad} ML\ (mem) \tag{2.2}$$
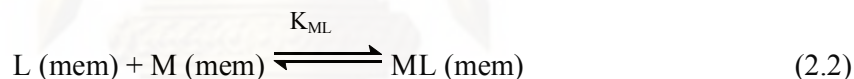
where D_M is distribution coefficient of analyte M between phase and K_{ML} is complexation constant in membrane phase by ionophore (L).

Recently, a novel analytical methodology called rapid flow-through analysis (RFA) based on bulk optode detection was proposed. RFA has been currently expanded for application to neutral ionophore-based ion-selective optodes [21-27]. The theoretical response equation for RFA is basically derived from Fick's law of diffusion and Lambert-Beer's law, which is further modified with the sample dispersion parameter in the flow system and the optode membrane phase, where these two parameters are experimentally obtained in certain systems, for the prediction of real flow-analytical response [3].

The concept of mass transfer is the movement of matter from a higher concentration to a lower concentration. This movement of the material is called diffusion. Diffusion can be represented by a basic equation that is referred to as Fick's law.

Fick's first law defines as a relationship wherein the diffusive flux to the concentration field that the flux goes from regions of high concentration to regions of low concentration:

$$J = -D\frac{\partial \phi}{\partial x}$$

(2.3)

where J is the diffusion flux, D is the diffusion coefficient (m^2 s^{-1}), ϕ is the concentration (mol m^{-3}) and x is the position (m). J measures the amount of substance that will flow through a small area during a small time interval (mol $m^{-2}s^{-1}$).

Fick's second law predicts how diffusion causes the concentration field to change with time:

$$\frac{\partial \phi}{\partial t} = D\frac{\partial^2 \phi}{\partial x^2}$$

(2.4)

where t is time.

2.4 Determination of lead

There is a great deal of demand for monitoring low level of lead in the environment because this toxic metal is now widespread, contaminating virtually the whole biosphere. The majority of lead determinations at the ppm-ppb levels are performed by using atomic absorption spectrometry (AAS) [28-30], inductively coupled plasma atomic emission spectrometry (ICP-AES) [31-32], inductively coupled plasma-mass spectrometry (ICP-MS) [33-36]. Although these techniques offer high sensitivity and high accuracy but which require well-controlled experimental conditions and are high cost and sophisticated performance. Thus, the numbers of preconcentration followed by spectrophotometric and flow injection spectrometric for determination of lead with

reported detection limit in range of ppb have been employed [37-47]. However, preconcentration step is tedious sample preparation and time-consuming.

In addition to the existing classical methods, a number of potentiometric ion-selective electrodes (ISEs) based on neutral compounds containing oxygen, nitrogen and sulphur donor atoms in molecule used as ionophores for lead selective electrodes have been published [48-59]. However, most of electrodes keep one or two or in some cases or all of the following drawbacks (i) high detection limit, (ii) narrow working concentration range and (iii) serious interferences from various cations such as Ag^+ Pb^{2+} and Cu^{2+} [60, 76].

Meanwhile, the development of bulk optode technique for determination of heavy metal ions include lead ion is one of the techniques in analytical chemistry which offer several advantages such as simple preparation and procedure, wide response range, reasonable selectivity and high sensitive, relatively fast response and no need for separate reference devices [4, 61].

2.5 Bulk optode for determination of selected metal ions

A multitude of optode membranes with a PVC matrix have been designed for many analytically relevant ions. The optical sensors for determination of heavy metal ions based on fundamentally different spectroscopic techniques have been presented (Table 2.3). Furthermore, the designs of optode membrane for determination of other metal ions have been presented [62, 66-67, 69-70, 72, 78, 80-81]. Unfortunately, since in 1992 Lerchi et al. [82] designed a new optode membrane that is the first report on lead-selective optode, a few of papers on bulk optode technique for determination of Pb^{2+} have been published so far (Table 2.4). The structure of selective ionophore for heavy metal ions were shown in figures 2.3 and figure 2.4 illustrated selective ionophore for lead ion.

Table 2.3 Ionophore and some characteristics of optode membrane for selected heavy meal ions in plasticized PVC membrane

Ionophore	Fluoroionophor Chromoionophore	Lipophilic ionic site	Plasticizer	Metal ion	Response time	Working range (mol L^{-1})	Detection limit (mol L^{-1})	Measured signal	Application or Remark	Ref.
-	TTBB	KTpClPB	NPOE	Ni^{2+}	<40 sec	10^{-8}-10^{-3} at pH 5.5	8.0×10^{-9} at pH 5.5	Fluorescence	Edible oil, water samples	[10]
MTZ	ETH5294	NaTPB	NPOE	Hg^{2+}	< 40 sec	$10^{-9.7}$-$10^{-4.8}$	5.0×10^{-11} at pH 7.0	Absorbance (batch and flow-through system)	Water samples	[27]
T-pico	ETH 5418	KTFPB	DOS	Hg^{2+}	10 min	$10^{-6.3}$-$10^{-3.3}$	5.0×10^{-7} at pH 4.7	Absorbance (batch system)	Interference with Ag^+, Pb^{2+},Cu^{2+},Ni^{2+}	[60]
MBDiBDTC	ETH 5418	KTm(CF$_3$)$_2$PB	DOS	Ag^+	500 min	$10^{-8.5}$-$10^{-4.5}$	2.5×10^{-9} at pH 4.7	Absorbance (flow-through system)	Interference with Hg^{2+},Environment sample	[63]
MBTBA	ETH 5418	KTm(CF$_3$)$_2$PB	DOS	Ag^+	-	$10^{-6.9}$-10^{-6}	1.6×10^{-9} at pH 4.5	Absorbance (flow-through system)	Interference with Ca^{2+} , water samples	[64]
L2	L2	PTCPB	TOP	Ag^+	2 min	$10^{-4.3}$-10^{-2}	5.0×10^{-6} at pH 5.9	Absorbance (flow-through system)	Interference with Hg^{2+}	[65]

Table 2.3 Ionophore and some characteristics of optode membrane for selected heavy meal ions in plasticized PVC membrane (continue)

Ionophore	Fluoroionophore Chromoionophore	Lipophilic ionic site	Plasticizer	Metal ion	Response time	Working range (mol L^{-1})	Detection limit (mol L^{-1})	Measured signal	Application or Remark	Ref.
HT18C6	Chromoionophore V	NaTPB	DOS	Hg^{2+}	5 min	$10^{-6.7}$-$10^{-3.9}$	2.0×10^{-7} at pH 4.0	Absorbance	Water samples	[68]
-	H2tpp	KTpClPB	DOS	Hg^{2+}	4 min	$10^{-6.6}$-$10^{-4.3}$	4.0×10^{-8} at pH 8.0	Fluorescence	Water samples	[73]
-	AQ	KTpClPB	NPOE	Cu^{2+}	40 sec	10^{-6}-10^{-2}	7.5×10^{-7} at pH 5.5	Fluorescence	Black tea samples	[74]
-	[12]aneNS$_3$	KTpClPB	NPOE	Ag$^+$	40 sec	$10^{-6.3}$-$10^{-1.8}$	1.0×10^{-7} at pH 6.5	Fluorescence	Medical radiological film, photographical, bleaching solution and water sample	[75]
-	L	NaTPB	DES	Co^{2+}	5 min	$10^{-6.3}$-$10^{-1.7}$	1.0×10^{-7} at pH 5.0	Fluorescence	Vitamin B12, cobalt cake ,alloy, water sample	[76]
-	Cu-I	NaTFPB	DOS	Ag^{1+}	15 min	10^{-12}-10^{-8}	4.0×10^{-11} at pH 7.4	Fluorescence (microsphere)	Pond water	[77]
-	H$_3$(tpfc)	NaTPB	DOS	Hg^{2+}	5 min	$10^{-6.9}$-10^{-4}	-	Fluorescence	Water samples	[79]

AQ =	1-hydroxy-2-(prop-2'-enyl)-4-(prop-2'-enyloxy)-9,10-anthraquinone		$KTm(CF_3)_2PB$ =	Potassium tetrakis [3,5-bis(trifluoromethyl)-phenyl]borate
[12]aneNS$_3$ =	1-(dansylamidopropyl)-1-aza-4,7,10-trithiacyclo dodecane		L =	7-[(5-chloro-8-hydroxy-7-quinolinyl)methyl]-5,6,7,8,9, 10-hexa hydro-2H-1,13,4,7,10 benzodioxa triazacyclo pentadecine-3,11 (4H,12H)-dione
Cu-I =	*o*-xylylenebis(*N*,*N*-diisobutyldithiocarbamate)		L2 =	9-(4-diethylamino-2-octadecyloxystyryl)acridine
DES =	Diethyl sebacate		MBDiBDTC =	Methylene bis(diethyldithiocarbamate)
DOS =	Bis (2-ethylhexyl)sebacate		MBTBA =	Methylene bis(2-thiobenzothiazole)
ETH 5294 =	9-(diethylamino)-5-(octadecanoylimino)-5H-benzo[a] phenoxazine		MTZ =	2-mercapto-2-thiazoline
ETH 5315 =	4-(octadecylamino)azobenzene		NaTFPB =	Sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate
ETH 5418 =	9-(diethylamino)-5-[4-(15-butyl-1,13-dioxo-2,14-dioxano decyl) henylimino] benzo [a]phenoxazine		NaTPB =	Sodium tetraphenylborate
H2tpp =	5,10,15,20 tetraphenylporphyrin		PTCPB =	Potassium tetrakis-4-(chlorophenyl)borate
H$_3$(tpfc) =	5,10,15-tris(pentafluorophorephenyl)corrole		TOP =	Tris-(2-ethylhexyl) sebacate
HT18C6 =	Hexathiacyclooctadecane		T-Pico =	Trityl-picolinamide
KTFPB =	Potassium tetrakis[3,5-bis(trifluoromethyl)phenyl] borate		TTBB =	2,5-thiophenylbis(5-*tert*-butyl-1,3-benzexazole)
KTpClPB =	Potassium tetrakis (4-chlorophenyl) borate			

Table 2.4 Ionophore and some characteristics of optode membrane for lead ion in plasticized PVC membrane

Ionophore	Chromoionophore Fluoroionophore	Lipophilic ionic site	Plasticizer	Response time	Working range (mol L^{-1})	Detection limit (mol L^{-1})	Measured signal	Application or Remark	Ref.
Lead IV	TMANB	KTpClPB	DOS	-	$10^{-6.7}$-$10^{-2.7}$ in 0.1 M HCl	-	Fluorescence	Interference with Ag$^+$ and Hg^{2+}, water samples	[14]
ETH 5493	ETH 5418	NaTFPB	DOS	15 min	$10^{-7.3}$-$10^{-4.0}$	3.0×10^{-9} at pH 5.7	Fluorescence	Interference with Cd^{2+}, Drinking water	[15]
-	TBHPP	PTCPB	DOP	4 min	$10^{-5.3}$-$10^{-3.4}$	6.0×10^{-8} at pH 7.0	Fluorescence	Soil samples	[16]
ETH 5435	ETH 5418	KTm(CF$_3$)$_2$PB	DOS	220 min	$10^{-8.3}$-$10^{-2.3}$	3.2×10^{-12} at pH 5.68	Absorbance (flow-through system)	Interference with Cd^{2+}, Ag$^+$ and Cu^{2+}, Hg^{2+} irreversible change in membrane	[82]
ETH 5493	ETH 2439	KTFPB	DOS	-	10^{-7}-10^{-2} at pH 5.0	-	Absorbance	Interference with Cd^{2+}, Cu^{2+} causes drifting signals	[83]
DBzDA18C6	PAN	NaTPB	NPOE	-	$10^{-8.0}$-$10^{-4.3}$	1.0×10^{-8} at pH 5.0	Absorbance	Use long time to regenerate	[84]

ETH 5435 = *N,N,N',N'*- tetradodecyl-3,6-dioxaoctanedithioamide

ETH 5493 = *N,N,N',N'*-tetradodecyl-3,6-dioxaoctane-1-thio-8-oxodiamide

ETH 2439 = 4-[[9-(dimethylamino)-5H-benzo[a]phenoxa-zine-5-ylidene] benzeneacetic acid 11-[(1-butyl-pentyl)oxy]-11-oxoundecyl ester

DOP = Bis-(2-ethylhexyl)phthalate)

DBzDA18C6 = 1,10-dibenzyl-1,10-diaza-18-crown-6

PAN = 1-(2-pyridylazo)-2-naphthol

TMANB = 3,3',5,5'-tetramethyl-*N*-(9-anthrylmethyl)benzidine

NaTFPB = Sodium tetrakis[3,5-bistrifluoromethyl] borate

Lead IV = *tert*-butylcalix[4]arene-tetrakis(*N,N*-dimetylthioacetamide

TBHPP = 5,10,15,20-tetra-(3-bromo-4-hydroxyphenyl)porphyrin

MBDiBDTC

MBTBA

MTZ

HT18C6

T-Pico

H2tpp

[12]aneNS₃

L2

H₃tpfc

TTBB

AQ
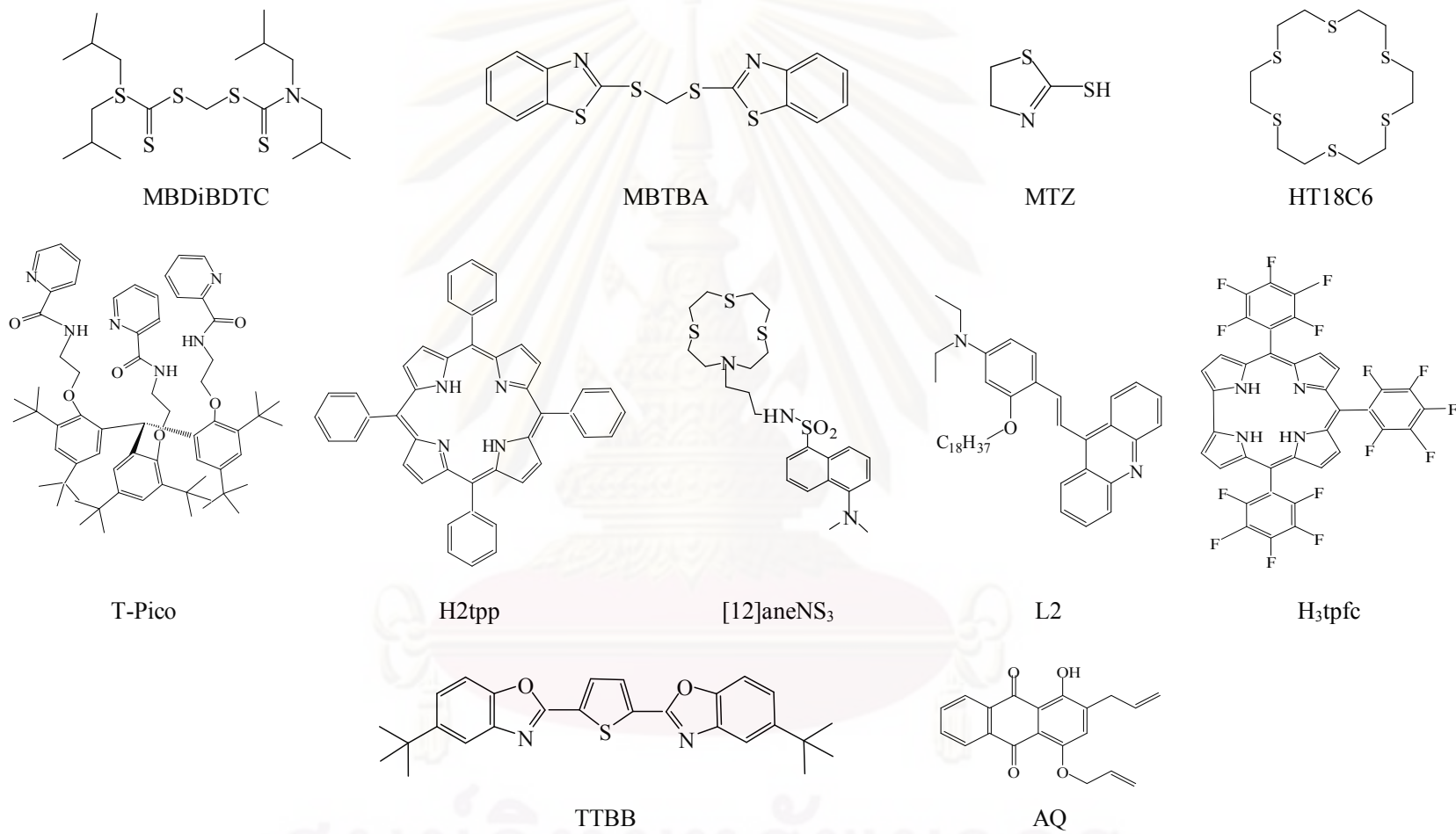
Figures 2.3 Some ionophores for heavy metal ions.
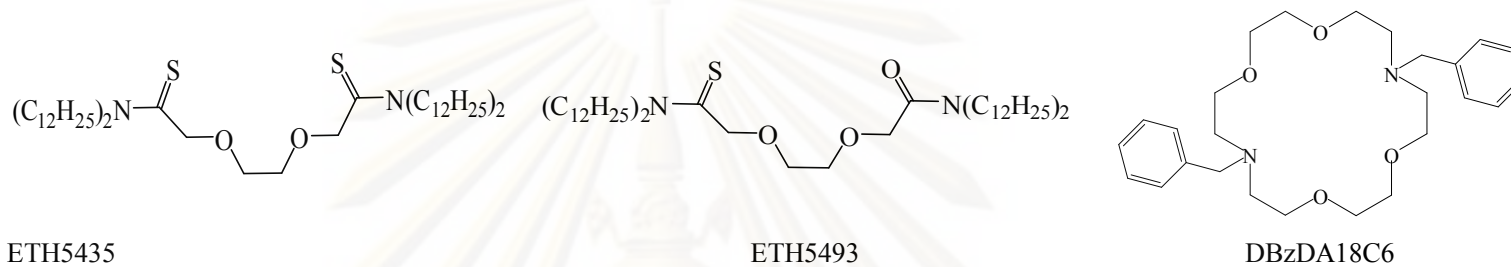
ETH5435

ETH5493

DBzDA18C6

TBHPP

R= CH₂C(S)N(CH₃)₂
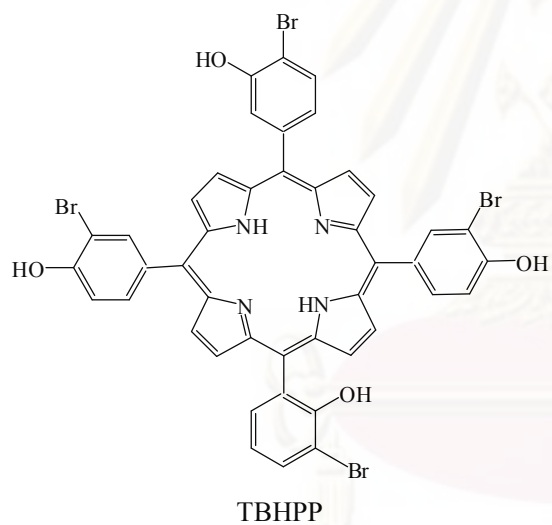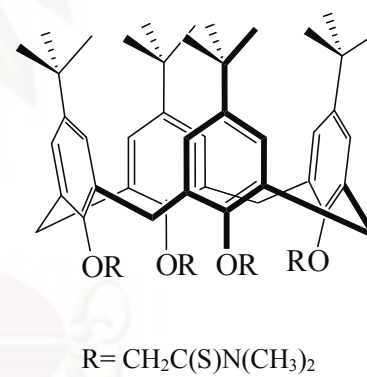
Lead IV

Figures 2.4 Some ionophores for lead ion

Usually, a design of ion-selective optode membrane is focused on the choice of ionophore/fluoroionophore and its optimization. However, one of shortcoming of bulk optode technique for determination of metal ions and Pb^{2+} is their long response time in highly diluted sample solutions and suffers from poor selectivity. Therefore, the high selective optode membrane for determination of Pb^{2+} in a short response time is required. The use of better designed optode membrane and the use of flow-through system for determination of analyte ion are proposed to reduce the response time meanwhile the detection limit will be improved.

In this work, the selective optode membrane incorporating *tert*-butylcalix[4]arene-tetrakis (*N,N*-dimetylthioacetamide) with sulphur-containing recognition site as lead-selective ionophore, ETH 5294 as proton-selective chromoionophore and potassium tetrakis (4-chlorophenyl) borate as lipophilic anionic sites plasticized in PVC membrane was designed. Then prepared optode membrane was used as sensing device for determination of Pb^{2+} in various water samples in batch and flow-through system. The optode membrane was placed in flow-through measuring cell for improving response time and detection limit of lead. These compositions of lead-selective optode membrane and its application have never been studied.

CHAPTER III

PREPARATION OF OPTODE MEMBRANE
AND ITS RESPONSES

3.1 Components of optode membrane

The major components of optode membrane in this work consist of *tert*-butylcalix[4]arene-tetrakis(*N,N*-dimetylthioacetamide) as ion-selective ionophore, 9-(diethylamino)-5-(octadecanoylimino)-5H-benzo[a]phenoxazine (ETH 5294) as a proton-selective chromoionophore and potassium tetrakis (4-chlorophenyl) borate (KTpClPB) as cation exchanger (or lipophillic anionic sites).

tert-Butylcalix[4]arene-tetrakis(*N,N*-dimetylthioacetamide) is of interest as lead-selective ionophore because the sulphur recognition sites are selective to Pb^{2+} corresponding well-established ionophores used in potentiometric ion-selective electrodes. Malinowska *et al*. [85] investigated lead-selective electrodes based on thioamide functionalized calix[4]arenes as ionophore. The obtained result showed the electrode based on tetrathioamide functionalized calix[4]arenes or *tert*-butyl calix[4]arene-tetrakis(*N,N*-dimetylthioacetamide) had good selectivity for Pb^{2+} in the presence of some transition, alkali, alkaline earth and metal ions. Wroblewski and Brzozka [86] used thioamidecalix[4]arene derivative to prepare potentiometric anion and cation selective membrane. The membrane without added lipophilic salts showed high selectivity for perchlorates over other anions while the added lipophilic salts gave good selectivity towards Pb^{2+}. Furthermore, Ceresa and Pretsch [87] determined formal complex formation constants of seven ionophores with Pb^{2+} and a series of cations interfering. The result revealed the calix[4]arene ionophore or *tert*-butyl calix[4]arene-tetrakis(*N,N*-dimetylthioacetamide) formed a very strong 1:1 complex with Pb^{2+}.

In order to produce satisfactory characteristics of the optode membrane, the chromoionophore must meet a number of requirements [88]:

- It must be as liphophilic as possible in order to minimize loss through leaching into the aqueous phase (Log P_{TLC} > 10.6).

- It must have a large molar extinction coefficient (ideally of the order of 100,000 l cm^{-1} M^{-1}) to allow for sufficient sensitive response from the thin membranes used in optode membrane.

- It must be chemically and photochemically stable under the conditions employed in the application in order to ensure adequate lifetime for sensor membranes.

- Finally, it must not bind cations other than protons so that any UV-VIS spectral changes can be ascribed solely to the proton exchange reaction.

9-(Diethylamino)-5-(octadecanoylimino)-5H-benzo[a]phenoxazine or ETH 5294 is one of phenoxazine dye derivatives that is the most commonly employed as chromoionophore in optode membrane [8, 27, 62, 66]. ETH 5294 is not only chromophore but also proton-selective ionophore. The colors of the protonated form and the neutral form of the chromoionophore are different. Its color change occurs when the metal ions are extracted into membrane while the protons are released from chromoionophore, resulting in a change of the optical properties.

There are two types of lipophillic ionic sites that can be used for preparation optode membrane. Lipophillic cationic sites (R^{+}) used in anion-selective optode membrane are commonly lipophilic tetraammonium salts while tetraphenylborate derivatives as lipophillic anionic sites (R^{-}) are employed for cation- selective system. The lipophillic anionic sites provide the optode membrane with necessary ion-exchange properties, because both the chromoionophore and ionophore are neutral therefore which cannot function as ion exchanger.

Potassium tetrakis (4-chlorophenyl) borate or KTpClPB is added to make the membrane be permeable only the one charge (positive charge in this work). TpClPB^{-} acts as lipophilic anionic sites in order to maintain the electroneutrality condition within the membrane phase. It is used to allow ionophore extraction of cationic analyte and inhibition of extraction of anions [68]. Furthermore, it has been reported

that the addition of a salt with a highly lipophilic anion (such as potassium tetrakis (4-chlorophenyl) borate) in the membrane phase may ensure a sufficiently high amount of cation in the membrane phase [63].

The nature of the plasticizer is also well known to affect the dynamic concentration range, selectivity behavior of optode membrane and facilitate the transport of analyte ions. In order to have homogenous membrane, plasticizer must be physically compatible with the polymer used in membrane preparation. The appropriate plasticizer must be selected so as to obtain a transparent and flexible membrane, which has the maximum response to the analyte.

Bis(2-ethylhexyl) sebacate or DOS is one of potential plasticizers for preparing optode membrane that has the best response toward analyte ion when compared to the other plasticizers [11, 60, 68, 73].

The choice of matrix for the optode membrane is governed by the parameters such as permeability for analyte, mechanical stability and suitability for plasticization of the major components and extractant [89].

Poly (vinyl chloride) or PVC is the most commonly used polymer for optode membrane because it forms fairly stable sensor layers and acts as a good solvent for both chromoionophore and ion exchanger [90].

The best membrane characteristic is usually obtained at ratio 2:1 (w/w) of plasticizer/PVC [10-11, 14, 72-74, 91].

All chemicals used were of selectophore® grade and were purchased from Fluka.

The structure of major components of lead-selective optode membrane is shown in Figure 3.1.

R= CH₂C(S)N(CH₃)₂

Lead-selective ionophore   ETH 5294

KTpClPB
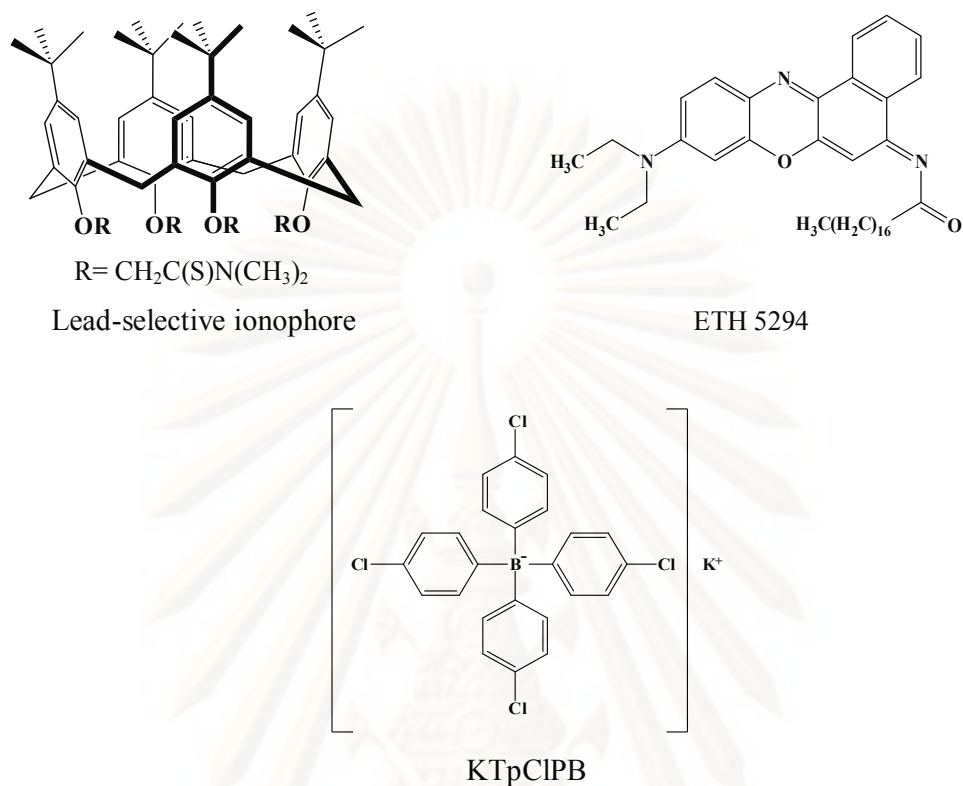
Figure 3.1 Major components of lead-selective optode membrane in this work.

3.2 Lead-selective optode membrane preparation

The cocktail solution was prepared as described by Wygladacz et al [92] and Ngeontae et al. [93]. The procedure is as follows:

0.95 mg of *tert*-butylcalix[4]arene-tetrakis(*N,N*-dimetylthioacetamide), 0.22 mg of KTpClPB, 0.12 mg of ETH 5294, 29.57 mg of PVC and 59.14 mg of DOS were dissolved in 2.0 mL of tetrahydrofuran (THF) in a glass vial. The mixture was immediately shaken to obtain clear homogeneous solution. The concentrations of three components are shown in Table 3.1. A typical membrane composition for the optode membrane is always kept in the order of $C < R^- < L$ [91].

Table 3.1 The concentration of major components of lead-selective optode membrane

Components	Symbol	mmol kg^{-1}
Lead-selective ionophore	L	10.0
Cation exchanger	R$^-$	5.0
Proton-selective chromoionophore	C	2.5

An aliquot of 50 µL cocktail solution was spread on a square microscope cover glass (22×22 mm) using micropipette at 23±1 °C and 40±4 % humidity. The homogenous, transparent and pinkish purple membranes were obtained. Prior to spread, the microscope cover glasses were cleaned with THF to remove organic impurities and dust. The membranes were dried at room temperature for at least 30 min before use and the resulting membranes were kept in a box. The lead-selective optode membrane preparation steps are illustrated in Figure 3.2.

| prepared cocktail solution | pipetted and spread | dried at room temperature | kept in box |
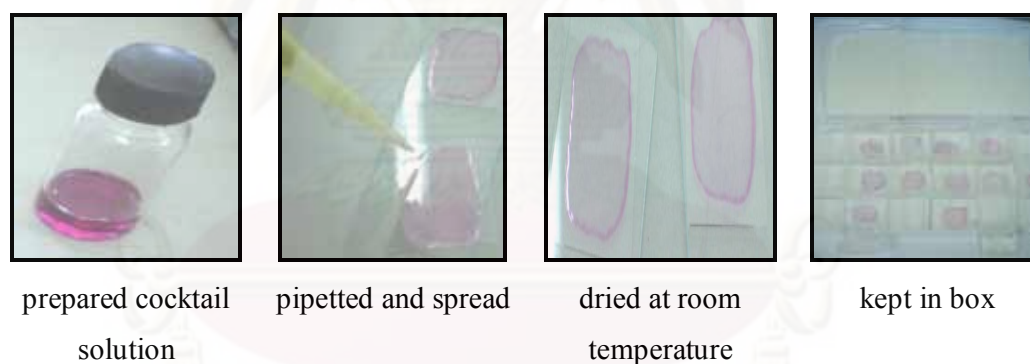
Figure 3.2 Lead-selective optode membrane preparation steps.

The absorption measurement of lead-selective optode membrane was carried out on a UV-VIS spectrophotometer (HP 8453, Hewlett-Packard). The optode membrane was conditioned by immersing in 0.01 mol L^{-1} HCl solution for 5 min in order to transform chromoionophore in membrane to completely protonated form resulting in a change color from pinkish purple to blue. The characteristic absorption bands of protonated chromoionophore were found at 616 and 660 nm as shown in Figure 3.3 A. The fully deprotonated form of chromoionophore was prepared by

immersing an optode membrane in a 0.01 mol L^{-1} NaOH solution for 5 min. The maximum absorption at 545 nm (Figure 3.3 B) was found.
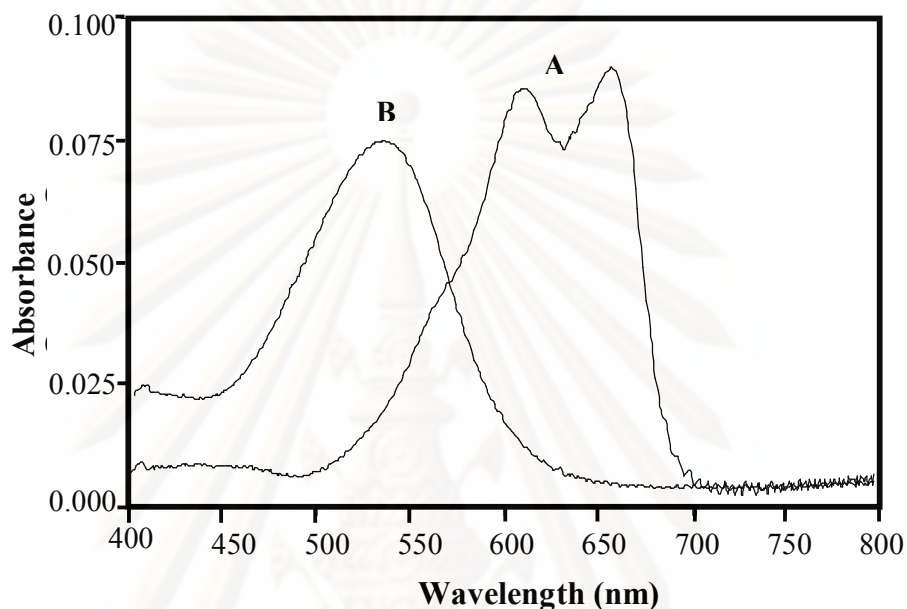
Figure 3.3 Absorption spectra of lead-selective optode membrane in fully protonated form (A) and fully deprotonated form (B) of chromoionophore.

3.3 Optode membrane response

The optode membrane for determination of Pb^{2+} in this work based on absorption spectrophotometry technique. A microscope cover glass without membrane was used as blank reference for absorbance measurement between 400-800 nm.

The optode membrane response can be defined as

$$\alpha = \frac{[C]}{[C]_{tot}} \qquad (3.1)$$

$$(1-\alpha) = \frac{[CH^+]}{[C]_{tot}} \qquad (3.2)$$

where $[C]$ is the concentration of deprotonated chromoionphore, $[CH^+]$ is the concentration of protonated chromoionphore and $[C]_{tot}$ is the total concentration of chromoionphore. The degree of protonation chromoionphore (α) is used to present the response function of optode membrane in absorbance mode. The measured absorbance at 660 nm corresponding to the protonated chromoionophore is directly related to the membrane response. If the optode membrane complies with Beer's law [94]:

$$\alpha = \frac{A_{prot} - A}{A_{prot} - A_{deprot}} \qquad (3.3)$$

where A is the absorbance of the chromoionophore for a giving equilibrium, A_{prot} and A_{deprot} are the absorbance values of fully protonated ($\alpha = 0$) and fully deprotonated ($\alpha = 1$) chromoionophore, respectively.

CHAPTER IV

RESULTS AND DISCUSSION

In this chapter, the optimization and influence parameters of optode membrane response were studied in batch and flow-through systems. Furthermore, the analytical performance of proposed optode and the application for determination of Pb^{2+} in real water samples under suitable conditions were evaluated.

4.1 Chemicals and apparatus

All chemical used were analytical-reagent grade and were used without further purification. The chemicals list is shown in Table 4.1

Table 4.1 Chemicals list

Chemicals	Supplier
Citric acid monohydrate, $C_6H_8O_7 \cdot H_2O$	MERCK
Ethylenediaminetetraacetic acid disodium salt dihydrate, $C_{10}H_{14}O_8N_2Na_2 \cdot 2H_2O$	MERCK
Hydrochloric acid 37%, HCl	MERCK
Lead nitrate, $Pb(NO_3)_2$	CARLO ERBA
Magnesium nitrate , $Mg(NO_3)_2 \cdot 6H_2O$	FLUKA
Mercury nitrate, $Hg(NO_3)_2$	MERCK
Nitric acid 65%, HNO_3	MERCK
Pb standard solution (1000 mg L^{-1})	MERCK
Potassium nitrate, KNO_3	BDH
Silver nitrate, $AgNO_3$	BDH
Sodium acetate, CH_3COONa	CARLO ERBA
Sodium hydroxide, NaOH	CARLO ERBA
Sodium nitrate, $NaNO_3$	CARLO ERBA
Tris(hydroxymethyl) aminomethane, $C_4H_{11} NO_3$	MERCK
Tri sodium citrate, $Na_3C_6H_5O_7 \cdot 2H_2O$	FISHER

The visible spectra and absorbance measurements were recorded on a Hewlett Packard diode array spectrophotometer, model 8453 (USA). Inductively coupled plasma optical emission spectrometric measurements were performed on Perkin Elmer, model PLASMA-1000 (USA). The pH values of sample solution were determined with a pH glass electrode, Orion 2 star, model 9162BNWP and a pH meter, Orion, OR3557 (Taiwan).

4.2 Preparation of chemical solutions

All aqueous solutions were prepared using ultrapure water from Milli-Q purify-cation system (Millipore). 1.0×10^{-3} mol L^{-1} of buffer solutions used were: (1) TRIS buffer (Tris (hydroxymethyl)-aminomethane), (2) acetate buffer (sodium acetate) and (3) citrate buffer (Tri sodium citrate), were prepared and adjusted to appropriate pHs with 0.01 mol L^{-1} HCl or 0.01 mol L^{-1} NaOH or 0.01 mol L^{-1} citric acid.

A stock solution of 1.0×10^{-2} mol L^{-1} Pb^{2+} was prepared by dissolving an appropriate amount of $Pb(NO_3)_2$ with Milli-Q water in an appropriate volumetric flask. Test solutions of Pb^{2+} were prepared by dilution of Pb^{2+} stock solution with buffer solution. 1.0×10^{-6} mol L^{-1} Pb^{2+} solution was diluted to concentration below 1.0×10^{-7} mol L^{-1} with same buffer solution. The Pb^{2+} solutions were buffered in order to provide nearly constant ionic strength. The low level concentration of measuring ions was studied therefore the activity coefficients in the aqueous solution were assumed to be constant so that the concentration of measuring ions was used for calculations.

Glasswares were pretreated with 5% HNO_3 overnight before used.

4.3 Extraction study in batch system

The influence of several parameters such as pH, type of buffer solution, concentration of conditioning and regenerating solutions, time of condition and regeneration, and response time were investigated.

4.3.1 General procedure for absorbance measurements

The absorbance measurements of the optode membrane were performed in three steps as following:

Step 1. An optode membrane was conditioned by immersing in a 0.01 mol L^{-1} HCl, as conditioning solution, for 5 min to obtain fully protonated chromoionophore and then rinsed with Milli-Q water. The absorbance of the conditioned optode membrane was recorded at 660 nm (A_{prot}) when a cover glass without membrane was used as blank [63, 66, 82].

Step 2. The conditioned optode membrane was rinsed with Milli-Q water and then exposed to a Pb^{2+} solution for 30 min or until equilibrium is achieved and then rinsed with Milli-Q water. The absorbances of the optode membrane were measured over the wavelength range of 400-800 nm.

Step 3. This optode membrane was regenerated by immersing in a regenerating solution to elute Pb^{2+} from the membrane and then rinsed with Milli-Q water. This membrane can be used in the first step. The same process was repeated for 3 times.

A fully deprotonated chromoionophore membrane was prepared by immersing in a 0.01 mol L^{-1} NaOH for 5 min and then rinsed with Milli-Q water. The absorbance of this optode membrane was also recorded at 660 nm (A_{deprot}).

The measured absorbance is directly related to the membrane response, thus $\alpha = (A_{prot} - A)/(A_{prot} - A_{deprot})$ [94]. The characteristic function of optode membrane showed a sigmoidal shape as predicted by theory which relate to response of optode membrane as (1-α) or α versus logarithm of concentration of Pb^{2+}. The experimental data was fitted on response curve of optode membrane by varying value of ion-exchange constant (K_{exch}^{opt}).

4.3.2 Effect of pH

One of the effective variables on response of optode membrane is pH of solution because the responses characteristics of optode membrane such as sensitivity, response concentration range and detection limit depend on the solution pH [83].

The effect of pH on response of optode membrane was carried out over the pH range of 3.0 - 9.0 which contained 3.16×10^{-6} mol L^{-1} Pb^{2+}. The pH of buffer solutions was adjusted by either 0.01 mol L^{-1} HCl or 0.01 mol L^{-1} NaOH. The absorbance of optode membrane was recorded at 660 nm after equilibration for 30 min. The experiments were performed in triplicate.

Figure 4.1 illustrated the degree of deprotonation (α) or the response as a function of pH. The degree of deprotonation increased from pH 3.0 to pH 7.0. The maximum response was obtained at pH 7.0. At pH > 7.0, the reduced response may be due to hydrolysis of Pb^{2+}. Moreover, the pK_a of chromoionophore is 11.41 ± 0.03 in DOS-PVC membrane [95], resulting in a partial deprotonation of chromoionophore at high pH and formation of $Pb(OH)_x$ species. The decreased response at low pH due to the extraction of proton (H^+) from the aqueous solution into the membrane.
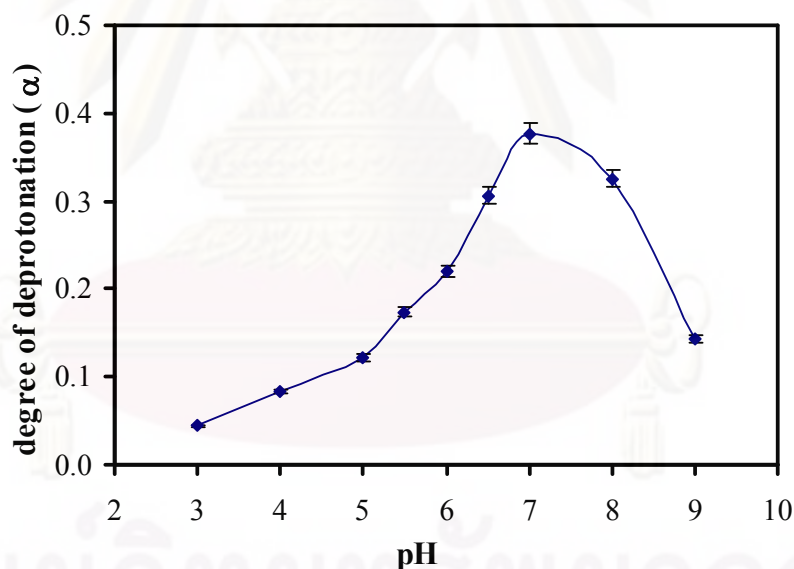
Figure 4.1 The effect of pH on the response of optode membrane at 660 nm in the presence of 3.16×10^{-6} mol L^{-1} of Pb^{2+}.

Subsequently experiment, the responses of optode membrane to different concentrations of Pb^{2+} (1.00×10^{-3} to 3.16×10^{-8} mol L^{-1}) at pH 5.0, 6.0, 7.0 were carried out because the degree of deprotonation sharply increased in these pH ranges. The absorbance of optode membrane was recorded at 660 nm after equilibration for 30 min. The experiments were performed in triplicate.

The response curve, as shown in Figure 4.2, shifted from lower to higher α value as pH of solution increased from 5.0 to 7.0. The best result was obtained at pH 7.0 because provide lower working concentration range and wide response range. Therefore, pH 7.0 was chosen as the working pH.
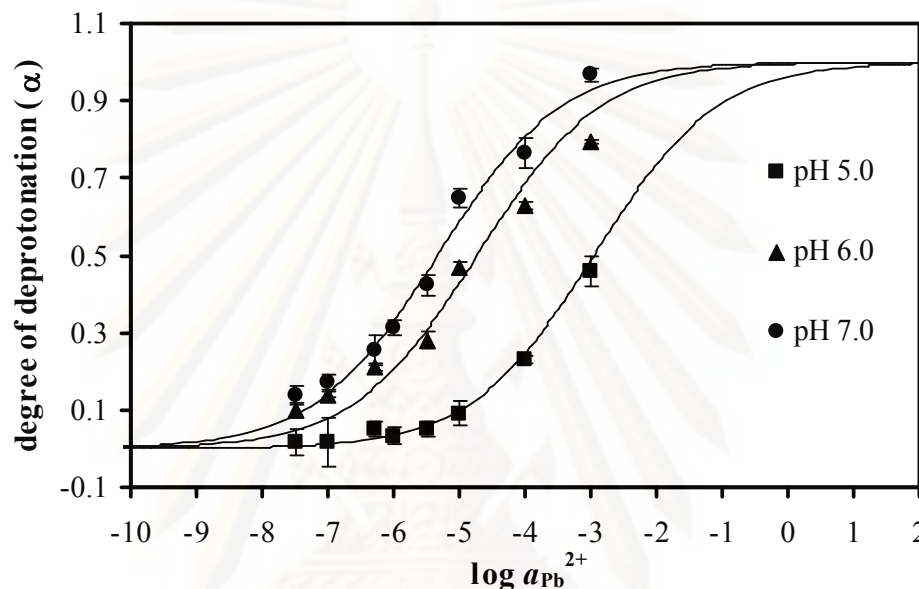
Figure 4.2 The response of lead-selective optode membrane at pH 5.0, 6.0, 7.0 in various concentrations of Pb^{2+}.

4.3.3 Type of buffer

The response of optode membrane is based on Pb^{2+} and H^+ exchange between membrane and aqueous phases. So the pH of test solution has to be kept constant by buffering. Thus, the used buffer solution in the experiment should not interference the measurement of Pb^{2+} system.

Type of buffer solution was investigated by using 1.00×10^{-3} mol L^{-1} of acetate, citrate and TRIS buffers adjusted pH by 0.01 mol L^{-1} acetic or citric or HCl or 0.01 mol L^{-1} NaOH to pH 7.0. The concentrations of Pb^{2+} in the range of 1.00×10^{-3} - 3.16×10^{-8} mol L^{-1} were used. The absorbance of optode membrane was recorded at 660 nm after equilibration for 30 min. The experiments were performed in triplicate.

Figure 4.3 showed the responses of optode membrane in different buffer solutions at pH 7.0. The lower response in citrate buffer was observed probably due to

the complex formation between Pb^{2+} and citrate [96]. Although the response in acetate buffer was good enough as in TRIS buffer but the pH of acetate buffer should be in the range of 3.0-6.2 [97]. The response of optode membrane in TRIS buffer had greater response range than those of acetate buffer and citrate buffer. Therefore, TRIS buffer was selected as buffer solution for further studies.
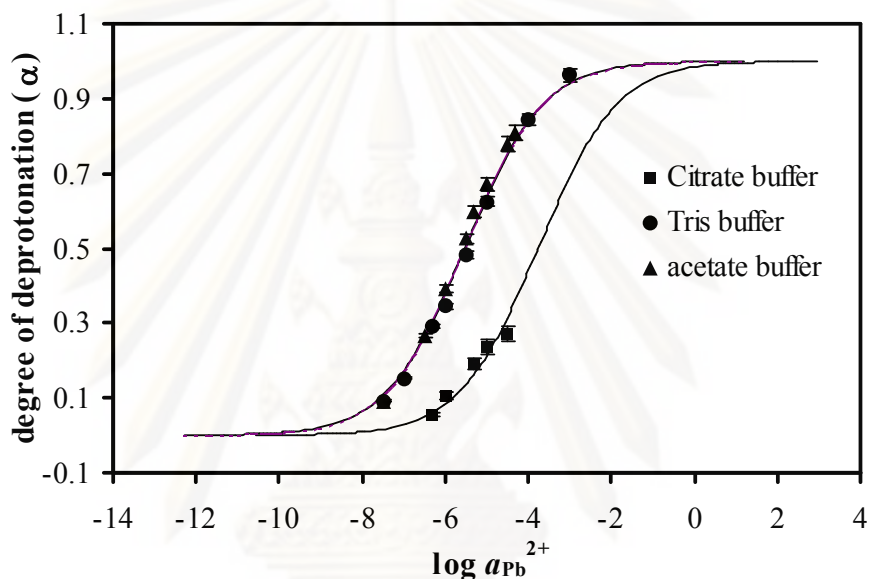
Figure 4.3 The different types of buffer solutions on response of optode membrane.

4.3.4 Response time

One of the important parameters of optode membrane is its response time. The response time of the optode membrane was controlled by the time required for analyte to diffuse from bulk of solution toward the membrane adducting by ionophore. The response time of the proposed optode membrane was defined as the time required to reach 99% (t_{99}) of steady signal absorbance. It would be desirable for the optode membrane to have a short response time. The response time of the optode membrane depended on the membrane thickness, membrane composition, activity of the measuring ion and pH of the measurement [88].

The response time of optode membrane was investigated by using TRIS buffer solution at pH 7.0 containing 1.00×10^{-5} or 3.16×10^{-5} mol L^{-1} Pb^{2+}. The absorbance of

optode membrane was recorded at 545 nm over a period of 60 min by recoding every 5 min intervals. This wavelength was chosen because the change of absorption increased when the concentration of Pb^{2+} increased. In addition, it was easy to observe the steady signal absorbance at 99%. The experiments were performed in triplicate.

Figure 4.4 illustrated the change of absorption at 545 nm of optode membrane with different concentrations of Pb^{2+} versus response time. The observation of 99% steady signal absorbance was found within 15 and 30 min for 1.00×10^{-5} and 3.16×10^{-5} mol L^{-1} Pb^{2+}, respectively. Therefore, the response time of 30 min was chosen for batch system.
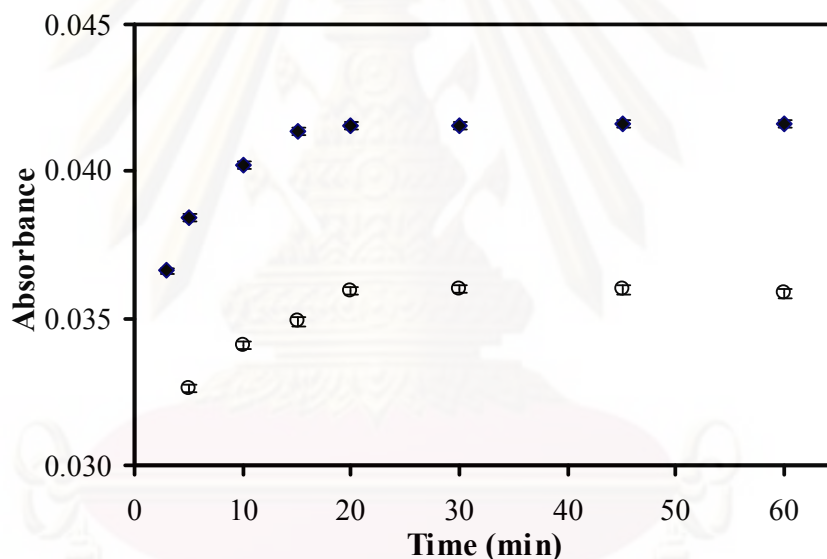
Figure 4.4 Response time of optode membrane in the presence of ∘1.00×10^{-5} and ♦ 3.16×10^{-5} mol L^{-1} of Pb^{2+}.

4.3.5 Type of conditioning solution

Conditioning solution was investigated by using 1.00×10^{-3} mol L^{-1} TRIS buffer and 0.01 mol L^{-1} HCl in order to obtain fully protonated chromoionophore. The absorbance of conditioned optode membrane was recorded at 660 nm between 5-60 min. The experiments were performed in triplicate.

Figure 4.5 showed the absorbance of optode membrane in function of time with different conditioning solutions. It was found that 0.01 mol L^{-1} HCl was suitable

conditioning solution because it provided a short time to reach maximum absorbance within 5 min.
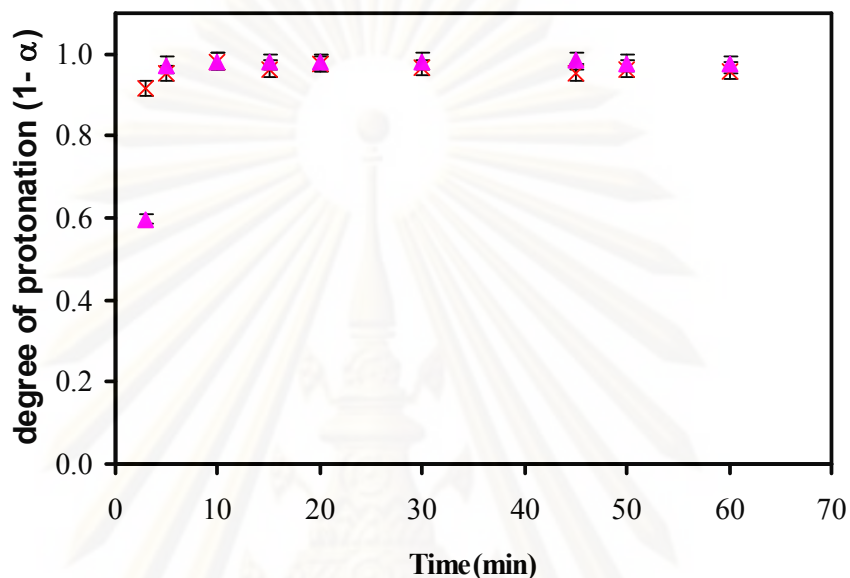
Figure 4.5 The response of optode membrane at 660 nm as a function of time in different conditioning solutions; ▲1.00×10^{-3} mol L^{-1} Tris buffer, × 0.01 mol L^{-1} HCl.

4.3.6 Type of regenerating solution

Several reagents such as HCl, HNO$_3$, and EDTA were used as regenerating reagents. HCl and HNO$_3$ were considered because Pb^{2+} was less extracted by optode membrane at low pH (obtained result in Figure 4.1). EDTA was chosen as regenerating solution because the formation constant between EDTA and Pb^{2+} is high [98]. The efficiency of the regenerating solution was considered as the regeneration time which was defined as the time taken for reaching the baseline signal (the signal observed in 0.01 mol L^{-1} HCl as conditioning solution), where the minimum absorbance has been reached at the wavelength of 545 nm.

The experiment of regenerating step was studied by soaking an optode membrane in TRIS buffer solution at pH 7.0 containing 3.16×10^{-5} mol L^{-1} Pb^{2+} and then rinsed with Milli-Q water. After that the optode membrane was immersed in 0.01 mol L^{-1} of the chosen regenerating solution. The absorbances of optode membranes in

different regenerating solutions were recorded at 545 nm versus time. The experiments were performed in triplicate. The results were shown in Figure 4.6.
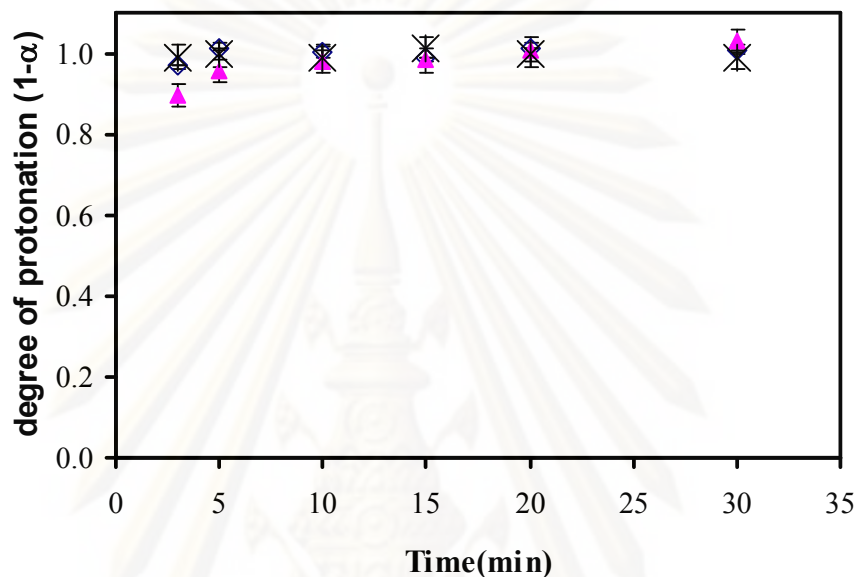
Figure 4.6 The response of optode membrane at 545 nm as a function of time in different regenerating solutions; ♦ 0.01 mol L^{-1} HCl, ▲ 0.01 mol L^{-1} EDTA, × 0.01 mol L^{-1} HNO$_3$.

It was found that all reagents could fully regenerate optode membrane within 3-5 min. In case of HCl solution, the color of the regenerated membrane did not change after immersing in Pb^{2+} solution at any response time. Therefore, HCl solution was not suitable to use as a regeneration solution. In case of EDTA and HNO$_3$ solutions, EDTA took longer than HNO$_3$ reagent at the same concentration. Thus, HNO$_3$ solution was used as regenerating solution. However, while the concentration of HNO$_3$ was increased up to 1.00 mol L^{-1} for accelerating and completing regenerated optode membrane, the result of regeneration of membrane remained the same. Therefore, 0.01 mol L^{-1} HNO$_3$ was chosen as regenerating solution within 3 min.

4.3.7 Response behavior

The diversities of designed optode membrane in plasticized PVC membrane with high selectivity for Pb^{2+} have been described [82]. Their response behavior was based on a cation-exchange system.

In this work, the proposed optode membrane contained *tert*-butyl calix[4]arene-tettrakis (*N,N*-dimetylthioacetamide) (L) as lead-selective and ETH 5294 (C) as proton-selective chromoionophore. In order to keep the electroneutrality of the membrane, KTpClPB cation exchanger (R^-) was also incorporated into membrane. The response of the proposed optode membrane was based on cation-exchange mechanism. The response process can be described by the followings:

The extraction of Pb^{2+} from sample solution into membrane phase and its complexation by ionophore (L) with the subsequent release of hydrogen ion from chromoionophor (CH^+) into aqueous sample phase. Therefore, the absorption spectra of the optode membrane were changed after equilibration in conditioning solution, TRIS buffer solution (pH 7.0) containing different concentrations of Pb^{2+} in the range of 3.16×10^{-8} - 5.00×10^{-3} mol L^{-1}. The absorption spectra of the optode membrane were recorded in comparison with the absorption spectrum of fully protonated chromoionophore (0.01 mol L^{-1} HCl) and the absorption spectrum of fully deprotonated chromoionophore (0.01 mol L^{-1} NaOH). The results were shown in Figure 4.7.
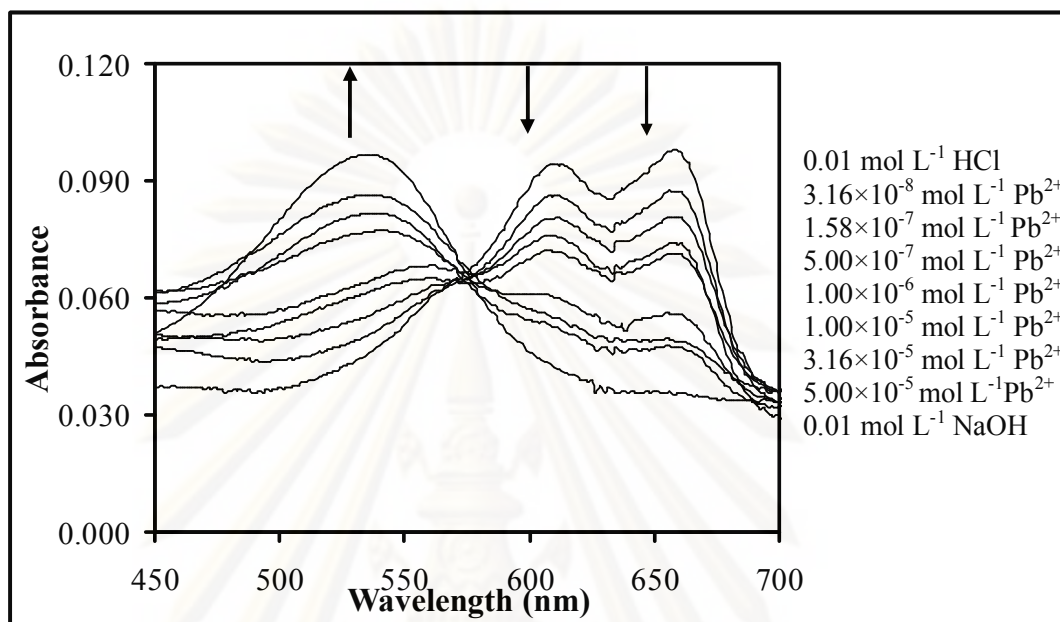
Figure 4.7 Absorption spectra of the proposed optode membranes after equilibration in TRIS buffer (pH 7.0) solution containing different concentrations of Pb^{2+}.

The absorption spectrum of the fully protonated membrane showed two absorption bands at 616 and 660 nm which correspond to protonated form of chromoionophore (CH^+) and the color of membrane was blue. When the concentration of Pb^{2+} increased, the deprotonation of chromoionophore occurred resulting in a change form blue to pink purple. Thus, a reduction in the absorption band at 616 and 660 nm and an increase in the absorption band at 545 nm were occurred.

If 1:1 ratio was assumed for the complexation of Pb^{2+} and ionophore (L) in the membrane phase, the over all equilibrium between membrane phase (mem) and aqueous solution (aq) can be described by Equation (4.1):

$$Pb^{2+}_{(aq)} + L_{(mem)} + CH^{+}_{(mem)} + R^{-}_{(mem)} \overset{K^{opt}_{exch}}{\rightleftharpoons} PbL^{2+}_{(mem)} + C_{(mem)} + H^{+}_{(aq)} + R^{-}_{(mem)} \qquad (4.1)$$
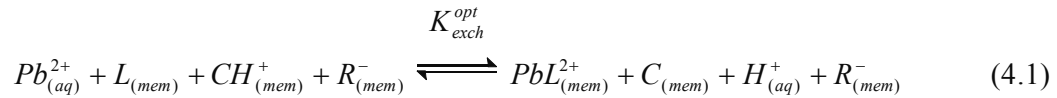
The ion-exchange constant (K^{opt}_{exch}) corresponding to the upper equilibrium was expressed by Equation (4.2):

$$K^{opt}_{exch} = \frac{[PbL^{2+}][H^{+}][C]}{[Pb^{2+}][CH^{+}][L]} \qquad (4.2)$$

where PbL^{2+} and CH^{+} represented the ionophore-lead complex and the protonated chromoionophore, respectively. The concentration term referred to the concentration of each species expressed in Equation (4.1). For electroneutrality reasons, the sum of the concentration of two cations must be equal to the total concentration of the accessible cation exchanger $[R^{-}]_{tot}$ in the membrane,

$$[R^{-}]_{tot} = [PbL^{2+}] + [CH]^{+} \qquad (4.3)$$

According to α value which may be defined as the ratio of concentration deprotonated chromoionphore, [C], in relation to the total amount present in membrane, $[C]_{tot}$, thus, [94]

$$\alpha = \frac{[C]}{[C]_{tot}}; \ (1-\alpha) = \frac{[CH^{+}]}{[C]_{tot}} \qquad (4.4)$$

In fact the detectable species in the membrane phase was the chromoionophore. Its absorbance was determined at the wavelength of the maximum absorption of its protonated form. Typically, the degree of protonation of chromoionophore was used to present the response function of optode membrane in absorbance mode. The measured absorbance was directly related to the membrane response, if the optode membrane complies with Beer's law: [94]

$$\alpha = \frac{A_{prot} - A}{A_{prot} - A_{deprot}} \tag{4.5}$$

where A was the absorbance of the chromoionophore for a giving equilibrium, A_{prot} and A_{deprot} were the absorbance values at the fully protonated ($\alpha=0$) and fully deprotonated ($\alpha=1$) chromoionophore, respectively. The response function for Pb^{2+} can be derived as follows in Equation (4.6): [6]

$$a_{Pb^{2+}} = \frac{1}{K_{exch}^{opt}} \left(\frac{\alpha\, a_{H^+}}{1-\alpha} \right)^z \left[\frac{R_{tot}^- - (1-\alpha)C_{tot}}{z(L_{tot} - \frac{n}{z}\{R_{tot}^- + (1-\alpha)C_{tot}\})^n} \right] \tag{4.6}$$

where z was the charge of Pb^{2+} (z = 2) and n was the ion-ionophore complex stoichiometry ($n = 1$). The logarithmic form of Equation (4.6) showed the dependence between the activity of Pb^{2+} and the degree of protonation of chromoionophore ($1-\alpha$) since all the other terms were constant for each analytical system. Plotting ($1-\alpha$) versus log a_{Pb}^{2+} a sigmoidal curve was obtained. All calculated curves were fitted to the experimental data by varying K_{exch}^{opt} in Equation (4.6). This confirmed the validity of Equation (4.1) in explaining the response mechanism of the proposed optode membrane toward Pb^{2+} and stoichiometry obtained in the solution phase. The response of the optimized optode membrane of Pb^{2+} was shown in Figure 4.8.
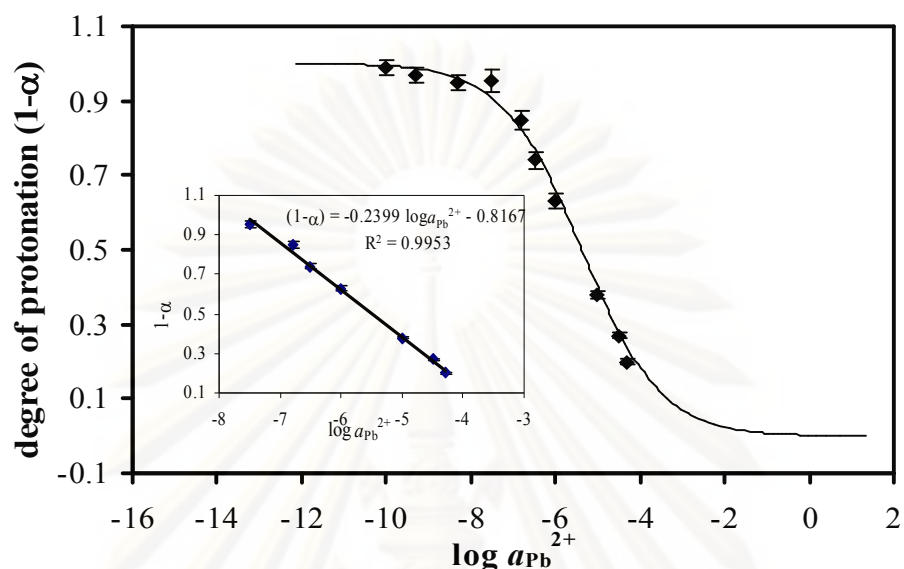
Figure 4.8 Response of the optode membrane in TRIS buffer solution (pH 7.0) containing $3.16.0 \times 10^{-8}$-5.0×10^{-5} mol L^{-1} of Pb^{2+}.

The corresponding response calculated with Equation (4.6) and $\log K_{exch}^{opt}$ = -9.2 as the function of the degree of protonation $(1-\alpha)$ values obtained with $\alpha = (A_{prot} - A)/(A_{prot} - A_{deprot})$ from the absorbance at 660 nm were obtained as a curve with linear regression equation of $(1 - \alpha = -0.2399 \log a_{Pb}{}^{2+} - 0.8167)$. The curve can serve as the calibration for determination of Pb^{2+} concentration. Moreover, the dynamic range was clearly obtained from 3.16×10^{-8} to 5.00×10^{-5} mol L^{-1} Pb^{2+}.

4.3.8 Repeatability and reproducibility

Optode repeatability and reproducibility are two of their important characteristic features, both of which were investigated. The repeatability of the optode sensor was performed by repetitive exposing the single optode membrane, which was prepared from the same cocktail solution. The single optode membrane was conditioned in 0.01 mol L^{-1} HCl then immersed in TRIS buffer solution (pH 7.0) containing 3.16×10^{-5} mol L^{-1} Pb^{2+} under the optimum conditions. The absorption of optode membrane was recorded at 545 nm. The optode membrane was regenerated in 0.01 mol L^{-1} HNO_3 solution after each measurement. The obtained result for

absorbance measurement at 545 nm and the relative standard deviation (R.S.D.) values of single optode membrane were summarized in Table 4.2 (n = 10).

Table 4.2 Absorbance from single optode membrane in TRIS buffer solution (pH 7.0) containing 3.16×10^{-5} mol L^{-1} Pb^{2+} under the optimum conditions

No. of replicate	Absorbance at 545 nm
1	0.090
2	0.090
3	0.089
4	0.087
5	0.087
6	0.087
7	0.087
8	0.085
9	0.084
10	0.084
mean	0.087
SD	0.002
% R.S.D	2.3

The relative standard deviation (R.S.D.) of absorbance was 2.3 % (n=10), indicated that the optode sensor have good repeatability.

The reproducibility was evaluated by measuring absorbance of twelve membranes, which were prepared in the same batch of the cocktail solution. The membranes were conditioned in 0.01 mol L^{-1} HCl after that they were exposed in TRIS buffer solution (pH 7.0) containing 3.16×10^{-5} mol L^{-1} Pb^{2+} at optimized conditions. Each membrane was performed in triplicate. The absorbance of the optode membranes were recorded at 545 nm. The results were shown in Table 4.3.

Table 4.3 Absorbance from different optode membranes in TRIS buffer solution (pH 7.0) containing 3.16×10^{-5} mol L^{-1} Pb^{2+} under the optimum conditions

No. of membrane	Mean absorbance at 545 nm
1	0.048
2	0.050
3	0.050
4	0.047
5	0.048
6	0.049
7	0.047
8	0.049
9	0.051
10	0.052
11	0.051
12	0.048
mean	0.049
SD	0.002
% R.S.D	4.1

The relative standard deviation (R.S.D.) of absorbance was 4.1% (n=12), indicated the acceptable reproducibility of the procedure used for preparation of optode membranes.

4.3.9 Short term stability and life time

The *short-term stability* of the optode membrane was defined as its stability of absorbance of optode membrane for at least 6 h in solution. [12, 60, 75] The optode membrane was immersed in TRIS buffer at pH 7.0, then the absorbance of this membrane was recorded at 660 nm over a period of 6 h by recoding every 30 min intervals (n=12). The results obtained were shown in Figure 4.9.
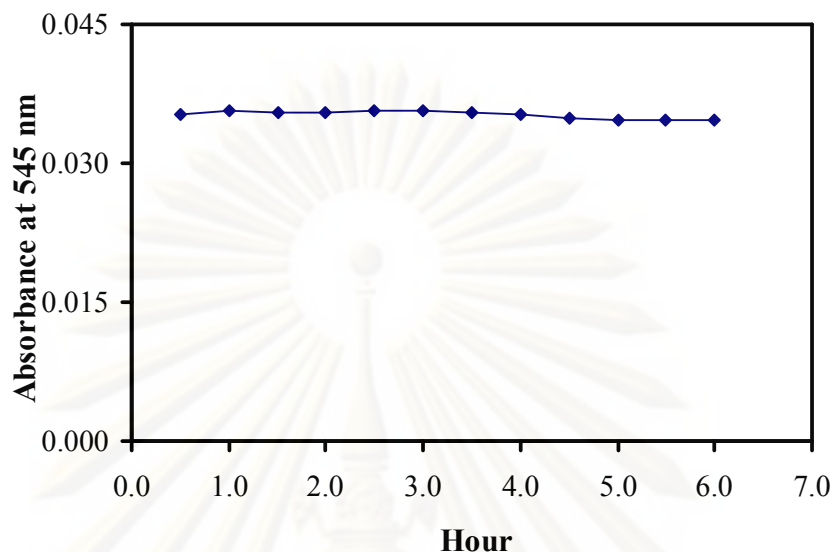
Figure 4.9 The short term stability of optode membrane in TRIS buffer solution at pH 7.0 over a period of 6 h.

The results showed that R.S.D. was only 1.0 % and a good short-term stability at least 6 h.

The *life time* of the optode membrane described by its stability of absorbance of optode membrane for at least a period of 30 days when the optode membrane was not in use (membrane was kept in ambient condition) [12, 60, 76] The absorbance of the optode membrane was daily recorded at 545 nm for a period of 30 days in comparison with the absorption values of a freshly prepared optode membrane. The optode membrane should keep in dark for long term study. The results were illustrated in Figure 4.10.
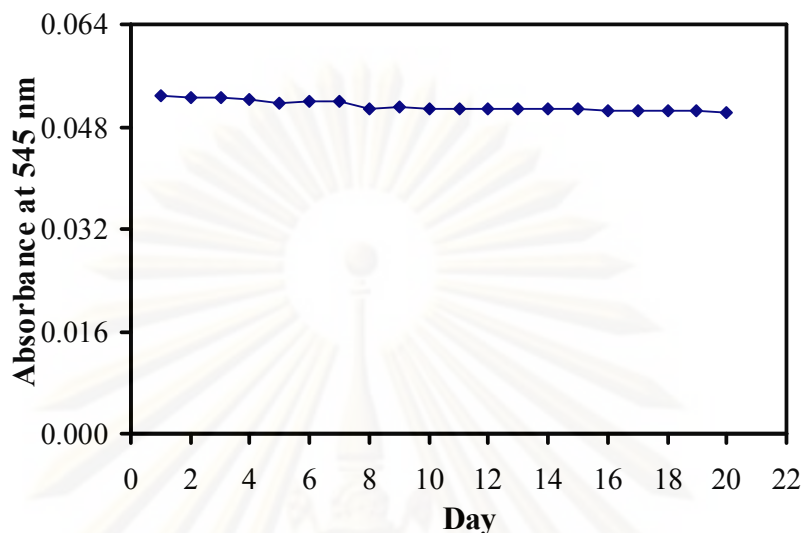
Figure 4.10 The life time of optode membrane kept in ambient condition
for a period of 20 days.

The observed absorption changed by 1.7, 3.9, 4.8 and > 10 % (with respect to
the first day) after 7, 15, 20 and 30 days, respectively. The R.S.D was 1.6 % for 20
absorption values. Therefore, the life time of optode membrane which stored in
ambient condition was at least 20 days when not in use. However, the prepared optode
membrane would be used in the practice within 7 days.

4.3.10 Selectivity

The selectivity behavior was obviously one of the most important
characteristics of an ion-selective optical sensor, which was the relative response of
optode membrane for the analyte ion over the other interfering ions present in
solution. Thus, the influence of the common interfering ions on response of the
proposed Pb^{2+} optode membrane was investigated. The selectivity coefficient depends
on pH and degree of protonation of chromoionophore [83]. The common interfering
ions were induced to study of selectivity by the relative stabilities of the ion-
ionophore complexes [87]. The optical selectivity of the proposed optode membrane
over common interfering ions was carried out by separated sample solution method
(SSM) [94]. The response of optode membrane in the presence of different
concentrations of each interfering ion was measured and selectivity coefficients

($K_{Pb,M}^{opt}$) determined by graphically plotting the corresponding ($1-\alpha$) versus log concentration of interfering ions (log C_M) followed by Equation (4.7).

$$K_{Pb,M}^{opt} = \frac{z^M}{2} \frac{K_{exch}^M}{K_{exch}^{Pb}} \left(\frac{\alpha a_{H^+}}{1-\alpha}\right)^{2-z^M} \frac{\left([L_{tot}]-\frac{n_M}{z^M}\{[R_{tot}^-]-(1-\alpha)[C_{tot}]\}\right)^{n_M}}{\left([L_{tot}]-\frac{n_{Pb}}{2}\{[R_{tot}^-]-(1-\alpha)[C_{tot}]\}\right)^{n_{Pb}}} \tag{4.7}$$

where z^M, z^{Pb} were the charge of the ion interfering ion M and Pb^{2+}, n_M, n_{Pb} were the ion interfering-ionophore complex stoichiometry and lead-ionophore complex stoichiometry, respectively.

The response of optode membrane of interfering ions was evaluated by preparing different concentrations of metal ions in TRIS buffer solution at pH 7.0 as follows:

3.16×10^{-8} to 1.00×10^{-3} mol L^{-1} Pb^{2+}

3.16×10^{-8} to 1.00×10^{-5} mol L^{-1} Ag^+

3.16×10^{-8} to 1.00×10^{-5} mol L^{-1} Hg^{2+}

1.00×10^{-6} to 3.16×10^{-4} mol L^{-1} Cd^{2+}

3.16×10^{-4} to 1.00 mol L^{-1} Mg^{2+}

0.01 to 1.00 mol L^{-1} Na^+

0.01 to 1.00 mol L^{-1} K^+

The optode membrane was conditioned in 0.01 mol L^{-1} HCl for 5 min then rinsed with Milli-Q water. The conditioned optode membrane was immersed in TRIS buffer solution (pH 7.0) containing different concentrations of interfering ions as mentioned above. The absorbance of each optode membrane in different concentrations of interfering ions was recorded at 660 nm. The response of optode membranes were illustrated in Figure 4.11.
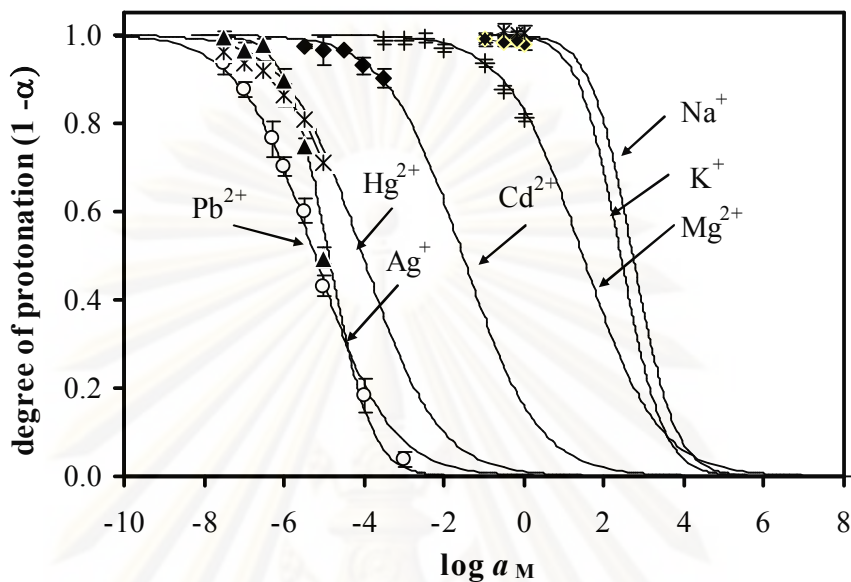
Figure 4.11 Response curves of the optode membrane in various interfering ions at pH 7.0.

The selectivity coefficients given in Table 4.4 were calculated at the highest sensitivity ($\alpha = 0.5$). The Pb^{2+} show high selectivity over hard alkaline metals: Na^+, K^+, Mg^{2+} and transition metals: Cd^{2+}, Hg^{2+}, Ag^+ due to its specific binding capability from ionophore results [87].

Table 4.4 The ion-exchange constant (K_{exch}^{opt}) and selectivity coefficents ($K_{Pb,M}^{opt}$) of the proposed optode membrane in TRIS buffer solution (pH 7.0) containing various concentrations of metal ions

Interfering ions	$\log K_{exch}^{M}$	$\log K_{Pb,M}^{opt}$
Pb^{2+}	-9.30	0
Ag^+	-2.20	-0.35
Hg^{2+}	-10.50	-1.20
Cd^{2+}	-13.00	-3.70
Mg^{2+}	-9.80	-6.70
K^+	-9.50	-7.65
Na^+	-16.00	-7.95

4.4 Extraction study in flow-through system

One of drawbacks for Pb^{2+} determination at lower concentration using bulk optode technique was relatively long response time. The time limiting step was not the diffusion within the membrane phase but the convective mass transport from the bulk of sample solution until the required absolute of analyte ion was reached [82]. The shorter response time was feasible by replacing the batch system to a continuous flow system [6]. Moreover, the use of better designed measuring cell and the use more than two but thinner membranes in contact with the sample solution or fluorescence measurements will reduce the response time [82]. So the flow-through measuring cell was designed in order to decrease the time required to reach equilibrium of lower concentration of Pb^{2+}.

The influence parameters such as response time and flow rate were investigated in flow-through system. The absorption measurement was similar to batch system, but the optode membrane was placed in flow-through cell and connected to peristaltic pump.

4.4.1 Home made flow-through cell

The home made flow-through cell (Figure 4.12 and Figure 4.13) consisted of the rectangular 38 mm × 30 mm acrylic block of 10 mm height with cylindrical opening of 12 mm diameter in the middle (a), rectangular 38 mm × 30 mm acrylic block of 10 mm height as optical widow (b) with inlet (c) and outlet tubes (d), rectangular 20 mm × 25 mm vacuum plastic sheet as seal gasket with elliptical opening of 17 mm (e), cover glass slide as support for optode membrane (f). All of part of flow-through cell was fixed screws for clamping the block (g). The flow-through cell system consists of a peristaltic pump Ismatec model ISM 827 (h), home made flow-through cell and connecting Tygon tubing R3607 (2.79 mm i.d.) (i), Teflon FEP tubing (0.50 mm i.d.) (j).

The arrangement of conditioned experiment was performed by setting the flow-through cell in optical path area of the spectrophotometer. The reference of flow-through cell consisted of a glass slid without membrane. Prior to analysis, flow-

through cell system was cleaned by 0.01 mol L^{-1} HNO$_3$ and followed by Milli-Q water for 5 min.
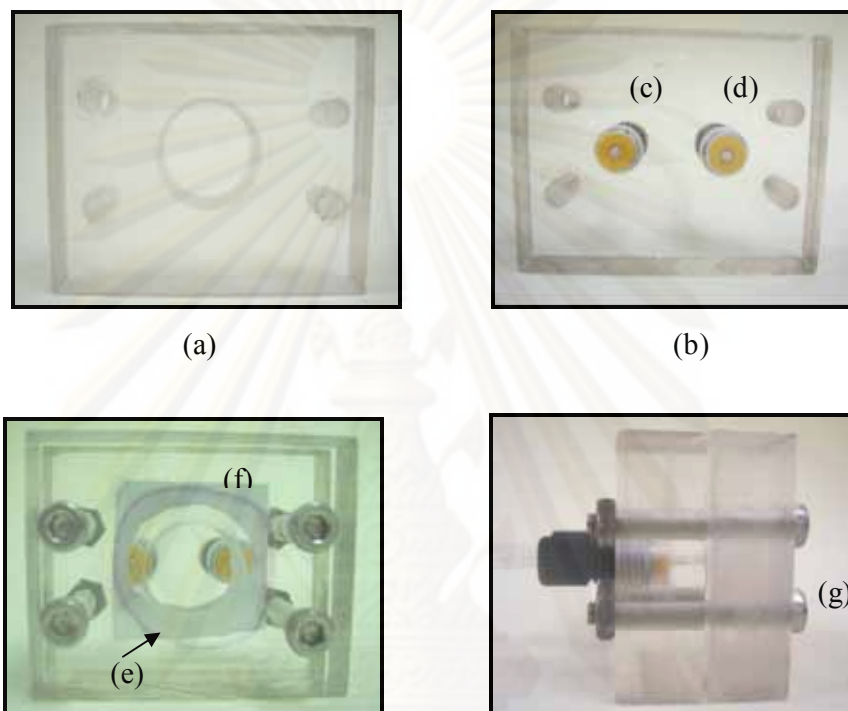
Figure 4.12 Parts of home made flow-through cell (a) acrylic block; (b) optical widow; inlet (c) and outlet tubes (d); seal gasket (e); optode membrane (f); fixing scews (g).
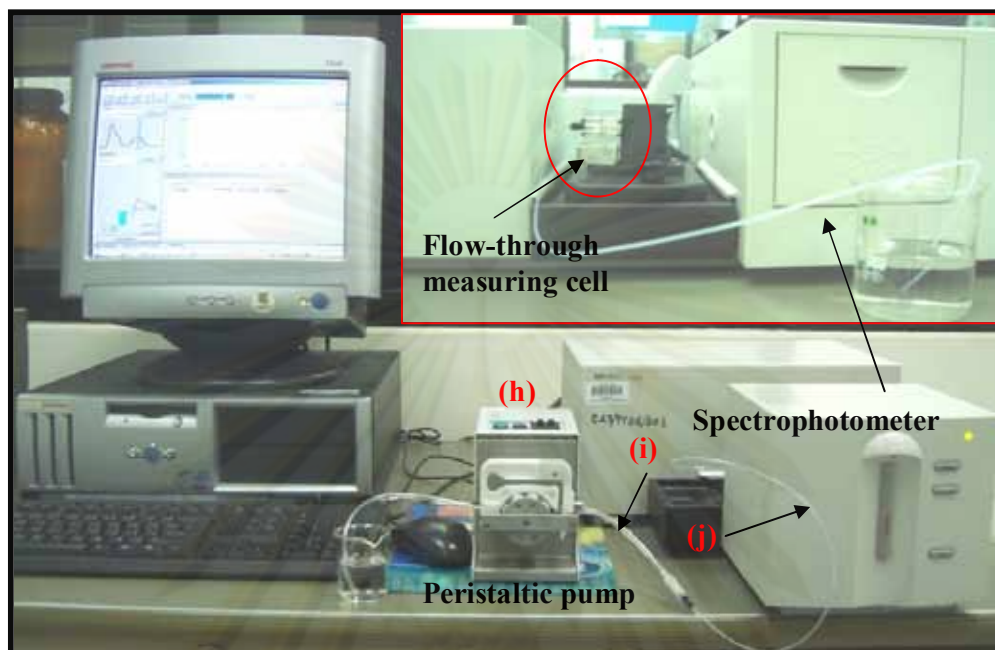
Figure 4.13 The arrangement of flow-through cell system; peristaltic pump (h); home made flow-through cell and connecting Tygon tubing (i), Teflon FEP tubing (j) in optical path area of the spectrophotometer.

4.4.2 Effect of flow rate

The flow rate of solution was an important parameter in flow-through system that affected equilibration of Pb^{2+} from solution into membrane.

A membrane was placed in the flow-through measuring cell. 0.01 mol L^{-1} HCl was passed through the membrane at a flow rate of 1.6 mL min^{-1} for 3 min in order to let chromoionophore be fully protonated form then milli-Q water was passed through the membrane at a flow rate of 1.6 mL min^{-1} for 3 min. TRIS buffer solution at pH 7.0 containing 1.00×10^{-6} mol L^{-1} Pb^{2+} was passed through the membrane at different flow rates from 0.9 to 7.5 mL min^{-1} for 30 min. The absorbance of each optode membrane was recorded at 660 nm. The response (α) of membrane in various flow rates was determined. The response of optode membrane versus difference of flow rate of solution was illustrated in Figure 4.14.
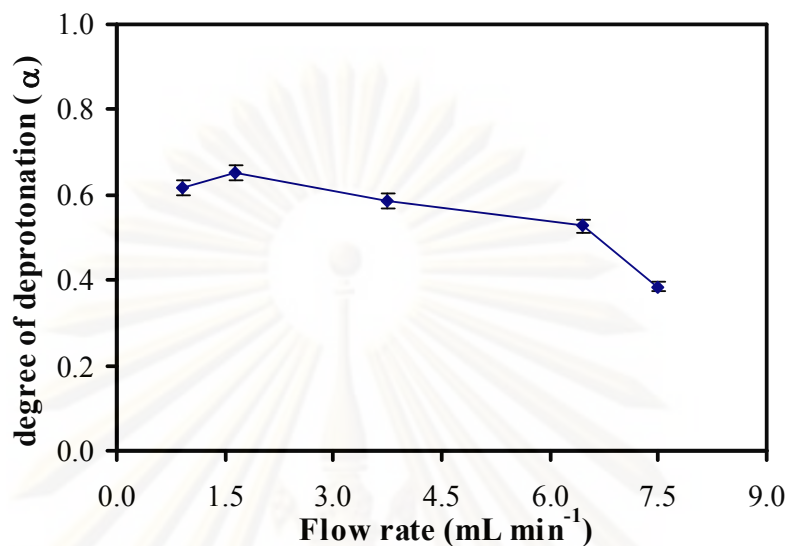
Figure 4.14 The response of optode for 1.00×10^{-6} mol L^{-1} Pb^{2+} at different flow rates of 0.9-7.5 mL min^{-1}.

The response of optode membrane decreased as the flow rate increased in the range of $3.8 - 7.5$ mL min^{-1} because of less contact of Pb^{2+} with recognition sites of ionophore in optode membrane. The maximum degree of deprotonation was obtained at a flow rate of 1.6 mL min^{-1}. Therefore, a flow rate of 1.6 mL min^{-1} was chosen due to high response and less time-consuming of sampling solutions.

4.4.3 Response time

A membrane was placed in the flow-through measuring cell. 0.01 mol L^{-1} HCl was passed through the membrane at a flow rate of 1.6 mL min^{-1} in order to let chromoionophore be fully protonated form then milli-Q water was passed through the membrane for 3 min. TRIS buffer solution at pH 7.0 containing 3.16×10^{-6} mol L^{-1} Pb^{2+} was passed through the membrane at a flow rate of 1.6 mL min^{-1}. The absorbance of the optode membrane was recorded at 545 nm. The experiments were performed in triplicate. The response time of the optode membrane was shown in Figure 4.15.
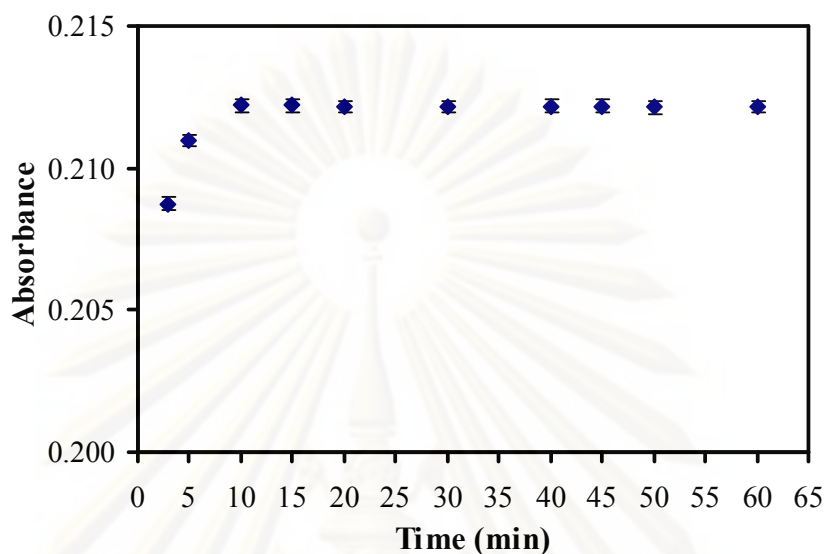
Figure 4.15 Response time of the optode membrane in Tis buffer solution (pH 7.0) containing 3.16×10^{-6} mol L^{-1} Pb^{2+} at a flow rate of 1.6 mL min^{-1}.

The response time in flow-through system was improved in comparison with batch system. The response time of the optode membrane to reach 99% of steady signal absorbance was within 15 min. It probably due to the better diffusion of Pb^{2+} from sample solution to membrane phase and the convective mass extraction in a continuous flow-through system which can be described by using well-known Fick's law.

4.4.4 Repeatability and reproducibility

A membrane was placed in the flow-through measuring cell. 0.01 mol L^{-1} HCl was passed through the membrane at a flow rate of 1.6 mL min^{-1} in order to let chromoionophore be fully protonated form for 3 min and milli-Q water was then passed through the membrane for 3 min. TRIS buffer solution at pH 7.0 containing 3.16×10^{-6} mol L^{-1} Pb^{2+} was passed through the membrane at a flow rate of 1.6 mL min^{-1} for 15 min. The absorbance of the optode membrane was recorded at 545 nm. After each measurement, the regenerating step was studied by pass in 0.01 mol L^{-1} HNO_3 at a flow rate of 1.6 mL min^{-1} and of milli-Q water was then passed through the membrane for 3 min. The absorbance of the optode membranes was recorded at

545 nm. The relative standard deviation (R.S.D.) values of the optode membrane were summarized in Table 4.5 (n=10).

Table 4.5 Absorbances of single optode membrane in TRIS buffer solution (pH 7.0) containing 3.16×10^{-5} mol L^{-1} Pb^{2+} at a flow rate of 1.6 mL min^{-1}

No. of replicate	Absorbance at 545 nm
1	0.087
2	0.087
3	0.086
4	0.087
5	0.086
6	0.086
7	0.086
8	0.086
9	0.086
10	0.086
mean	0.086
SD	0.001
% R.S.D	1.2

The reproducibility was evaluated by measuring absorbance of nine membranes, which were prepared in the same batch of the cocktail solution. The experiment was performed as the same manners of the repeatability (each membrane was performed in triplicate). The obtained results were shown in Table 4.6.

Table 4.6 Absorbances of several optode membranes in TRIS buffer solution (pH 7.0) containing 3.16×10^{-5} mol L^{-1} Pb^{2+} at a flow rate of 1.6 mL min^{-1}

No. of membrane	Mean absorbance at 545 nm
1	0.235
2	0.222
3	0.220
4	0.223
5	0.220
6	0.228
7	0.234
8	0.221
9	0.230
mean	0.226
SD	0.006
% R.S.D	2.7

The relative standard deviation (R.S.D.) of absorbance for single optode and several optode membranes were 1.2 % (n=10) and 2.7 % (n=9), respectively. The results indicated that repeatability and reproducibility of optode sensor were acceptable.

The optimized conditions and the analytical parameters of the optode membrane for Pb^{2+} were summarized in Table 4.7.

Table 4.7 Specifications of optode membrane for determination of Pb^{2+}

Parameter	Value or range	
	Batch	**Flow**
pH and type of buffer	TRIS buffer of pH 7.0	TRIS buffer of pH 7.0
Conditioned condition	0.01 mol L^{-1} HCl for 5 min	0.01 mol L^{-1} HCl for 3 min
Response time	30 min	15 min
Regenerated condition	0.01 mol L^{-1} HNO_3 for 3 min	0.01 mol L^{-1} HNO_3 at 1.6 mL min^{-1} for 3 min
Sample flow rate	-	1.6 mL min^{-1}
Repeatability	2.3% R.S.D. (n=10) with 3.16×10^{-5} mol L^{-1} Pb^{2+}	1.2% R.S.D. (n=10) with 3.16×10^{-5} mol L^{-1} Pb^{2+}
Reproducibility	4.1% R.S.D. (n=12) with 3.16×10^{-5} mol L^{-1} Pb^{2+}	2.7% R.S.D. (n=9) with 3.16×10^{-5} mol L^{-1} Pb^{2+}
Stability (life time)	Optode membrane kept in ambient condition, ~ 20 days	

4.5 Analytical performance of the proposed method

The extended of working range for determination of analyte ion using bulk optode technique depends on pH, membrane composition, charge of analyte and stoichiometry of complexation in membrane [91]. The linearity part of the sigmoidal response curve is usually employed for analysis, defined as linearity between a lower and an upper detection limit. The detection limit or the lower detection limit can be estimated from different approximates: (i) the loss of sensitivity due to the sigmoidal shape of the response function at low analyte concentrations and (ii) the interference from other ions present [7, 91]. Using the first approximation, it has been defined through: (a) the standard deviation of background signal [82]; (b) the variation of a given fraction of the maximum slope of response function [27, 63] and (c) the intersection point of two linear functions of maximum and minimum slope [100]. The upper detection limit can be described in analogy with previous definitions (b) and (c).

In this work, the detection limit was obtained by using the approximation of the loss of sensitivity which was defined in (c). [70, 101-103]. This approximation offered two advantages; a simple calculation and an interpolation with less error [102]. In order to determine the detection limit, two series of Pb^{2+} standard solutions were prepared. One maximum slope zone (8 standards, 3.16×10^{-8} to 5.00×10^{-5} mol L^{-1}) with the obtained linear functions was $(1-\alpha) = -0.2346 \log a_{Pb}^{2+} - 0.8243$, and another one in the minimum slope zone of lower concentration (3 standards, 1.26×10^{-10} to 3.16×10^{-9} mol L^{-1}) with the obtained linear functions was $(1-\alpha) = -0.0104 \log a_{Pb}^{2+} + 0.8803$ (as shown in Figure 4.16). The lower detection limit was defined as the concentration that corresponded to the intersection of two linear functions of maximum slope and minimum slope. The interception of both functions gave a lower detection limit (DL) of 2.49×10^{-8} mol L^{-1}. A practical upper detection limit obtained from the intercept of the linear calibration function with the axis of abscise was found at 1.0×10^{-3} mol L^{-1}. The central zone of sigmoidal response curve showed a straight line that was a dynamic range. Dynamic range of 3.16×10^{-8} to 5.00×10^{-5} mol L^{-1} in batch system was obtained.
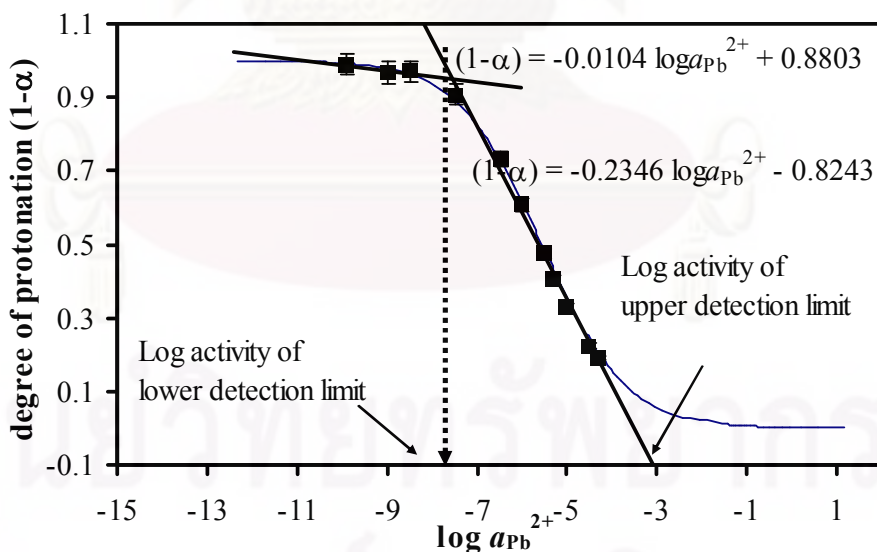
Figure 4.16 Sigmoidal response curve of the optode membrane with the intersection of two linear functions for determination of detection limit in batch system.

In flow-through system, two series of Pb^{2+} standard solutions were prepared. One in the maximum slope zone (7 standards, 1.26×10^{-8} to 3.16×10^{-5} mol L^{-1}) with the obtained linear function was $(1-\alpha) = -0.1994 \log a_{Pb}^{2+} - 0.6294$, and another one in the minimum slope zone of lower concentrations (3 standards, 1.00×10^{-10} to 5.00×10^{-9} mol L^{-1}) with the obtained linear function was $(1-\alpha) = -0.0111 \log a_{Pb}^{2+} + 0.8783$ (as shown in Figure 4.17). The interception of both functions gave a lower detection limit (DL) of 8.97×10^{-9} mol L^{-1} and the obtained value of upper limit was 3.16×10^{-3} mol L^{-1}. A dynamic range of 1.26×10^{-8} to 3.16×10^{-5} mol L^{-1} was obtained.
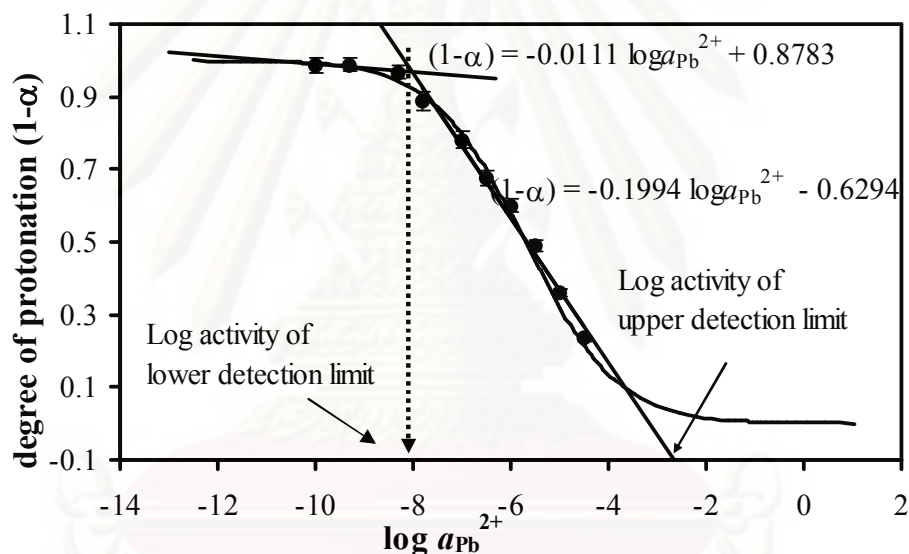
Figure 4.17 Sigmoidal response curve of the optode membrane with the intersection of two linear functions for determination of detection limit in flow-through system.

4.6 Real water samples analysis

The practical application of the proposed optode membrane to determine Pb^{2+} in real water samples was demonstrated under the optimum conditions. The results obtained were compared with the results determined by ICP-OES (inductively coupled plasma optical emission spectrometry).

The water samples (pond water and tap water) were collected in the polyethylene bottles and adjusted to pH 2 with nitric acid. Pond water sample was filtered to remove particles before use. The spiked method was evaluated to determine Pb^{2+} in water samples. Standard Pb^{2+} at different concentrations was spiked into 50 mL of water sample. The water samples were diluted with TRIS buffer to pH 7.0 in a final volume of 100 mL The absorbance measurements of the optode membrane were performed in both batch and flow-through systems under the optimum conditions as summarized in Table 4.7. The experiments were performed in triplicate.

The results of batch system were illustrated in Figure 4.18 and Figure 4.19 for pond water and tap water, respectively and for flow-through systems, the results were illustrated in Figure 4.20 and Figure 4.21 for pond water and tap water, respectively. The determination of Pb^{2+} in pond water and tap water by batch and flow-through measurements were summarized in Table 4.8
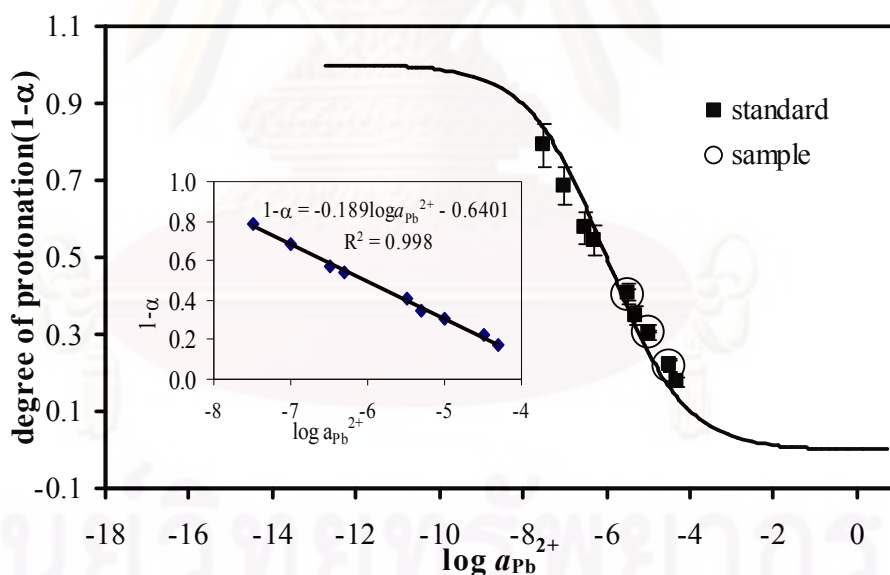
Figure 4.18 The response curve plot of the degree of protonation versus log activity of Pb^{2+}. The inset figure showed a calibration curve used to determine Pb^{2+} in pond water sample in batch system.
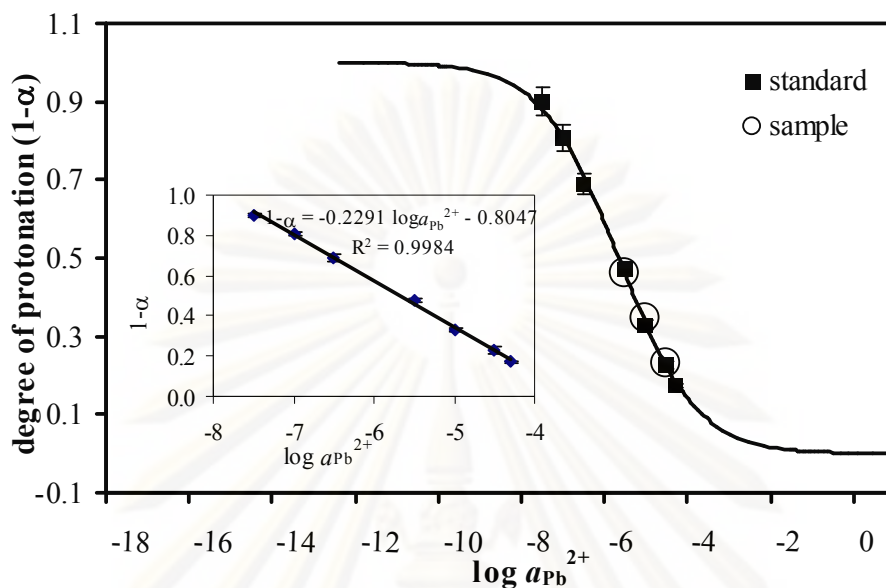
Figure 4.19 The response curve plot of degree of protonation versus log activity of Pb^{2+}. The inset figure showed a calibration curve used to determine Pb^{2+} in tap water sample in batch system.
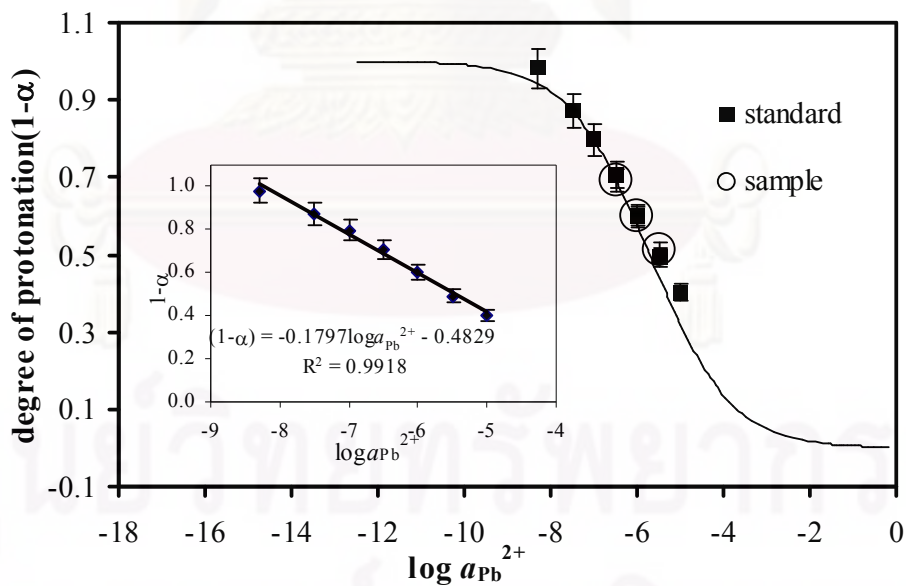
Figure 4.20 The response curve plot of degree of protonation versus log activity of Pb^{2+}. The inset figure showed a calibration curve used to determine Pb^{2+} in pond water sample in flow-through system.
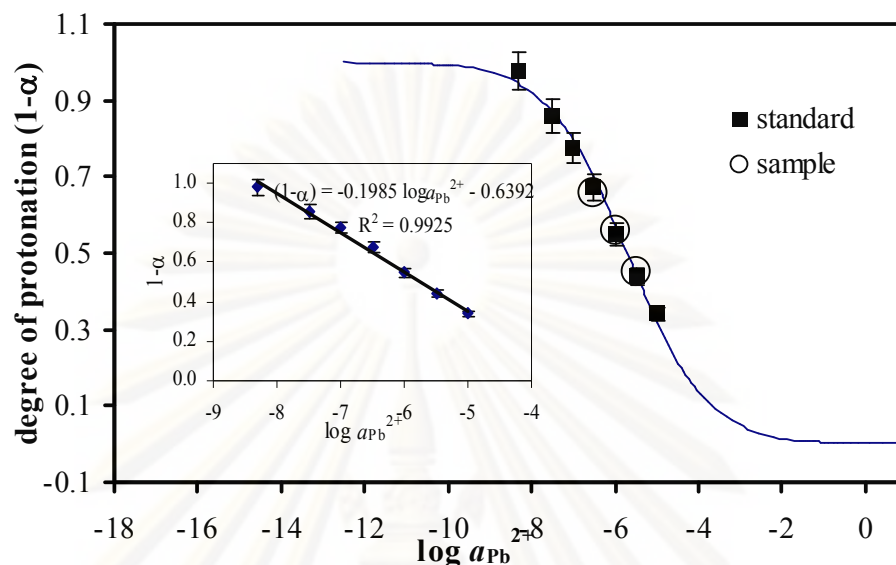
Figure 4.21 The response curve plot of degree of protonation versus log activity of Pb^{2+}. The inset figure showed a calibration curve used to determine Pb^{2+} in tap water sample in flow-through system.

Table 4.8 The determination of Pb^{2+} in real water samples in batch system and flow-through system

Sample	Batch system			Flow-through system		
	Added (mg L^{-1})	Found ± SD (mg L^{-1})^a	Recovery (%)	Added (mg L^{-1})	Found ± SD (mg L^{-1})^a	Recovery (%)
Pond water	-	< DL	-	-	< DL	-
	3.16×10^{-6}	$(3.06 \pm 0.02)\times10^{-6}$	97	3.16×10^{-7}	$(2.88 \pm 0.01)\times10^{-7}$	91
	1.00×10^{-5}	$(0.97 \pm 0.01)\times10^{-5}$	97	1.00×10^{-6}	$(0.93 \pm 0.01)\times10^{-6}$	93
	3.16×10^{-5}	$(2.95 \pm 0.02)\times10^{-5}$	93	3.16×10^{-6}	$(2.88 \pm 0.01)\times10^{-6}$	91
Tap water	-	< DL	-	-	< DL	-
	3.16×10^{-6}	$(3.02 \pm 0.01)\times10^{-6}$	96	3.16×10^{-6}	$(2.87 \pm 0.01)\times10^{-6}$	91
	1.00×10^{-5}	$(0.96 \pm 0.01)\times10^{-5}$	96	1.00×10^{-5}	$(0.91 \pm 0.01)\times10^{-5}$	91
	3.16×10^{-5}	$(3.05 \pm 0.01)\times10^{-5}$	97	3.16×10^{-5}	$(3.19 \pm 0.01)\times10^{-5}$	101

^{a} mean value of three determinations   DL = Detection limit

The proposed optode membrane was successfully applied to determine Pb^{2+} in different real water samples under the optimum conditions either batch system or flow-through system with the satisfied recoveries. Sample throughput in flow-through system was 4 samples h^{-1}. The statistical t-test was used to compare the experimental means obtained from the proposed optode membrane and ICP-OES. The results were summarized in Table 4.9. No significant different value ($t_{critical}$ = 4.30 > t_{exp} = 1.00-3.42, n=3, 95% of confidence level) was found between both methods.

Table 4.9 The comparison results of the proposed bulk optode technique and ICP-OES for determination of Pb^{2+} in real water samples

| Sample | Added | Bulk optode | | ICP-OES | t-staistic ($t_{0.05,3}$ = 4.30) |
		Found $\pm$ SD (mol L^{-1}) [a]	Recovery (%)	found$\pm$ SD (mol L^{-1}) [a]	
Drinking water (1)	-	< DL	-	< DL	-
	3.16×10^{-6}	$(3.19 \pm 0.01) \times 10^{-6}$	101	$(3.16 \pm 0.02) \times 10^{-6}$	2.61
	5.01×10^{-6}	$(4.82 \pm 0.01) \times 10^{-6}$	96	$(4.66 \pm 0.01) \times 10^{-6}$	1.86
Drinking water (2)	-	< DL	-	< DL	-
	3.16×10^{-6}	$(3.13 \pm 0.01) \times 10^{-6}$	99	$(3.36 \pm 0.03) \times 10^{-6}$	1.90
	5.01×10^{-6}	$(4.99 \pm 0.02) \times 10^{-6}$	97	$(5.06 \pm 0.02) \times 10^{-6}$	1.00
Pond water	-	< DL	-	< DL	-
	1.00×10^{-6}	$(0.92 \pm 0.01) \times 10^{-6}$	92	$(1.01 \pm 0.02) \times 10^{-6}$	3.24
	3.16×10^{-6}	$(2.89 \pm 0.01) \times 10^{-6}$	91	$(3.25 \pm 0.02) \times 10^{-6}$	3.42
	1.00×10^{-5}	$(9.33 \pm 0.01) \times 10^{-6}$	93	$(9.75 \pm 0.02) \times 10^{-6}$	1.06
Tap water	-	< DL	-	< DL	-
	1.00×10^{-6}	$(0.91 \pm 0.02) \times 10^{-6}$	91	$(1.08 \pm 0.02) \times 10^{-6}$	1.11
	3.16×10^{-6}	$(2.87 \pm 0.02) \times 10^{-6}$	91	$(3.18 \pm 0.02) \times 10^{-6}$	1.01
	1.00×10^{-5}	$(9.66 \pm 0.02) \times 10^{-6}$	97	$(9.55 \pm 0.02) \times 10^{-6}$	1.74

[a] mean value of three determination

CHAPTER V

CONCLUSION

The objectives of this work were focused on the development of selective determination of Pb^{2+} using bulk optode technique and the improvement of response time. The sensitive optode membrane based on *tert*-butyl calix[4]arene-tettrakis (*N,N*-dimetylthioacetamide) as lead-selective ionophore, ETH 5294 as proton-selective chromoionophore and KTpClPB as cation exchanger in plasticized PVC membrane was fabricated. The preparation of the membrane was simple and easy. The determination of Pb^{2+} via cation-exchange mechanism in batch and flow-through measurement was studied with absorption spectrospotometry technique. The optode membrane responded to Pb^{2+} by changing color from blue to pink purple after equilibration in TRIS buffer (pH 7.0) solution containing different concentrations of Pb^{2+} and can be regenerated with dilute nitric acid solution. The optode membrane showed high selectivity towards Pb^{2+} over Na^+, K^+, Mg^{2+}, Cd^{2+}, Hg^{2+} and Ag^+.

The analytical parameters of the optimized optode membrane used for the determination of Pb^{2+} were summarized in Table 5.1.

Table 5.1 Analytical parameters of the optimized optode membrane used for determination of Pb^{2+}

Parameter	Value or range	
	Batch	**Flow**
pH and type of buffer	TRIS buffer of pH 7.0	TRIS buffer of pH 7.0
Conditioned condition	0.01 mol L^{-1} HCl in 5 min	0.01 mol L^{-1} HCl in 3 min
response time	30 min	15 min
Regenerated condition	0.01 molL^{-1} HNO$_3$ for 3 min	0.01 molL^{-1} HNO$_3$ at 1.6 mL min^{-1} for 3 min
sample flow rate	-	1.6 mL min^{-1}
Dynamic range	3.16×10^{-8} to 5.0×10^{-5} mol L^{-1} of Pb^{2+}	1.26×10^{-8} to 3.16×10^{-5} mol L^{-1} of Pb^{2+}
Detection limit (DL)	lower DL 2.49×10^{-8} mol L^{-1} upper DL 1.0×10^{-3} mol L^{-1}	lower DL 8.97×10^{-9} mol L^{-1} upper DL 3.16×10^{-3} mol L^{-1}
Repeatability	2.3% R.S.D. (n=10) with 3.16×10^{-5} mol L^{-1}	1.2% R.S.D. (n=12) with 3.16×10^{-5} mol L^{-1}
Reproducibility	4.1% R.S.D. (n=10) with 3.16×10^{-5} mol L^{-1}	2.7% R.S.D. (n=9) with 3.16×10^{-5} mol L^{-}
Stability (life time)	Optode membrane kept in ambient condition ~ 20 days	

Furthermore, the proposed method can be applied to determine Pb^{2+} in real water samples with high accuracy and the results were compared with well-established inductively coupled plasma optical emission spectrometry (ICP-OES). No significant different value ($t_{critical}$ = 4.30 > t_{exp} = 1.00-3.42, n=3, 95% of confidence level) was found between both methods.

Finally, we success to improve of the response time and detection limit for determination of Pb^{2+} using lead-selective optode membrane coupling with flow-through cell.

Suggestion in the future works

The response time and the detection limit for determination of Pb^{2+} can be improved by

- using the spin-on device to cast thinner optode membrane,
- varying the ratio of the composition of major components,
- optimizing temperature and humidity during preparation of optode membrane,
- modifying flow-through cell,
- using more than one optode membrane in both batch and flow-through systems,
- using fluorescence measurement,
- fabricating the optode sensors as single functional micrometer-sized particles

REFERENCES

[1] Carson, B. L.; Ellis, H. V. and McCann, J. L. Toxicology and biological monitoring of metals in humans. Michigan: Lewis, **1987**.

[2] WHO's guidelines for drinking-water quality, 2006. [Online]. (n.d.) Available from: http://www.whoint/watersanitationhealth/ dwq/gdwq0506.pdf [2008, May 6].

[3] Hisamoto, H. and Suzuki, K., Ion-selective optodes: current developments and future prospects. *TrAC Trends in Analytical Chemistry* **1999,** 18, 513-524.

[4] Wang, K. M. Theories and methods of optical chemical sensors Changsha, China: Human Education Press, **1995**.

[5] Jantana, J.; Josowicz, M.; Vanysek, P. and DeVney, D. M., Chemical sensors, Anal Chem. **1998**, 70, 179-208.

[6] Seiler, K. and Simon, W., Theoretical aspects of bulk optode membranes. *Analytica Chimica Acta* **1992,** 266, 73-87.

[7] Seiler, K. and Simon, W., Principles and mechanisms of ion-selective optodes. *Sensors and Actuators B: Chemical* **1992,** 6, 295-298.

[8] Zeng, H. and Dureault, B., Cesium-selective optode membrane based on the lipophilic calix[4]biscrown in the 1,3-alternate conformation. *Talanta* **1998,** 46, 1485-1491.

[9] Sanchez-Pedreo, C.; Ortuno, J. A.; Albero, M. I.; Garcia, M. S. and Valero, M. V., Development of a new bulk optode membrane for the determination of mercury(II). *Analytica Chimica Acta* **2000,** 414, 195-203.

[10] Shamsipur, M.; Poursaberi, T.; Karami, A. R.; Hosseini, M.; Momeni, A.; Alizadeh, N.; Yousefi, M. and Ganjali, M. R., Development of a new fluorimetric bulk Optode membrane based on 2,5-thiophenylbis(5-tert-butyl-1,3-benzexazole) for nickel(II) ions. *Analytica Chimica Acta* **2004,** 501, 55-60.

[11] Zhang, X.-B.; Peng, J.; He, C.-L.; Shen, G.-L. and Yu, R.-Q., A highly selective fluorescent sensor for Cu^{2+} based on 2-(2'-hydroxyphenyl)benzoxazole in a poly(vinyl chloride) matrix. *Analytica Chimica Acta* **2006,** 567, 189-195.

[12] Safavi, A. and Sadeghi, M., A PVC-membrane bulk optode for gallium (III) ion determination. *Talanta* **2007,** 71, (1), 339-343.

[13] Sadeghi, S. and Doosti, S., Novel PVC membrane bulk optical sensor for determination of uranyl ion. *Sensors and Actuators B: Chemical* **2008,** 135, 139-144.

[14] Chan, W.-H.; Yang, R.-H.; Mo, T. and Wang, K.-M., Lead-selective fluorescent optode membrane based on 3,3',5,5'-tetramethyl-N-(9-anthrylmethyl)benzidine. *Analytica Chimica Acta* **2002,** 460, 123-132.

[15] Telting-Diaz, M. and Bakker, E., Mass-produced ionophore-based fluorescent microspheres for trace level determination of lead ions. *Analytical Chemistry* **2002,** 74, 5251-5256.

[16] Malinowska, E.; Brzozka, Z.; Kasiura, K.; Egberink, R. J. M. and Reinhoudt, D. N., Lead selective electrodes based on thioamide functionalized calix[4]arenes as ionophores. *Analytica Chimica Acta* **1994,** 298, 253-258.

[17] Bailey, R. A.; Clark, H. M.; Ferris, J. P. and Krause S.; Strong, R. L., Chemistry of the environment. 2nd edition, academic press, New York, **1986**.

[18] Takeno, N. Atlas of Eh-pH diagrams. [Online]. (2005). Available from: http://www.gsj.jp/GDB/openfile/files/no0419/openfile419e.pdf [2008, June 22].

[19] Stanley, E. M. Environmental chemistry. 4th edition, Boston: Willard grant press, **1984**.

[20] Optode [online]. (n.d.) Available from: http://en.wikipedia.org/wiki/Optode [**2008**, June 15]

[21] Hisamoto, H.; Watanabe, K.; Nakagawa, E.; Siswanta, D.; Shichi, Y. and Suzuki, K., Flow-through type calcium ion selective optodes based on novel neutral ionophores and a lipophilic anionic dye. *Analytica Chimica Acta* **1994**, 299, 179-187.

[22] Hisamoto, H.; Satoh, S.; Satoh, K.; Tsubuku, M.; Siswanta, D.; Shichi, Y.; Koike, Y. and Suzuki, K., Theory and practice of rapid flow-through analysis based on ion-selective optode detection and its application to sodium ion determination. *Analytica Chimica Acta* **1999**, 396, (2-3), 131-141.

[23] Sanchez-Pedreno, C.; Garĉa, M. S.; Ortuno, J. A.; Albero, M. I. and Exposito, R., Kinetic methods for the determination of cadmium(II) based on a flow-through bulk optode. *Talanta* **2002**, 56, 481-489.

[24] Albero, M. I.; Ortuno, J. A.; García, M. S.; Sanchez-Pedreno, C. and Exposito, R., Determination of zinc (II) in pharmaceuticals based on a flow-through bulk optode. *Journal of Pharmaceutical and Biomedical Analysis* **2002**, 29, 779-786.

[25] Ortuno, J. A.; Exposito, R.; Martnez, F.; Sanchez-Pedreno, C. N.; García, M. S. and Albero, M. I., Linear and nonlinear approaches to study transient signals of a flow-through bulk optode. *Sensors and Actuators B: Chemical* **2006**, 115, 584-588.

[26] Safavi, A.; Rostamzadeh, A. and Maesum, S., Wide range pH measurements using a single H^+-selective chromoionophore and time-based flow method. *Talanta* **2006**, 68, 1469-1473.

[27] Amini, M. K.; Khezri, B. and Firooz, A. R., Development of a highly sensitive and selective optical chemical sensor for batch and flow-through determination of mercury ion. *Sensors and Actuators B: Chemical* **2008**, 131, 470-478.

[28] Freschi, G. P. G.; Dakuzaku, C. S.; de Moraes, M.; Nobrega, J. A. and Gomes Neto, J. A., Simultaneous determination of cadmium and lead in wine by electrothermal atomic absorption spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy* **2001**, 56, 1987-1993.

[29] Turunen, M.; Perniemi, S.; Ahlgrn, M. and Westerholm, H., Determination of trace elements in heavy oil samples by graphite furnace and cold vapour atomic absorption spectrometry after acid digestion. *Analytica Chimica Acta* **1995**, 311, 85-91.

[30] Sreenivasa Rao, K.; Balaji, T.; Prasada Rao, T.; Babu, Y.; Naidu, G. R. K., Parsons, P. J. and Slavin, W., Electrothermal atomization atomic absorption spectrometry for the determination of lead in urine: results of an inter laboratory study. *Spectrochimica Acta Part B: Atomic Spectroscopy* **1999**, 54, 853-864.

[31] Sreenivasa Rao, K.; Balaji, T.; Prasada Rao, T.; Babu, Y. and Naidu, G. R. K., Determination of iron, cobalt, nickel, manganese, zinc, copper, cadmium and lead in human hair by inductively coupled plasma-atomic emission

spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy* **2002**, 57, 1333-1338.

[32] Chew, L. T.; Bradley, D. A.; Mohd, A. Y. and Jamil, M. M., Zinc, lead and copper in human teeth measured by induced coupled argon plasma atomic emission spectroscopy (ICP-AES). *Applied Radiation and Isotopes* **2000**, 53, 633-638.

[33] White, W. M.; Albařde, F. and Tlouk, P., High-precision analysis of Pb isotope ratios by multi-collector ICP-MS. *Chemical Geology* **2000**, 167, 257-270.

[34] Barbaste, M.; Halicz, L.; Galy, A.; Medina, B.; Emteborg, H.; C. Adams, F. and Lobinski, R., Evaluation of the accuracy of the determination of lead isotope ratios in wine by ICP-MS using quadrupole, multicollector magnetic sector and time-of-flight analyzers. *Talanta* **2001**, 54, 307-317.

[35] Larivìre, D.; Reiber, K. M.; Evans, R. D. and Cornett, R. J., Determination of 210Pb at ultra-trace levels in water by ICP-MS. *Analytica Chimica Acta* **2005**, 549, 188-196.

[36] Sahan, Y.; Basoglu, F. and Gucer, S., ICP-MS analysis of a series of metals (Namely: Mg, Cr, Co, Ni, Fe, Cu, Zn, Sn, Cd and Pb) in black and green olive samples from Bursa, Turkey. *Food Chemistry* **2007**, 105, 395-399.

[37] Wang, J.; Harald Hansen, E. and Gammelgaard, B., Flow injection on-line dilution for multi-element determination in human urine with detection by inductively coupled plasma mass spectrometry. *Talanta* **2001,** 55, 117-126.

[38] Di Nezio, M. S.; Palomeque, M. E. and Fernandez Band, B. S., A sensitive spectrophotometric method for lead determination by flow injection analysis with on-line preconcentration. *Talanta* **2004,** 63, 405-409.

[39] Mesquita, R. B. R.; Fernandes, S. M. V. and Rangel, A. N. O. S. S., A flow system for the spectrophotometric determination of lead in different types of waters using ion-exchange for pre-concentration and elimination of interferences. *Talanta* **2004,** 62, 395-401.

[40] Wang, Z.-H.; Zhang, Z.-P.; Wang, Z.-P.; Liu, L.-W. and Yan, X.-P., Acrylic acid grafted polytetrafluoroethylene fiber as new packing for flow injection on-line microcolumn preconcentration coupled with flame atomic absorption

spectrometry for determination of lead and cadmium in environmental and biological samples. *Analytica Chimica Acta* **2004,** 514, 151-157.

[41] Cankur, O.; Korkmaz, D. and Ataman, O. Y., Flow injection-hydride generation infrared spectrophotometric determination of Pb. *Talanta* **2005,** 66, 789-793.

[42] Dadfarnia, S.; Haji Shabani, A. M.; Tamaddon, F. and Rezaei, M., Immobilized salen (N,N'-bis (salicylidene) ethylenediamine) as a complexing agent for on-line sorbent extraction/preconcentration and flow injection-flame atomic absorption spectrometry. *Analytica Chimica Acta* **2005,** 539, 69-75

[43] Tuzen, M.; Parlar, K. and Soylak, M., Enrichment/separation of cadmium(II) and lead(II) in environmental samples by solid phase extraction. *Journal of Hazardous Materials* **2005,** 121, 79-87.

[44] Hu, L. N. and Ren, Z. G., Separation, preconcentration and detern\mination of Pb(II) in water sample using microcrystalline triphenylmethane loaded with quinoline-8-olate. *Chinese Chemical Letters* **2009**, 20, 334-338.

[45] Gama, E. M.; da Silva Lima, A. and Lemos, V. A., Preconcentration system for cadmium and lead determination in environmental samples using polyurethane foam/Me-BTANC. *Journal of Hazardous Materials* **2006,** 136, 757-762.

[46] Baytak, S. and Turker, A. R., Determination of lead and nickel in environmental samples by flame atomic absorption spectrometry after column solid-phase extraction on Ambersorb-572 with EDTA. *Journal of Hazardous Materials* **2006,** 129, 130-136.

[47] Cespon-Romero, R. M. and Yebra-Biurrun, M. C., Flow injection determination of lead and cadmium in hair samples from workers exposed to welding fumes. *Analytica Chimica Acta* **2007,** 600, 221-225.

[48] Michalska, A.; Wojciechowski, M.; Bulska, E.; Mieczkowski, J. Z. and Maksymiuk, K., Poly(n-butyl acrylate) based lead (II) selective electrodes. *Talanta* **2009**, 79, 1247-1251.

[49] Mousavi, M. F.; Sahari, S.; Alizadeh, N. and Shamsipur, M., Lead ion-selective membrane electrode based on 1,10-dibenzyl-1,10-diaza-18-crown-6. *Analytica Chimica Acta* **2000**, 414, 189-194.

[50] Rouhollahi, A.; Reza Ganjali, M. and Shamsipur, M., Lead ion selective PVC membrane electrode based on 5,5'-dithiobis-(2-nitrobenzoic acid). *Talanta* **1998**, 46, 1341-1346**.**

[51] Su, C.-C.; Chang, M.-C. and Liu, L. K., New Ag^+- and Pb^{2+}-selective electrodes with lariat crown ethers as ionophores. *Analytica Chimica Acta* **2001**, 432, 261-267.

[52] Tavakkoli, N.; Khojasteh, Z.; Sharghi, H. and Shamsipur, M., Lead ion-selective membrane electrodes based on some recently synthesized 9,10-anthraquinone derivatives. *Analytica Chimica Acta* **1998**, 360, 203-208.

[53] Li, X.-G.; Ma, X.-L. and Huang, M.-R., Lead(II) ion-selective electrode based on polyaminoanthraquinone particles with intrinsic conductivity. *Talanta* **2009**, 78, 498-505.

[54] Vassilev, V.; Tomova, K. and Boycheva, S., Pb(II)-ion-selective electrodes based on chalcogenide glasses. *Journal of Non-Crystalline Solids* **2007**, 353, 2779-2784.

[55] Gupta, V. K.; Jain, A. K. and Kumar, P., PVC-based membranes of N,N'-dibenzyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane as Pb(II)-selective sensor. *Sensors and Actuators B: Chemical* **2006**, 120, 259-265.

[56] Chen, L.; Zhang, J.; Zhao, W.; He, X. and Liu, Y., Double-armed calix[4]arene amide derivatives as ionophores for lead ion-selective electrodes. *Journal of Electroanalytical Chemistry* **2006**, 589, 106-111.

[57] Hassan, S. S. M.; Abou Ghalia, M. H.; Amr, A.-G. E. and Mohamed, A. H. K., New lead (II) selective membrane potentiometric sensors based on chiral 2,6-bis-pyridinecarboximide derivatives. *Talanta* **2003**, 60, 81-91.

[58] Lu, J.; Chen, R. and He, X., A lead ion-selective electrode based on a calixarene carboxyphenyl azo derivative. *Journal of Electroanalytical Chemistry* **2002**, 528, 33-38.

[59] Ohki, A.; Kim, J. S.; Suzuki, Y.; Hayashita, T. and Maeda, S., Lead-selective poly(vinyl chloride) membrane electrodes based on acyclic dibenzopolyether diamides. *Talanta* **1997**, 44, 1131-1135.

[60] Kuswandi, B.; Nuriman; Dam, H. H.; Reinhoudt, D. N. and Verboom, W., Development of a disposable mercury ion-selective optode based on trityl-picolinamide as ionophore. *Analytica Chimica Acta* **2007**, 591, 208-213.

[61] Wolfbeis, O. S., Fiber-optic chemical sensors and biosensors. *Analytical Chemistry* **2000,** 72, 81-90.

[62] Morf, W. E.; Seiler, K.; Rusterholz, B. and Simon, W., Design of a novel calcium-selective optode membrane based on neutral ionophores. *Analytical Chemistry* **1990**, 62, 738-742.

[63] Lerchi, M.; Reitter, E.; Simon, W.; Pretsch, E.; Chowdhury, D. A. and Kamata, S., Bulk optodes based on neutral dithiocarbamate ionophores with high selectivity and sensitivity for silver and mercury cations. *Analytical Chemistry* **1994**, 66, 1713-1717.

[64] Lerchi, M.; Orsini, F.; Cimerman, Z.; Pretsch, E.; Chowdhury, D. A. and Kamata, S., Selective optical sensing of silver ions in drinking water. *Analytical Chemistry* **1996**, 68, 3210-3214.

[65] Murkovic, I.; Oehme, I.; Mohr, G. J.; Ferber, T. and Wolfbeis, O. S., Optode membrane for continuous measurement of silver ions. *Mikrochimica Acta* **1995**, 121, 249-258.

[66] Bochenska, M., Design and characterization of Li-selective optical sensor. *Journal of Inclusion Phenomena and Molecular Recognition in Chemistry* **1995**, 22, 269-275.

[67] Safavi, A. and Sadeghi, M., Design and evaluation of a thorium (IV) selective optode. *Analytica Chimica Acta* **2006**, 567, 184-188.

[68] Ensafi, A. A. and Fouladgar, M., A sensitive and selective bulk optode for determination of Hg(II) based on hexathiacyclooctadecane and chromoionophore V. *Sensors and Actuators B: Chemical* **2009**, 136, 326-331.

[69] Zare-Shahabadi, V.; Akhond, M.; Tashkhourian, J. and Abbasitabar, F., Characterization of a new uranyl selective bulk optode; utilizing synergistic effect in optical sensor. *Sensors and Actuators B: Chemical* **2009**, 141, 34-39.

[70] Yari, A. and Papi, F., Highly selective sensing of mercury(II) by development and characterization of PVC-based optical sensor. *Sensors and Actuators B: Chemical* **2009**, 138, 467-473.

[71] He, H.; Li, H.; Mohr, G.; Kovacs, B.; Werner, T. and Wolfbeis, O. S., Novel type of ion-selective fluorosensor based on the inner filter effect: an optrode for potassium. *Analytical Chemistry* **2002**, 65, 123-127.

[72] Yang, X.; Wang, K.; Xiao, D.; Guo, C. and Xu, Y., Development of a fluorescent optode membrane for sodium ion based on the calix[4]arene and tetraphenylporphine. *Talanta* **2000**, 52, 1033-1039.

[73] Chan, W. H.; Yang, R. H. and Wang, K. M., Development of a mercury ion-selective optical sensor based on fluorescence quenching of 5,10,15,20-tetraphenylporphyrin. *Analytica Chimica Acta* **2001,** 444, 261-269.

[74] Shamsipur, M.; Poursaberi, T.; Avanes, A. and Sharghi, H., Copper(II)-selective fluorimetric bulk optode membrane based on a 1-hydroxy-9,10-anthraquinone derivative having two propenyl arms as a neutral fluorogenic ionophore. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **2006**, 63, 9-14.

[75] Shamsipur, M.; Alizadeh, K.; Hosseini, M.; Caltagirone, C. and Lippolis, V., Selective optode membrane for silver ion based on fluorescence quenching of the dansylamidopropyl pendant arm derivative of 1-aza-4,7,10-trithiacyclododecane ([12]aneNS$_3$). *Sensors and Actuators B: Chemical* **2006**, 113, 892-899.

[76] Shamsipur, M.; Sadeghi, M.; Alizadeh, K.; Sharghi, H. and Khalifeh, R., An efficient and selective flourescent optode membrane based on 7-[(5-chloro-8-hydroxy-7-quinolinyl)methyl]-5,6,7,8,9,10-hexahydro-2H-1,13,4,7,10 benzo dioxatriazacyclopentadecine-3,11(4H,12H)-dione as a novel fluoroionophore for determination of cobalt(II) ions. *Analytica Chimica Acta* **2008**, 630, 57-66.

[77] Wygladacz, K.; Radu, A.; Xu, C.; Qin, Y. and Bakker, E., Fiber-optic microsensor array based on fluorescent bulk optode microspheres for the trace analysis of silver ions. *Analytical Chemistry* **2005**, 77, 4706-4712.

[78] Xu, C.; Wygladacz, K.; Qin, Y.; Retter, R.; Bell, M. and Bakker, E., Microsphere optical ion sensors based on doped silica gel templates. *Analytica Chimica Acta* **2005**, 537, 135-143.
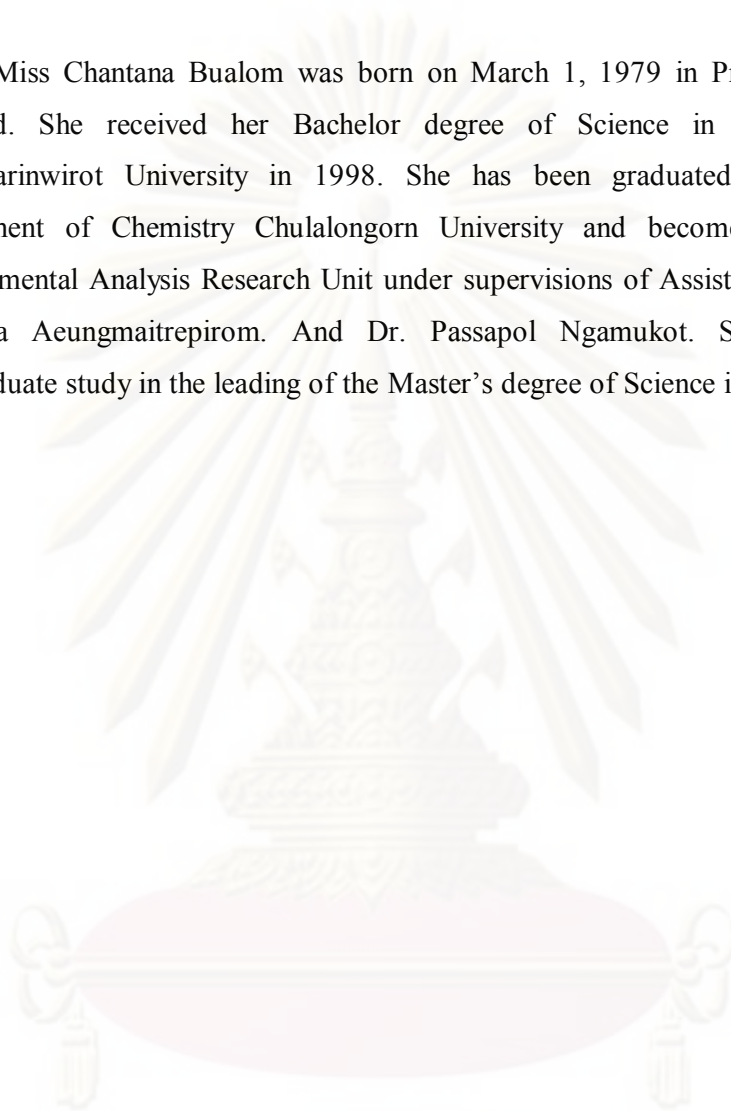
[79] He, C.-L.; Ren, F.-L.; Zang, X.-B. and Han, Z.-X., A fluorescent chemical sensor for Hg(II) based on corrole derivative in a PVC matrix. *Talanta* **2006**, 70, 364-369.

[80] Rivera, L.; Puyol, M.; Villuendas, F. and Alonso, J., Miniaturized setup for fluorescence sensing with optodes: Characterization of a new hemicyanine ion-selective-based membrane. *Sensors and Actuators B: Chemical* **2008**, 134, 863-868.

[81] Ganjali, M. R.; Hosseini, M.; Hariri, M.; Faridbod, F. and Norouzi, P., Novel erbium (III)-selective fluorimetric bulk optode. *Sensors and Actuators B: Chemical* **2009**, 142, 90-96.

[82] Lerchi, M.; Bakker, E.; Rusterholz, B. and Simon, W., Lead-selective bulk optodes based on neutral ionophores with subnanomolar detection limits. *Analytical Chemistry* **1992,** 64, 1534-1540.

[83] Antico, E.; Lerchi, M.; Rusterholz, B.; Achermann, N.; Badertscher, M.; Valiente, M. and Pretsch, E., Monitoring Pb^{2+} with optical sensing films. *Analytica Chimica Acta* **1999,** 388, 327-338.

[84] Alizadeh, N.; Moemeni, A. and Shamsipur, M., Poly(vinyl chloride)-membrane ion-selective bulk optode based on 1,10-dibenzyl-1,10-diaza-18-crown-6 and 1-(2-pyridylazo)-2-naphthol for Cu^{2+} and Pb^{2+} ions. *Analytica Chimica Acta* **2002**, 464, 187-196.

[85] Malinowska, E.; Brzozka, Z.; Kasiura, K.; Egberink, R. J. M. and Reinhoudt, D. N., Lead selective electrodes based on thioamide functionalized calix[4]arenes as ionophores. *Analytica Chimica Acta* **1994,** 298, 253-258.

[86] Wroblewski, W. and Brzozka, Z., Switching of ion selectivity of membranes by lipophilic ionic sites. *Analytica Chimica Acta* **1996,** 326, 163-168.

[87] Ceresa, A. and Pretsch, E., Determination of formal complex formation constants of various Pb^{2+} ionophores in the sensor membrane phase. *Analytica Chimica Acta* **1999,** 395, 41-52.

[88] O'Neill, S.; Conway, S.; Twellmeyer, J.; Egan, O.; Nolan, K. and Diamond, D., Ion-selective optode membranes using 9-(4-diethylamino-2-octadecanoatestyryl)-acridine acidochromic dye. *Analytica Chimica Acta* **1999,** 398, 1-11.

[89] Oehem, I.; Prattes, S.; Wolfbeis, O. S. and Mohr, G. J., The effect of polymeric supports and methods of immobilization on the performance of an optical copper(II)-sensitive membrane based on the colourimetric reagent Zincon, *Talanta* **1998**, 47, 595-604.

[90] Mohr, G. J. and Wolfbeis, O. S., Effects of the polymer matrix on an optical nitrate sensor based on a polarity-sensitive dye. *Sensors and Actuators B: Chemical* **1996,** 37, 103-109.

[91] Bakker, E.; Buhlmann, P. and Pretsch, E., Carrier-Based Ion-Selective Electrodes and Bulk Optodes. 1. General Characteristics. *Chemical Reviews* **1997,** 97, 3083-3132.

[92] Wygladacz, K. and Bakker, E., Imaging fiber microarray fluorescent ion sensors based on bulk optode microspheres. *Analytica Chimica Acta* **2005,** 532, 61-69.

[93] Ngeontae, W.; Xu, C.; Ye, N.; Wygladacz, K.; Aeungmaitrepirom, W.; Tuntulani, T. and Bakker, E., Polymerized Nile Blue derivatives for plasticizer-free fluorescent ion optode microsphere sensors. *Analytica Chimica Acta* **2007,** 599, 124-133.

[94] Bakker, E. and Simon, W., Selectivity of ion-sensitive bulk optodes. *Analytical Chemistry* **1992,** 64, 1805-181.

[95] Qin, Y. and Bakker, E., Quantitive binding constants of H^+-selective chromoionophores and anion ionophores in solvent polymeric sensing membranes. *Talanta* **2002**, 58, 909-918.

[96] Bottari, E. and Vicedomini, M., On the complex formation between lead(II) and citrate ions in alkaline solution. *Journal of Inorganic and Nuclear Chemistry* **1973**, 35, 2447-2453

[97] Useful pH range of buffer solution [Online]. (n.d.) Avarible from: http://www.sigmaaldrich.com/life-science/core-bioreagents/biological. [**2008**, May 6].

[98] Garrett, S. J. Formation constant for EDTA metal ion complexes [Online]. (n.d.) Available from: http://www.cem.msu.edu./~cem333/EDTATable.html. [**2009**, August 25].

[99] Bakker, E.; Willer, M.; Lerchi, M.; Seiler, K. and Pretsch, E., Determination of complex formation constants of neutral cation-selective ionophores in solvent polymeric membranes. *Analytical Chemistry* **1994,** 66, 516-521.

[100] Bakker, E.; Willer, M. and Pretsch, E., Detection limit of ion-selective bulk optodes and corresponding electrodes. *Analytica Chimica Acta* **1993**, 282, 265-271.

[101] Capitan-Vallvey, L. F.; Lapresta-Fernandez, A.; Fernandez-Ramos, M. D. and Cuadros-Rodríguez, L., Establishment of wide-range linear response curves in bulk optode sensors for cations based on ion exchange. *Sensors and Actuators B: Chemical* **2006**, 117, 27-34.

[102] Capitan-Vallvey, L. F.; Fernandez Ramos, M. D. and Alvarez de Cienfuegos-Galvez; P., Opical test stip for calcium determination based on neutral ionophore. *Analytica Chimica Acta* **2002**, 451, 231-241.

[103] Cano-Raya, C.; Fernandez-Ramose, M. D.; Gomez-Sanchez, J. and Capitan-Vallvey, L. F., Irreversibleboptical sensor for mercury determination based on tetraarylborate decomposition. *Sensors and Actuators B: Chemical* **2006**, 117, 135-142.

VITA

Miss Chantana Bualom was born on March 1, 1979 in Prachuapkhirikhan, Thailand. She received her Bachelor degree of Science in Chemistry from Srinakharinwirot University in 1998. She has been graduated student at the Department of Chemistry Chulalongorn University and become a member of Environmental Analysis Research Unit under supervisions of Assistant Professor Dr. Wanlapa Aeungmaitrepirom. And Dr. Passapol Ngamukot. She finished her postgraduate study in the leading of the Master's degree of Science in Academic Year 2009.