

**PREPARATION OF OPTICAL SENSING MEMBRANE  
BASED ON DYE IMMOBILIZATION FOR THE  
DETERMINATION OF COBALT(II) ION**

**Sudarat Sombatsri**

**A Thesis Submitted in Partial Fulfillment of the Requirements for the  
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Suranaree University of Technology  
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การเตรียมแผ่นเยื่อตรวจวัดเชิงแสงด้วยวิธีการตรึงสี้อมสำหรับวิเคราะห์  
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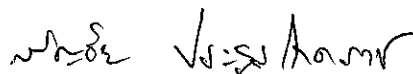
Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for Master's Degree.

Thesis Examining Committee



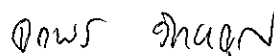
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ศุภรัตน์ สมบัติศรี : การเตรียมแผ่นเยื่อตรวจวัดเชิงแสงด้วยวิธีการตรึงสี้อมสำหรับ  
วิเคราะห์ไอออนโคบอลต์ (PREPARATION OF OPTICAL SENSING MEMBRANE  
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ในงานวิจัยนี้เสนอวิธีการอย่างง่ายสำหรับเตรียมแผ่นเยื่อตรวจวัดเชิงแสงสำหรับตรวจวัด  
ไอออนโคบอลต์ในสารละลายในน้ำ โดยการตรึงไดโซเดียม-1-ไนโตรโซ-2-แนพทอล-3,6-  
ไดซัลโฟเนต หรือเกลือไนโตรโซ-อาร์ ลงในแผ่นเยื่อไคโตซานที่ได้ดัดแปร ไคโตซานน้ำหนัก  
โมเลกุลสูงได้รับเลือกนำมาใช้เป็นเมทริกซ์รองรับ และโครงสร้างของไคโตซานได้รับการดัดแปร  
ด้วยอีพิกโลโรไฮดรินเพื่อเพิ่มความเสถียรให้กับแผ่นเยื่อในสารละลายในน้ำ

เมื่อแผ่นฟิล์มตรวจวัดอยู่ในสารละลายไอออนโคบอลต์ สีของแผ่นเยื่อตรวจวัดเปลี่ยนจากสี  
เหลืองเป็นสีส้มแดง ซึ่งเป็นผลมาจาก การเกิดสารเชิงซ้อนระหว่างไอออนโคบอลต์กับเกลือ  
ไนโตรโซ-อาร์ วัดค่าความดูดกลืนรังสีของสารเชิงซ้อนที่ 525 นาโนเมตร การตอบสนองสูงสุดของ  
แผ่นฟิล์มตรวจวัด เกิดในสารละลายบัฟเฟอร์ฟอสเฟต พีเอช 5.0 และเวลาที่แช่แผ่นฟิล์มใน  
สารละลาย 15 นาที

เส้นตรงสำหรับการเทียบมาตรฐานในพิสัยความเข้มข้น 1-60 ส่วนในล้านส่วน และ 35-400  
ส่วนในพันล้านส่วนนั้น เกิดจากการใช้สารละลายปริมาตร 3.0 มิลลิลิตร และ 300 มิลลิลิตร  
ตามลำดับ ชิดจำกัดการตรวจหาในสารละลายปริมาตร 3.0 มิลลิลิตร คือ 0.82 ส่วนในล้านส่วน  
และในสารละลายปริมาตร 300 มิลลิลิตร คือ 12 ส่วนในพันล้านส่วน สารมลทินสองชนิดหลัก  
สำหรับแผ่นฟิล์มตรวจวัดนี้ คือ ไอออนคอปเปอร์ และไอออนนิกเกิล แผ่นฟิล์มตรวจวัดมีความ  
เสถียรที่ดีในระยะสองเดือนที่ศึกษา และมีความเที่ยงสูง การใช้แผ่นฟิล์มตรวจวัดหาปริมาณไอออน  
โคบอลต์ในน้ำตัวอย่างได้ผลเป็นที่น่าพอใจ

SUDARAT SOMBATSRI : PREPARATION OF OPTICAL SENSING  
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#### OPTICAL CHEMICAL SENSOR / CHITOSAN / COBALT/ NITROSO-R SALT

In this research, we have introduced a simple method for preparation of an optical chemical sensor for the determination of cobalt(II) ion in aqueous solution based on immobilized disodium-1-nitroso-2-naphthol-3,6-disulphonate or nitroso-R salt in a modified chitosan film. High molecular weight chitosan was chosen to use as a sensor matrix and its structure was modified with epichlorohydrin to enhance the stability of the film in aqueous solution.

In the presence of cobalt(II), color of the sensing film changed from yellow to reddish orange as a result of complex formation between cobalt(II) and nitroso-R salt. Absorbance measurement of the complex was made at 525 nm. An optimum response of the sensing film to cobalt(II) was obtained in phosphate buffer solution of pH 5.0 and the soaking time of 15 min.

Linear calibration curves over cobalt(II) concentration ranges of 1-60 ppm and 35-400 ppb were obtained with 3.0 and 300 mL solution, respectively. The limit of detection of 0.82 ppm was obtained with 3.0 mL solution and that of 12 ppb was obtained with 300 mL solution. Two major interference for the sensor are copper(II) and nickel(II). The sensing film exhibited good stability for two months and high

reproducibility. The sensing films were also applied for the determination of cobalt(II) in water samples with satisfactory results.

School of Chemistry

Academic Year 2008

Student's Signature\_\_\_\_\_

Advisor's Signature\_\_\_\_\_

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# CONTENTS

	<b>Page</b>
ABSTRACT IN THAI.....	I
ABSTRACT IN ENGLISH.....	II
ACKNOWLEDGEMENTS.....	IV
CONTENTS.....	V
LIST OF TABLES.....	IX
LIST OF FIGURES.....	X
LIST OF ABBREVIATIONS.....	XII
<b>CHAPTER</b>	
<b>I INTRODUCTION.....</b>	<b>1</b>
1.1 Chemical sensor.....	2
1.2 Research objectives.....	4
1.3 Scope and limitation of the study.....	4
<b>II LITERATURE REVIEWS.....</b>	<b>5</b>
2.1 Sensing reagents .....	6
2.1.1 Azo dyes.....	6
2.1.2 Nitro and nitroso dyes .....	7
2.1.3 Other class of dyes.....	9
2.2 Supporting matrix .....	10
2.2.1 Cellulose .....	10



## CONTENTS (Continued)

	<b>Page</b>
2.2.2 Agarose .....	11
2.2.3 Polyvinyl chloride.....	11
2.2.4 Chitosan.....	13
2.2.5 Nafion.....	14
<b>III EXPERIMENTAL</b> .....	17
3.1 Chemicals and materials.....	17
3.2 Apparatus and instruments.....	18
3.3 Spectrometric measurements.....	18
3.3.1 Measurement with UV-VIS spectrometer .....	18
3.3.2 Measurement with FT-IR spectrometer.....	19
3.3.3 Measurement with atomic absorption spectrometer.....	19
3.4 Preparation of aqueous solutions.....	19
3.5 Study of cobalt(II) complexing agents.....	20
3.6 Preparation of sensing film.....	20
3.6.1 Preparation of PVC based sensing film .....	20
3.6.2 Preparation of chitosan film.....	21
3.6.3 Preparation of crosslinked chitosan film.....	21
3.6.4 Preparation of chitosan based sensing film.....	22
3.6.5 Study of stability of chitosan film and chitosan based sensing films.....	22

## CONTENTS (Continued)

	<b>Page</b>
3.7 Response of sensing film .....	23
3.7.1 Response of sensing film to cobalt(II) ion .....	23
3.7.2 Interference study.....	23
3.7.3 Calibration studies.....	23
3.7.4 Water analysis.....	24
<b>IV RESULTS AND DISCUSSION.....</b>	<b>25</b>
4.1 Complexing agents for cobalt(II) ion .....	25
4.2 Supporting matrix and sensing film.....	28
4.2.1 PVC film.....	28
4.2.2 Chitosan film.....	28
4.2.3 Immobilization of organic dye in crosslinked chitosan film.....	30
4.3 Optical chemical sensor based on immobilized nitroso-R salt in crosslinked chitosan film.....	32
4.3.1 Study of immobilization time.....	34
4.3.2 Response of the sensing film to Co(II).....	35
4.3.3 Effect of pH.....	37
4.3.4 Calibration.....	38
4.3.5 Interference study.....	41
4.3.6 Reproducibility and lifetime.....	43

**CONTENTS (Continued)**

	<b>Page</b>
4.3.7 Water sample analysis.....	45
<b>V CONCLUSIONS</b> .....	47
REFERENCES.....	49
APPENDICES.....	56
APPENDIX A ABSORPTION SPECTRA OF SOME ORGANIC DYES AND THEIR COMPLEX WITH COBALT(II) ION.....	57
APPENDIX B STATISTIC TEST.....	63
CURRICULUM VITAE.....	66

## LIST OF TABLES

<b>Table</b>		<b>Page</b>
2.1	Summary of optical chemical sensor for the determination of Co(II).....	16
4.1	Color of organic dye solutions at various pH and their mixture with Co(II).....	26
4.2	Absorption characteristic of dye solutions and their complexes with Co(II) .....	27
4.3	Solubility studies of chitosan film and crosslinked chitosan film in various aqueous solutions.....	29
4.4	Response of sensing film to some metal ions.....	41
4.5	Absorbance of the sensing films with 10 and 60 ppm Co(II) solutions at 525 nm.....	44
4.6	Determination of Co(II) in water samples.....	46

## LIST OF FIGURES

Figure		Page
1.1	A simplified diagram of the components of a chemical sensor.....	3
2.1	Examples of azo compounds that can form complex with Co(II) .....	8
2.2	Chemical structure of nitroso-R salt.....	9
2.3	Chemical structure of cobalt-nitroso-R salt complex.....	9
2.4	Chemical structure of murexide .....	10
2.5	Chemical structure of polymers.....	12
4.1	FTIR transmittance spectra of uncrosslinked chitosan, glutaraldehyde-crosslinked chitosan, and epichlorohydrin-crosslinked chitosan.....	31
4.2	Absorption spectra of NSR solution and NRS-Co(II) complex in solution pH 5.0.....	33
4.3	Absorption spectra of the sensing film and the sensing film soaked in Co(II) solution at pH 5.0.....	34
4.4	The influence of time on the immobilization of NRS.....	35
4.5	Time dependence of the response signal at 525 nm of the sensing film with Co(II) at different concentrations.....	36
4.6	The effect of pH on the response signal of the sensing film to 50 ppm of Co(II).....	38
4.7	Calibration curve for Co(II) obtained with 3.0 mL solution .....	39

**LIST OF FIGURES (Continued)**

<b>Figure</b>		<b>Page</b>
4.8	Calibration curve for Co(II) obtained with 300 mL solution.....	40
4.9	Absorption spectra of sensing film and the sensing films with some interference ions.....	42
4.10	Absorbance of the sensing film at 435 nm.....	43
4.11	Stability of sensing films over a period of 60 days.....	45

## LIST OF ABBREVIATIONS

°C	degree celsius
μL	microliter
AAS	atomic absorption spectrometry
cm	centimeter
cm <sup>-1</sup>	wavenumber
conc.	concentrate
FT-IR	Fourier transform infrared
g	gram
h	hour
kg	kilogram
L	liter
M	molar
MW	molecular weight
mg	milligram
min	minute
mL	milliliter
mm	millimeter
nm	nanometer
ppb	part per billion
ppm	part per million

**LIST OF ABBREVIATIONS (Continued)**

s	second
v/v	volume by volume
w/v	weight by volume
w/w	weight by weight



# CHAPTER I

## INTRODUCTION

Heavy metal ions are growing interest in environmental and clinical analysis because of their toxicity to human health. Cobalt is one of the most important metal ions, which appears in many real and synthetic samples. Cobalt is an essential element in trace amounts for human and animal, for an example, it is part of vitamin B12 and plays a key role in the body's synthesis of this essential vitamin. Deficiency of cobalt can result in retarded growth rate, loss of appetite, and anemia in human. However, harmful health effects can also occur when too much metal is taken into the body. The World Health Organization (WHO, 2006) has recommended that exposure to cobalt in a concentration range from 0.04 to 0.14 mg/kg body weight per day for several years is leading to death. The Agency for Toxic Substances and Disease Registry (ATSDR, 2004) regulated maximum allowable level of cobalt in soil to be 40 ppm and the United States Environment Protection Agency (EPA, 2003) regulated the concentration of cobalt in groundwater not to exceed 2.2 ppm.

There are several techniques for the determination of cobalt such as flame and graphite furnace atomic absorption spectroscopy (AAS) (Ye et al., 2002; Matos et al., 2005), molecular absorption and fluorescence spectroscopy (Rezaei et al., 2001; Shar et al., 2004), voltammetry (Vega et al., 1997), energy dispersive X-ray fluorescence spectroscopy (EDXRF) (Jiang et al., 2005), and reversed-phase capillary high performance liquid chromatography (RP-Capillary-HPLC) (Chung and Chung, 2003).

These methods require expensive equipments, skilled operator, and sample pretreatment. Therefore, alternative methods such as optical chemical sensors which are simple to use and require less expensive instrument have been developed.

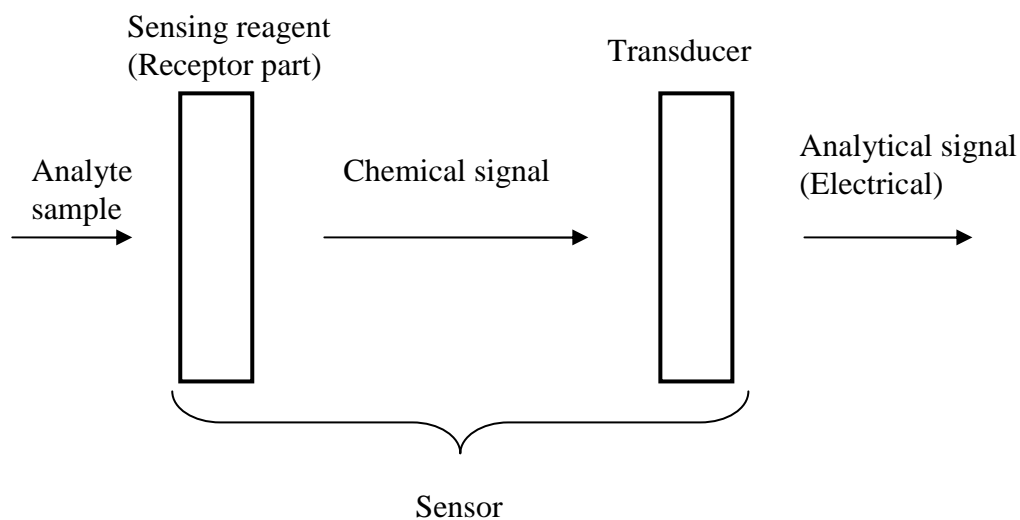
## 1.1 Chemical sensors

Sensor can be divided into two general groups. The first group is physical sensor that is used to measure parameters such as temperature and pressure changes of samples. The second group is chemical sensor which involves a response of an analyte such as inorganic cations and anions.

A chemical sensor can be defined as a device which responds to a particular analyte in a selective way through a chemical reaction. Chemical sensors contain two basic functional units which are a receptor part and a transducer part. Some sensors may include a separator which is, for example, a membrane. Figure 1.1 shows a simplified diagram of a chemical sensor device, when a receptor part responds to an analyte, a chemical signal such as color change, the production of heat or the emission of fluorescence light is produced and transformed into a form of energy by a transducer (Baldini et al., 2005).

Chemical sensors can be categorized into optical, electrochemical, mass-sensitive, and heat-sensitive, according to the types of transducer (Cattrall, 1997).

1. Optical sensors or optodes: The optical properties of the sensor are changed when the receptor part detects an analyte. A spectroscopic measurement is usually associated with a chemical reaction and conventional spectrometric method including absorption, fluorescence, and reflectance spectroscopy can be applied.



**Figure 1.1** A simplified diagram of the components of a chemical sensor.

2. Electrochemical sensors: A receptor surface of sensor upon binding an analyte alters the electrical characteristics: potential (ion-selective electrodes and field transistors, potentiometric cells), current at a given potential (voltammetric sensors), conductivity (conductometric and semiconductor gas sensors), and capacity of the receptor.

3. Mass-sensitive or piezoelectric sensor: The sensor responds to a change in the mass of a sensitive layer upon binding of an analyte with reagent molecules.

4. Heat-sensitive or calorimetric: The thermal effect of a chemical reaction involving an analyte is monitored with thermometer.

This thesis focuses on preparation of an optical chemical sensor for the determination of Co(II) where the receptor part of sensor has two main components including sensing reagent and supporting matrix.

## **1.2 Research objectives**

1. To investigate varieties of organic dyes that can be used as a chelating agent for Co(II).
2. To examine different types of polymers which are suitable for immobilization supporting matrix.
3. To construct sensing film for the determination of Co(II) in aqueous solution.
4. To determine Co(II) in real water samples with the sensing film.

## **1.3 Scope and limitation of the study**

1. Chitosan and PVC were investigated as supporting matrix for organic dye immobilization.
2. Dye, dye-metal complex and dye in supporting matrix were characterized by UV-VIS spectrometer.
3. Response of the developed sensors was studied by UV-VIS spectrometer.
4. Sensor performance characteristics such as response time, working range, detection limit, and lifetime were determined.

## **CHAPTER II**

### **LITERATURE REVIEWS**

Optical chemical sensors or optodes contain two main components including sensing reagent such as organic dyes and supporting matrix. For heavy metal ion optodes, a sensing reagent and a supporting matrix are usually based on complexing agent and polymer film or membrane. Organic ligands or organic dyes are commonly used as metal ion complexing agents, which are physically or chemically immobilized in a polymer matrix.

Immobilization of a sensing reagent in a polymeric membrane is an important step in the development of an optode. The immobilization techniques include entrapment, electrostatic interaction, and covalent attachment. Entrapment is a simple immobilization method, but a sensing reagent can be easily leached out of a polymer matrix. In electrostatic technique, a charged sensing reagent is retained in a polymer matrix by interacting with oppositely charged functional group of the matrix. This process is generally used with ion-exchange resins. The sensing reagent can be displaced from the polymer matrix by ionic species with similar charge to that of the sensing reagent under appropriate condition. For covalently immobilization, a sensing reagent is covalently bonded to a polymer matrix. With this method, the sensing reagent does not suffer from leaching and has a longer lifetime. However, the method often requires chemical modification of both the sensing reagent and the support matrix in order to facilitate the binding reaction.

## 2.1 Sensing reagent

Sensing reagents in optical sensor for the determination of heavy metal ions are usually based on organic indicator dyes such as pyridylazo resorcinol (PAR) (Yusof et al., 2002) for detection of Co(II) or for determination of Hg(II) (Ensafi and Fouladgar, 2006), pyrogallol red for determination of trace Co(II) (Ensafi et al., 2005), methyltrioctylammonium chloride (Rastegarzadeh and Moradpour, 2007) for Co(II) determination, 2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol (Br-PADAP) for detection of Ni(II) (Amini et al., 2004). This thesis focuses on investigation and application of organic indicator dyes as a sensing agent for the determination of Co(II). Organic dyes, for example, azo, carbonyl, phthalocyanine, arylcarbonium, sulphur, methane, and nitro dyes can be classified by chemical structures and functional groups. Some of the dyes that are used for Co(II) analysis are discussed here.

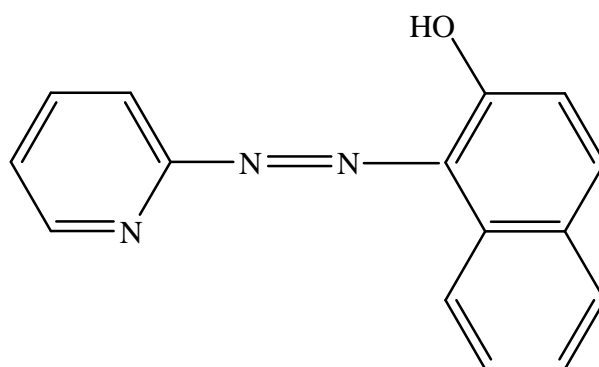
### 2.1.1 Azo dyes

The azo compound class accounts for 60% of all dyes in spectrophotometric analysis (Christic et al., 2002) and has been studied extensively. These dyes are characterized by chromophoric azo groups ( $-N=N-$ ) and have a wide spectrum of colors such as yellow, orange, and red which are more commercially supplied than any other colored dyes. The examples of the dyes in this group that have been proved to be the suitable ligands to complex with Co(II) are pyridylazo naphthol (PAN) (Matos et al., 2005; Shar et al., 2004), pyridylazo resorcinol (PAR) (Jiang et al., 2005; Yusof et al., 2002; Karipcin and Kabalcilar, 2007), thiazolylazo

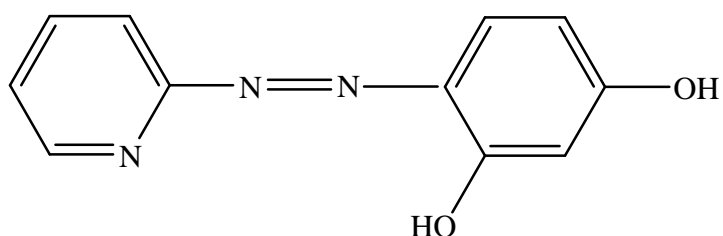
(Chung and Chung, 2003) and pyridylazo diethylaminophenol (PADAP) (Sozgen and Tutew, 2004) and their chemical structures are shown in Figure 2.1.

### 2.1.2 Nitro and nitroso dyes

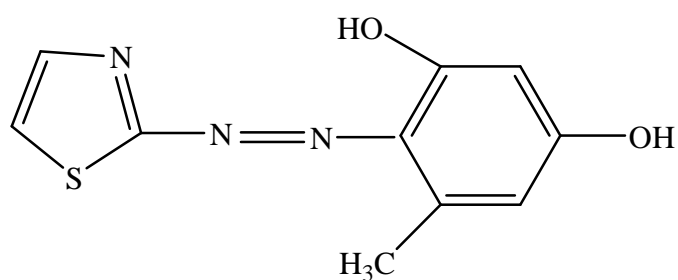
For this class, compounds containing  $-\text{NO}_2$  group or a  $-\text{NO}$  group are named only by means of the prefixes "nitro-" or "nitroso-", respectively. Nitroso-R salt or disodium-1-nitroso-2-naphthol-3,6-disulphonate, for example, was first proposed as a complexing agent for the determination of Co(II) ion by Van Klooster in 1921. The structure of the dye is shown in Figure 2.2. It is a derivative of 1-nitroso-2-naphthol which is a reagent for Co(II) ion analysis. The sulphonate groups of nitroso-R salt make cobalt complex soluble in water over a wide range of pH. Nitroso-R salt has been used as a complexing agent to determine cobalt spectrophotometrically in aqueous medium. Co-nitroso-R salt complex structure is shown in Figure 2.3. Nitroso-R salt was also used as a reagent for the determination some other ions such as Cu(II) (Jianzhong and Zhujun, 1995), Ni(II) (Issa et al., 1980; Ghasemi et al., 2004), Fe(II) and Fe(III) (Ivanov et al., 2006; Kruanetr et al., 2008).



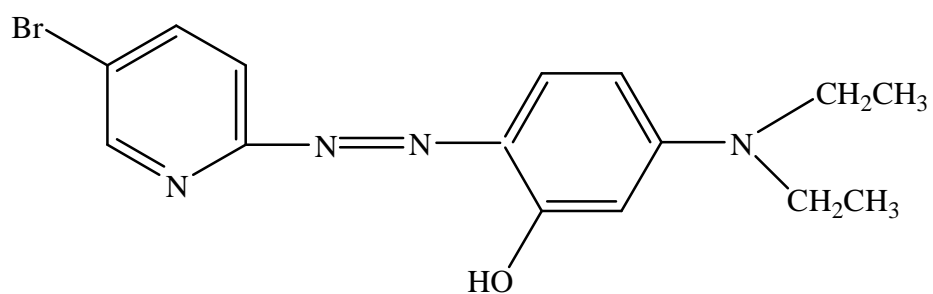
pyridylazo naphthol (PAN)



pyridylazo resorcinol (PAR)



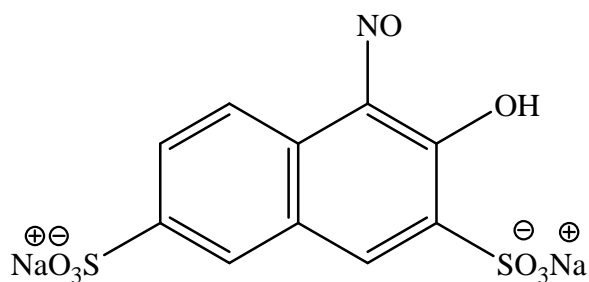
5-methyl-4-(2-thiazolylazo) resorcinol (5-Me-TAR)



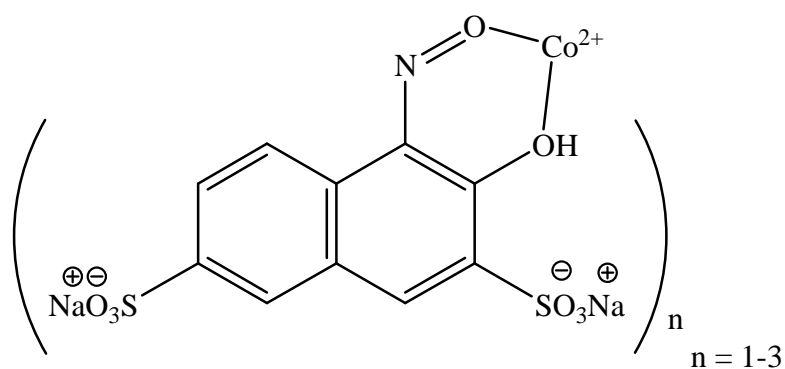
2-(4-diethylamino-2-hydroxyphenylazo)-5-bromopyridine

**Figure 2.1** Examples of azo compounds that can form complex with Co(II).





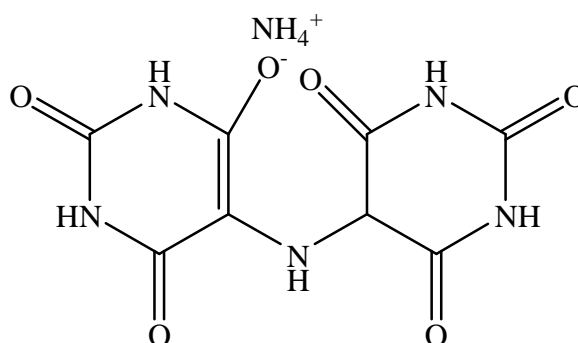
**Figure 2.2** Chemical structure of nitroso-R salt.



**Figure 2.3** Chemical structure of cobalt-nitroso-R salt complex.

### 2.1.3 Other classes of dyes

Other colorimetric reagent for determining cobalt(II), for example, is murexide or 5,5'-nitroindobarbituric acid monoammonium salt (Shamsipur and Alizadeh, 1992). The dye has a significant color change in the visible region when it complexes with cobalt(II). The chemical structure of murexide is shown in Figure 2.4. Murexide has long been used as a metallochromic indicator. However, the stability constants of its complexes are not very large which could limit the use of murexide as a metallochromic indicator in aqueous solution.



**Figure 2.4** Chemical structure of murexide.

## 2.2 Supporting matrix

Polymers are widely used as supporting matrix to entrap a sensing reagent in optical chemical sensors. They can function as a selective element which allows certain species to pass through or as a chemical transducer itself. Therefore, polymers can influence the performance characteristics of a sensor such as sensitivity and selectivity. Some requirements for a polymer to be used as a supporting matrix for optical chemical sensor are optically transparent, low intrinsic fluorescence or absorbance, homogeneous, compatible with sensing reagent, permeable to analyte, flexible, controllable film thickness, and thermally stable. Various polymers such as cellulose, polyvinyl chloride or PVC, chitosan, agarose, and nafion have been used in optical sensor and some polymers are discussed here.

### 2.2.1 Cellulose

Cellulose, Figure 2.5 (a), is a polysaccharide consisting of a linear polymer chain of  $\beta$ -(1 $\rightarrow$ 4)-D-glucopyranose units. The one primary and two secondary hydroxyl groups in each monomer unit in polymer structure are the groups

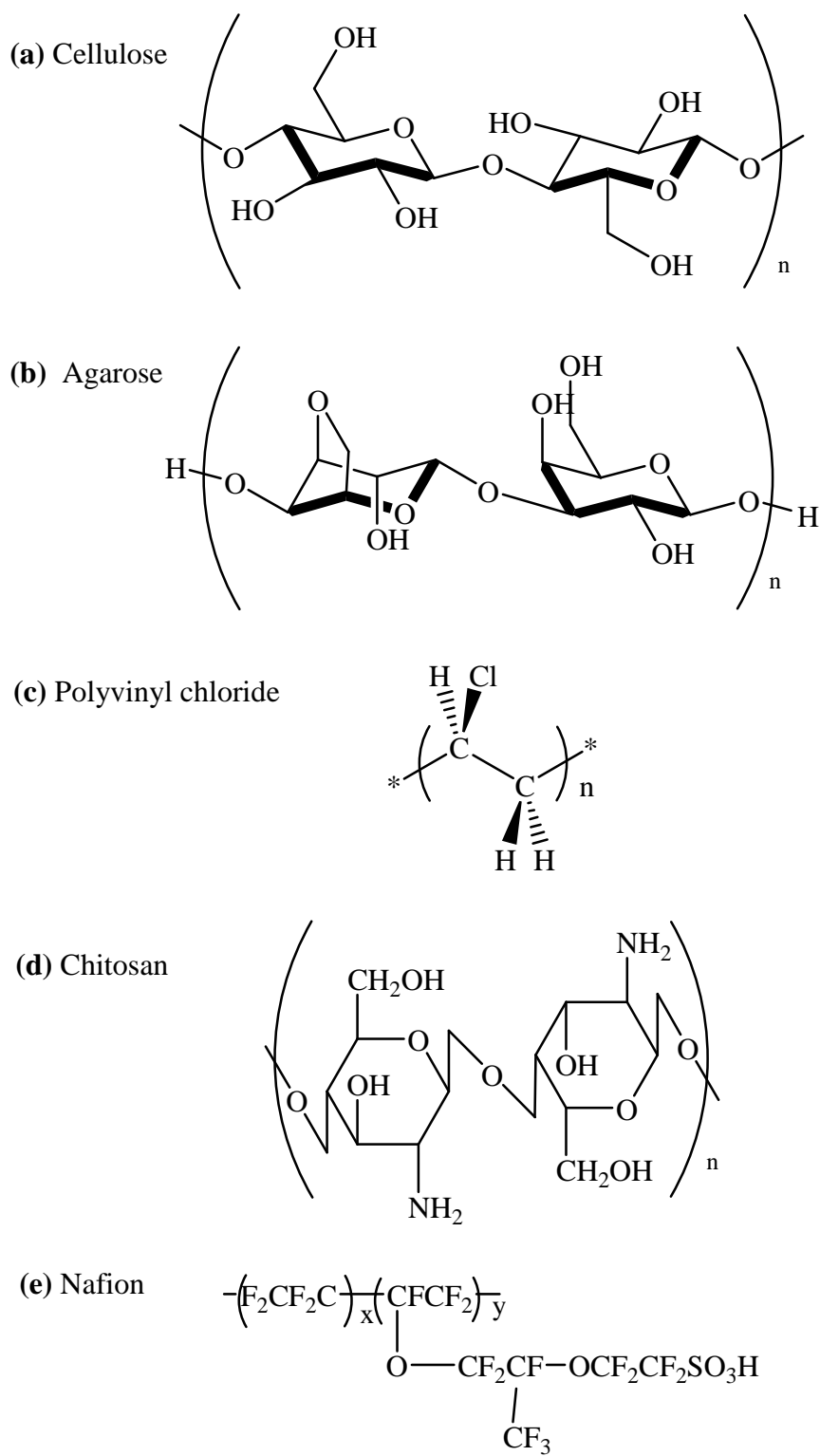
that can be modified for immobilization suitable reagent. Ensafi and Aboutalebi (2005) immobilized pyrogallol red on cellulose membrane and used the membrane to determine Co(II) in solution with pH 9.3 by monitoring the absorbance change at 550 nm. Rastegarzadeh and Moradpour (2007) used a cellulose membrane immobilized with methyltrioctylammonium chloride to spectrophotometrically determine Co(II) at 627 nm by forming an ion pair that changed a colorless membrane to a blue complex of  $[\text{Co}(\text{SCN})_4]^{2-}$  on the membrane at pH 5. However, cellulose is swollen in water and can be hydrolyzed by acids and bases. Consequently, its use in aqueous medium is limited.

### **2.2.2 Agarose**

Agarose, Figure 2.5 (b), is a gel-type hydrophilic support, chemically inert and microbiologically resistant material. It is quite stable in gel form and is the most widely used support in affinity chromatography. Agarose can be easily manufactured and simply activated and functionalized with an indicator. Hashemi et al. (2008) developed an optical sensor based on calmagite immobilized on a transparent agarose film for determination of Cu(II). The linear response of the sensor was in a concentration range of  $6.29 \times 10^{-9}$  -  $3.14 \times 10^{-6}$  M and limit of detection was  $1.10 \times 10^{-9}$  M.

### **2.2.3 Polyvinyl chloride**

Polyvinyl chloride or PVC, Figure 2.5 (c), is a useful polymer for sensor applications because it is optically transparent and hydrophobic which is stable in aqueous solution. However, a membrane made from PVC alone is relatively stiff



**Figure 2.5** Chemical structures of polymers.

and therefore, a suitable plasticizer is added to reduce the stiffness of the material. Ozturk et al. (2007) reported 4-(2-furylmethylene)-2-phenyl-5-oxazolone (PFO) embedded in PVC used for Fe(III) sensing in a concentration range of  $10^{-6}$ -  $10^{-4}$  M at pH 7 with limit of detection  $3.8 \times 10^{-6}$  M.

#### 2.2.4 Chitosan

Chitosan, Figure 2.5 (d), is a cationic natural polysaccharide which is nontoxic, hydrophilic, and resistant to many chemicals. Chitosan is soluble in aqueous acidic solution such as acetic, formic, or hydrochloric acid, to give a viscous solution. The amino groups in chitosan are fully protonated at pH~3, and then the polymer chains are positively charged along the polymer backbone in the solution, resulting in dissolution. Chitosan has various functional groups including amine and hydroxyl group on the saccharide backbone that can be modified and used to couple with sensing reagent under a mild reaction condition. Sensing reagent may be physically or chemically immobilized into the chitosan membrane. Adsorption of dyes on chitosan can be attributed to the interaction between the protonate amine group of chitosan ( $R-NH_2$ ) and the anionic group of the dye molecule. Dubas et al. (2006) immobilized anionic Nylosan dye onto cationic chitosan and used as an alcohol sensor. In case of chemical immobilization, covalent bonds are formed by chemical reaction between sensing reagent and functional groups in chitosan. For instance, Miao and Tan (2000) chemically bonded peroxidase with chitosan using glutaraldehyde as a bridging reagent.

Chitosan film can be formed by a casting technique and high molecular weight chitosan has been reported to have good film-forming properties

(Nunthanid et al., 2001). Yusof and Ahmad (2002) developed an optical sensor based on pyridylazo resorcinol (PAR) immobilized in chitosan membrane for the determination of Co(II) using flow injection analysis. The disadvantage of this optode was leaching of the sensing reagent in aqueous solution.

Active groups of chitosan which are amine and hydroxyl groups can form hydrogen bonds with water. Consequently, the membrane swells in an aqueous solution and the physical stability of the membrane is reduced. Chitosan membranes can be modified by crosslinking reaction to improve their stability. The hydroxyl and amino groups on glucosamine unit of chitosan provide the reactive sites for such modification. There are two types of crosslinking systems. One is chemical crosslinking, using reagents such as glutaraldehyde (GLA), glyoxal, and epichlorohydrin. Crosslinking chitosan polymer chains with glutaraldehyde (Monteiro et al., 1999; Beppu et al., 2007; Vieira and Beppu, 2006), epichlorohydrin (Wan et al., 2003; Vieira and Beppu, 2006) and glyoxal (Patel and Amiji, 1996; Yang et al., 2005) are examples of chemical modification. Another system is ionic crosslinking, using sulfuric acid or tripolyphosphate as crosslinking reagent (Cui et al., 2008).

### **2.2.5 Nafion**

Nafion, Figure 2.5 (e), is polyfluorinated hydrocarbon. The polymer consists of a polytetrafluoro ethylene backbone and perfluorovinyl ether pendent side chain with sulfonic acid group built into its structure. Nafion has become a polymer of choice for sensor applications because it is hydrophobic and resistant to most solvent, oxidant, and bases. In addition, nafion membrane is permeable for many

cations, but it is impermeable for anions and nonpolar compounds. Amini et al. (2004) reported an optical sensor based on 2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol (Br-PADAP) immobilized in nafion which was used for the determination of Ni(II). The sensor showed linear response to Ni(II) in a concentration range of  $8.52 \times 10^{-6}$ - $3.40 \times 10^{-4}$  M. The reagent leaching was not observed.

In this thesis, some dyes were investigated as the complexing agents for Co(II) in aqueous solution and only those that provided positive results were used to immobilize in chitosan and PVC. The dye immobilized films were studied spectrometrically for their response to Co(II). Optical chemical sensors reported for the determination of Co(II) are summarized in Table 2.1.

**Table 2.1** Summary of optical chemical sensor for the determination of Co(II).

<b>Sensing reagent</b>	<b>Supporting matrix</b>	<b>Linear range/ detection limit</b>	<b>Principle of detection</b>	<b>Response time</b>	<b>Regeneration time</b>	<b>References</b>
pyridylazo resorcinol	chitosan	$1.70 \times 10^{-8}$ - $0.17$ M  $/1.34 \times 10^{-10}$ M	reflectance  (760 nm)	60 s	5 min in 0.2M  HCl+ saturated KCl	Yusof and Ahmad  (2002)
pyrogallol red	cellulose	$1.70 \times 10^{-6}$ - $1.52 \times 10^{-4}$ M  $/3.6 \times 10^{-7}$ M	absorption  (550 nm)	120 s	10 s in 0.01M HCl	Ensafi and  Aboutalebi  (2005)
methyltriocetyl ammonium chloride	cellulose	$8.5 \times 10^{-6}$ - $1.3 \times 10^{-4}$ M  $/5.9 \times 10^{-6}$ M	absorption  (627 nm)	7 min	<1min in 0.02M  $\text{Na}_2\text{C}_2\text{O}_4$	Rastegarzadeh and Moradpour  (2007)



# CHAPTER III

## EXPERIMENTAL

### 3.1 Chemicals and materials

1. Chitosan with low molecular weight (MW ~ 150,000, Aldrich)
2. Chitosan highly viscous (MW > 600,000, Fluka)
3. Chemicals for preparation of acetate buffer: glacial acetic acid (99.9%, J.T.Baker), sodium acetate trihydrate (99.5%, BDH), and deionized water
4. Chemicals for preparation of phosphate buffer: phosphoric acid (85%, UNIVER), dibasic sodium phosphate (99.5%, Riedel-de Haën), and deionized water
5. Crosslinking reagent: glutaraldehyde solution (25% w/w, Unilab), glyoxal solution (40% in water, Fluka), and epichlorohydrin (99%, Aldrich)
6. Poly(vinyl chloride) high molecular weight (Fluka)
7. Tetrahydrofuran (99.8%, Carlo Erba)
8. Plasticizers : dioctyl phthalate (99.0%, Aldrich)
9. Indicator dyes: disodium-1-nitroso-2-naphthol-3,6-disulphonate (AR grade, Fluka) or nitroso-R salt, 5-methyl-4-(2-thiazolylazo)resorcinol (95.0%, Aldrich), 1-[[4-(phenylazo)phenyl]azo]-2-naphthalenol or sudan III (AR grade, Fluka), 5,5'-nitroindobarbituric acid monoammonium salt or murexide (AR grade, Carlo Erba), 1-(2-pyridylazo)-2-naphthol (97.0%, Fluka), 4-([2,4-dihydroxyphenyl]azo)benzenesulfonic acid sodium salt or

tropaeolin O indicator (AR grade, Riedel-de Haën), 4-(2-pyridylazo)-resorcinol (95.0%, Fluka), tri-sodium 5-hydroxy-1-(4-sulfonatophenyl-4-(4-sulfonatophenylazo)pyrazole-3-carboxylate or tartrazine (AR grade, Fluka).

10. Cobalt stock solution, 1000 mg/L (BDH)

## **3.2 Apparatus and instruments**

1. Apparatus for membrane preparation; glassware, glass slide, transparent film, magnetic stirrers, magnetic bars, micropipette, polypropylene (PP) bottles, and hot air oven.
2. CHEM4-Vis-fiber spectrometer (USB 4000, Ocean optics)
3. Fourier transform infrared (FT-IR) spectrometer (Model spectrum GX, Perkin- Elmer)
4. Flame-atomic absorption spectrometer (AAAnalyst 100, Perkin- Elmer)
5. pH meter (DELTA 320, Mettler Toledo)

## **3.3 Spectrometric measurements**

### **3.3.1 Measurement with UV-VIS spectrometer**

Absorbance of a sensing membrane was measured with a UV-VIS spectrometer. The absorbance measurement was made periodically while the sensing membrane was dipped in a solution which was contained in a 10-mm cuvette. The sensing film was first introduced in the UV-VIS cuvette filled with buffer and recorded as a blank. For the absorbance measurements, the sensing film was dipped

in 3.0 mL Co(II) solution or 3.0 mL water sample with continuous stirring. The absorbance of the sensing film was measured in the wavelength range of 350-700 nm.

### **3.3.2 Measurement with FT-IR spectrometer**

FT-IR spectrometer was used to obtain spectra of chitosan films. The sample film was prepared by detaching the film from the transparent film and cut into a suitable size. The sample film was placed onto a sample holder of the spectrometer before infrared spectra were recorded. The average thickness of the film was 0.01 mm. The spectra were obtained in the wavenumber range of 400-4000  $\text{cm}^{-1}$  and a resolution of 4  $\text{cm}^{-1}$ .

### **3.3.3 Measurement with atomic absorption spectrometer**

Concentrations of Co(II) ion in water samples were determined by atomic absorption spectrometer and parameters for the instrument setting are as follow;

Oxidant/Fuel: air/acetylene (2:5)

Wavelength: 240.7 nm

The only treatment of sample was digestion with nitric acid following a standard method for examination of water and wastewater (Greenberg et al., 1992).

## **3.4 Preparation of aqueous solutions**

1. Dye solutions (0.10% w/v) were prepared by dissolving 10.0 mg of dyes in a suitable solvent such as ethanol and water to produce 10 mL solutions.

2. 1% (v/v) acetic acid was prepared by diluting 1 mL of glacial acetic acid in deionized water and the volume was made up to 1 L.
3. Acetate buffer solution was prepared by mixing 0.1 M sodium acetate and 0.1 M acetic acid and the pH of the solution was adjusted with 1 M NaOH.
4. Phosphate buffer solution was prepared by dissolving 71.6 g of dibasic sodium phosphate in deionized water and mixed with phosphoric acid solution. pH of the solution was adjusted with 0.1 M NaOH or HCl.
5. 0.02 M epichlorohydrin solution was prepared by dissolving 0.4 g of epichlorohydrin in 200 mL of 0.067 M NaOH and the solution volume was adjusted to 250 mL.
6. Solutions of cobalt was prepared by diluting appropriate volume of the stock Co(II) solution and the solution volume was adjusted with buffer solution.

### **3.5 Study of cobalt(II) complexing agents**

The organic dye solution was mixed with 10 ppm Co(II) solution with the volume 1:1. The colored change of the solution was observed after mixing the solution for 30 min to 1 h. Absorption spectrum of the solution mixture was recorded by the UV-VIS spectrometer and compared to that of the dye solution.

### **3.6 Preparation of sensing films**

#### **3.6.1 Preparation of PVC based sensing film**

A mixture for membrane preparation was prepared by dissolving 120 mg of PVC, organic dye, and dioctyl phthalate (DOP) in 2 mL tetrahydrofuran (THF).

The resulting mixture was stirred for 5 min at room temperature. An aliquot of 250  $\mu\text{L}$  of this solution was poured on a glass slide and THF was evaporated at room temperature for overnight (12 h). The size of the film was 2.0 $\times$ 2.5 cm.

### **3.6.2 Preparation of chitosan film**

Chitosan solution was prepared by dissolving 1.00 g of high molecular weight chitosan in 100 mL 1% (v/v) acetic acid solution. The solution mixture was stirred at room temperature for 3 h and sonicated for 30 min to remove the air bubbles. To make a chitosan film, 250  $\mu\text{L}$  of the chitosan solution was casted onto a transparent film, dried in a hot air oven at 60°C for 24 h, and kept in a dessicator. The size of the film was 0.8 $\times$ 3.5 cm.

### **3.6.3 Preparation of crosslinked chitosan film**

Crosslinked chitosan films were prepared by either homogeneous or heterogeneous method. For homogeneous method glutaraldehyde or glyoxal was added into 1% (w/v) chitosan solution and the solution was stirred for a given time. The concentrations of glutaraldehyde and glyoxal in the solution were 0.75% and 4% wt, respectively. The solution mixture was pipetted (250  $\mu\text{L}$ ) and casted onto a transparent film, dried in a hot air oven at 60°C for 24 h, and kept in a dessicator.

For heterogeneous method, a chitosan film was crosslinked in glutaraldehyde, glyoxal, or epichlorohydrin solution.

For crosslinked in glutaraldehyde solution, a chitosan film was immersed in a solution of 0.75% (w/w) glutaraldehyde without agitation at room temperature for 2 h. After that, the film was washed with deionized water to remove

unreacted glutaraldehyde (Baroni et al., 2008). To crosslink chitosan with glyoxal, the chitosan film was immersed in 4% (w/w) glyoxal solution at 40°C without agitation for 30 min, and then the film were washed with deionized water (Yang et al., 2005).

In the epichlorohydrin crosslinking process, the chitosan film was immersed in 0.02 M epichlorohydrin at 40°C under continuous agitation for 2 h. After that, the film was washed with deionized water to remove unreacted crosslinking agent (Wan et al., 2003).

#### **3.6.4 Preparation of chitosan based sensing film**

The sensing film was prepared by immobilizing of organic dye onto the crosslinked chitosan film. In this process, the crosslinked chitosan films were soaked in 0.10% (w/v) of dye solutions under continuous agitation for 3 h at 27°C, washed with deionized water, dried, and kept in a dessicator. For NRS immobilized chitosan film, the chitosan film was immersed in 0.10% (w/v) of NRS solution with continuous stirring for various times.

#### **3.6.5 Study of stability of chitosan film and chitosan based sensing films**

Chitosan and crosslinked chitosan films were soaked in water, 1 M HCl and 1 M NaOH for 1 h to test the physical stability of the films. For the sensing films, the films were soaked in buffer solutions pH 3 to 8 for 1 h with acetate and with phosphate buffer systems.

### **3.7 Response of sensing film**

#### **3.7.1 Response of sensing film to cobalt(II) ion**

PVC and chitosan based sensing films were dipped into 3.0 mL Co(II) solution at pH 5.0 in a cuvette. The color changes of the sensing films were observed and the absorption spectra were recorded with the UV-VIS spectrometer in the wavelength range of 350-700 nm.

#### **3.7.2 Interference study**

The response of nitroso-R salt immobilized in chitosan film to other metal ion was measured by dipping the sensing film into 3.0 mL solution containing 50 ppm of the tested metal ions. The pH of the solutions was 5.0 and the soaking time was 15 min. The tested ions were K(I), Ca(II), Al(III), Fe(II), Fe(III), Cu(II), Zn(II), and Ni(II). The absorbance signals obtained with the tested metal ions were compared with that obtained with Co(II).

#### **3.7.3 Calibration studies**

Nitroso-R salt immobilized in the crosslinked chitosan films were used to obtain calibration curve for Co(II). The sensing films were submerged into 3.0 or 300.0 mL of Co(II) solution at different concentrations. The pH of the solutions and the soaking time were 5.0 and 15 min, respectively. For the measurement with 3.0 mL solution, the sensing film was dipped in the solution containing in the cuvette with continuous stirring. For the measurement with 300 mL solution, the sensing film was soaked in Co(II) solution which was contained in a 500-mL beaker with stirring for 15 min. Then, the sensing film was removed from the Co(II) solution,

rinsed with deionized water, and placed into a buffer solution in a cuvette to record the absorbance signal.

#### **3.7.4 Water analysis**

The sensing film was used to determine of Co(II) in the real samples. The water samples were collected from a reservoir and wastewater drainpipe located in Suranaree University of Technology (SUT), Nakhon Ratchasima, Thailand. The water samples were filtered and stored in polypropylene containers at 4°C.

For the measurement with the sensing film, the water samples were filtered and pH of the sample solutions was adjusted to 5 with a buffer solution.

The determination of Co(II) with the atomic absorbance spectrometer was done with the standard method for examination of water and wastewater (Greenberg et al., 1992). A sample of 50 mL was combined with 5 mL conc. HNO<sub>3</sub>. The solution was boiled gently and evaporated on a hot plate until volume of solution was 20 mL. If precipitation occurred, conc. HNO<sub>3</sub> was added and heating of the solution was continued until a clear solution was obtained. The digested solution was cooled down to room temperature and the volume of the solution was adjusted to 50 mL with deionized water.



## CHAPTER IV

### RESULTS AND DISCUSSION

#### 4.1 Complexing agents for cobalt(II) ion

Some organic dyes including 5-methyl-4-(2-thiazolyl)resorcinol (5-Me-TAR), pyridylazo resorcinol (PAR), sudan III, 1-(2-pyridylazo)-2-naphthol (PAN), disodium-2-nitroso-2-naphthol-3,6-disulphonate or nitroso-R salt (NRS), 4-([2,4-dihydroxyphenyl]azo)benzenesulfonic acid sodium salt or tropaeolin O, trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-(4-sulfonatophenylazo)pyrazole-3-carboxylate or tartrazine, and 5,5'-nitroindobarbituric acid monoammonium salt or murexide were investigated as a complexing agent for Co(II) in an aqueous solution. A solution of 0.10% (w/v) of organic dye was mixed with 10 ppm Co(II) solution in a buffer pH 5.0 and the observation was made for 30 min after mixing. The results are shown in Table 4.1.

Five organic dyes, 5-Me-TAR, PAN, PAR, murexide, and NRS, reacted with Co(II) in solution at pH 5.0 resulting in apparent changes of the colors of the organic dye solutions. Absorption spectra of the dye solutions and the dye solutions mixed with Co(II) were recorded by a UV-VIS spectrometer and are shown in appendix A. The wavelengths for absorption maximum of the dye solutions and the dye solutions mixed with Co(II) are given in Table 4.2. The color of tartrazine, sudan III, and tropaeolin O solution did not change when the dyes were mixed with Co(II) solution.

**Table 4.1** Color of organic dye solutions at various pH and their mixture with Co(II).

Organic dye	Color of mixture in solution pH 5	Color of dye solution			
		pH 3	pH 5	pH 8	pH >8
PAN	dark green	yellow	yellow	yellow	yellow
5-Me-TAR	red-brown	orange	orange	orange	orange
Sudan III	red	red	red	purple	-
PAR	red	orange	orange	reddish orange	-
Tartrazine	yellow	yellow	yellow	yellow-orange	-
Tropaeolin O	orange	orange	orange	orange	orange
Murexide	pink	colorless	purple	purple	-
Nitroso-R salt	reddish orange	yellow	yellow	yellow-green	yellow-green

**Table 4.2** Absorption characteristic of dye solutions and their complexes with Co(II).

Organic dye	Wavelength (nm)	
	Dye solution	Co(II) and dye mixture
PAN	480	465, 580, 620
5-Me-TAR	478	485, 585 (shoulder)
PAR	390	510
Murexide	520	515, 460 (shoulder)
Nitroso-R salt	372	383, 425

The influence of pH on the color change of the organic dyes was also studied by mixing the dye solutions with buffer solutions pH 3.0, 5.0, and 8.0. This study was conducted to clarify that the color change of the dyes solution was resulted from Co(II) or pH of the solution.

pH had no effect on the color change of the solutions of PAN, 5-Me-TAR, tropaeolin O, and NRS except for murexide, tartrazine, sudan III, and PAR. However, murexide solution exhibited a distinguished change of its color when the dye solution was mixed with Co(II) solution at pH 5.0. Within 1 h of observation, all solution mixtures had no considerable change of their colors. Therefore, PAR, PAN, 5-Me-TAR, murexide, and NRS were chosen for further studies as sensing reagents to immobilize in a suitable supporting matrix. In addition, pH of the solution for further studies was selected to be less than 8.0 because Co(II) precipitated in a basic solution.

## **4.2 Supporting matrix and sensing film**

### **4.2.1 PVC film**

Polyvinyl chloride (PVC) was initially chosen as a supporting matrix for dye immobilization because it is hydrophobic and optically transparent. It also has good mechanical and film forming properties.

PAN was dissolved in PVC solution and the solution was casted on a glass slide to obtain a PAN immobilized PVC film with a size of 2.0×2.5 cm. It was found that PAN immobilized in PVC slowly formed complex with Co(II) ion in aqueous solution. The green complex of Co(II) and PAN was observed ~1.5 h after the sensing film was immersed in Co(II) solution. Slow response of the sensing film to Co(II) could be resulted from low ion mobility in the hydrophobic PVC membrane. Other sensing reagents, 5-Me-TAR, PAR, murexide, and nitroso-R salt did not dissolve in PVC solution because they are hydrophilic dyes. Therefore, PVC polymer was not a suitable supporting matrix for these reagents.

### **4.2.2 Chitosan film**

Chitosan was investigated as a supporting matrix because it is optically transparent, resistant to many chemicals, and nontoxic. A film of chitosan was simply formed by a casting technique. However, the film swelled and dissolved in aqueous acidic solution, which limited its use. Swelling and dissolution of chitosan film resulted from the interaction of amine and hydroxyl groups of chitosan with water molecules. To reduce swelling of chitosan film, modification of a chitosan film via crosslinking reaction was studied with crosslinking reagent such as

glyoxal, glutaraldehyde (GA), and epichlorohydrin (ECH). The results are shown in Table 4.3.

Chitosan films crosslinked with epichlorohydrin, glutaraldehyde or glyoxal, were physically stable in acidic and alkali solutions as well as in distilled water for at least 1 h. Glutaraldehyde and glyoxal reacted with amino groups of chitosan while epichlorohydrin preferred to react with hydroxyl groups of chitosan (Vieira and Beppu, 2006). GA- and glyoxal- crosslinked chitosan films were more brittle than those crosslinked with ECH under the studied condition. Therefore, ECH was selected as a crosslinking reagent for the modification of chitosan film.

**Table 4.3** Solubility studies of chitosan film and crosslinked chitosan film in various aqueous solutions.<sup>a</sup>

Membrane	Solubility of chitosan film		
	1 M HCl	1 M NaOH	DI water
Chitosan	soluble	insoluble	swollen
Chitosan-GA	insoluble (swollen)	insoluble	insoluble
Chitosan-Glyoxal	Insoluble (swollen)	insoluble	insoluble
Chitosan-ECH	insoluble	insoluble	insoluble

<sup>a</sup> Crosslinked chitosan film were obtained under heterogeneous method.

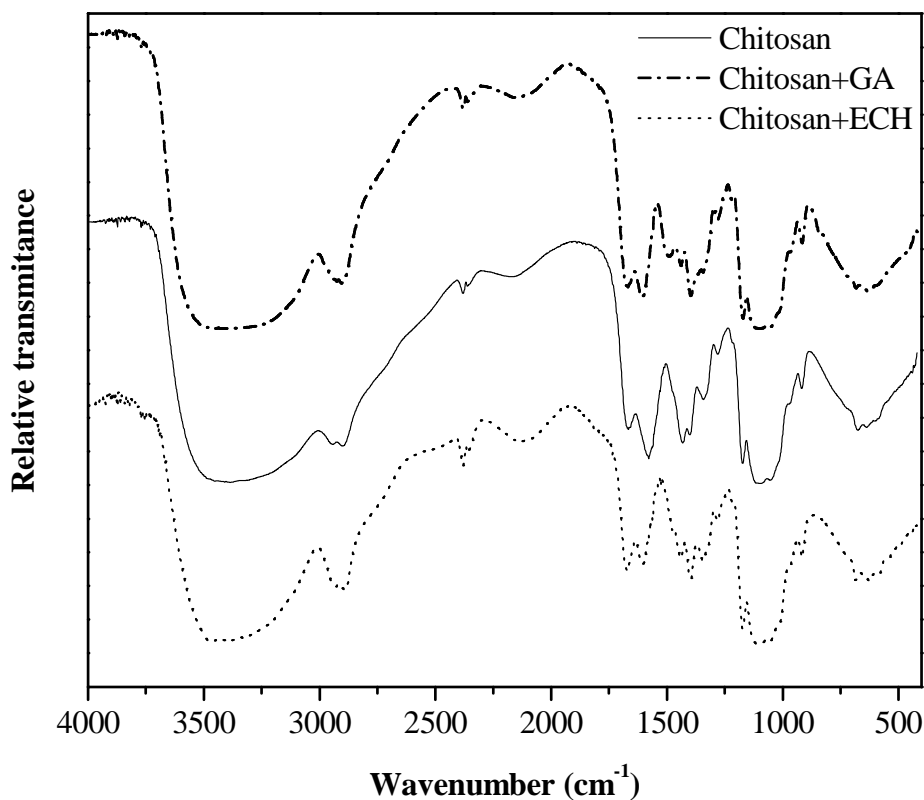
Pure chitosan films were clear and colorless to pale yellow. The chitosan film, especially those prepared from lower molecular weight chitosan became dark yellow after being stored for a few weeks at room temperature. Therefore, high molecular weight chitosan was selected for the preparation of chitosan film in this work.

The structure of modified chitosan was characterized by FT-IR as shown in Figure 4.1. Spectrum of chitosan film shows a broad and strong band ranging from 3300 to 3500  $\text{cm}^{-1}$  indicating the presence of  $-\text{OH}$  and  $-\text{NH}_2$  group. The peak at 2879  $\text{cm}^{-1}$  can be assigned to  $-\text{CH}_2$  group. The peak located at 1587  $\text{cm}^{-1}$  is characteristic of primary amine. The peak at 1377  $\text{cm}^{-1}$  corresponds to the C-N stretching.

The chitosan film crosslinked with GA shows an increase in the transmittance at 1665  $\text{cm}^{-1}$  because of imine bonds,  $\text{C}=\text{N}$ . For ECH-crosslinked chitosan film, decrease in transmittance between 1000 and 1300  $\text{cm}^{-1}$  was related to C-O-C stretching vibration, typical of epichlorohydrin-crosslinking and is in agreement with literature (Wan et al., 2003).

#### **4.2.3 Immobilization of organic dye in crosslinked chitosan film**

5-Me-TAR, PAR, murexide, and nitroso-R salt were used to immobilize in the chitosan film because they reacted with  $\text{Co(II)}$  producing colored product which could be monitored spectrometrically.



**Figure 4.1** FTIR transmittance spectra of uncrosslinked chitosan, glutaraldehyde-crosslinked chitosan, and epichlorohydrin-crosslinked chitosan.

5-Me-TAR reacted with Co(II) in solution at pH 5.0 resulted in a red-brown complex with absorption maximum at 485 nm. However, when 5-Me-TAR was immobilized in chitosan film, the dye leached out into the solution at pH 5.0.

PAR formed a red complex with Co(II) in solution at pH 5.0 with absorption maximum at 510 nm. When PAR was immobilized in chitosan film and exposed to Co(II) solution at pH 5.0, the color of the sensing film changed from

orange to red within 5 min. The absorption maximum was 520 nm. However, leaching of PAR from the sensing film into the solution was observed.

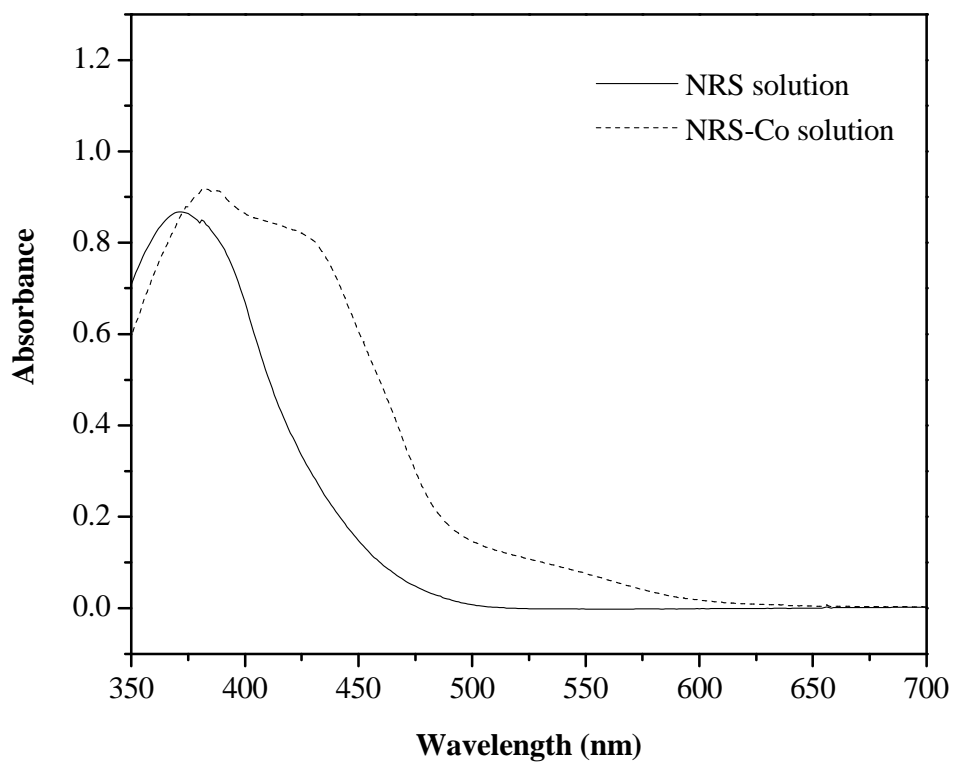
Murexide is the ammonium salt of purpuric acid which has been used as a complexing agent for a large number of metal in aqueous solution in a pH range of 5-8. When chitosan film immobilized with murexide was immersed in a Co(II) solution, the color of the sensing film changed from purple to colorless. The sensing film soaked in a solution of pH 5.0 resulted in slowly disappearing of the color of murexide within 1 h.

Nitroso-R salt is a common reagent that reacts with Co(II) ion forming a colored complex. In the presence of Co(II), the color of nitroso-R salt changed from yellow to reddish orange in buffer solution at pH 5.0. Similar color change was also observed with nitroso-R salt immobilized in chitosan film. Dye leaching from the sensing film into the solution was not observed in acidic solution because of the fact that protonated amino groups of chitosan interacted electrostatically with sulfonate groups of nitroso-R salt. Therefore, nitroso-R salt immobilized in crosslinked chitosan film was further investigated as an optical chemical sensor for the determination of Co(II).

### **4.3 Optical chemical sensor based on immobilized nitroso-R salt in crosslinked chitosan film**

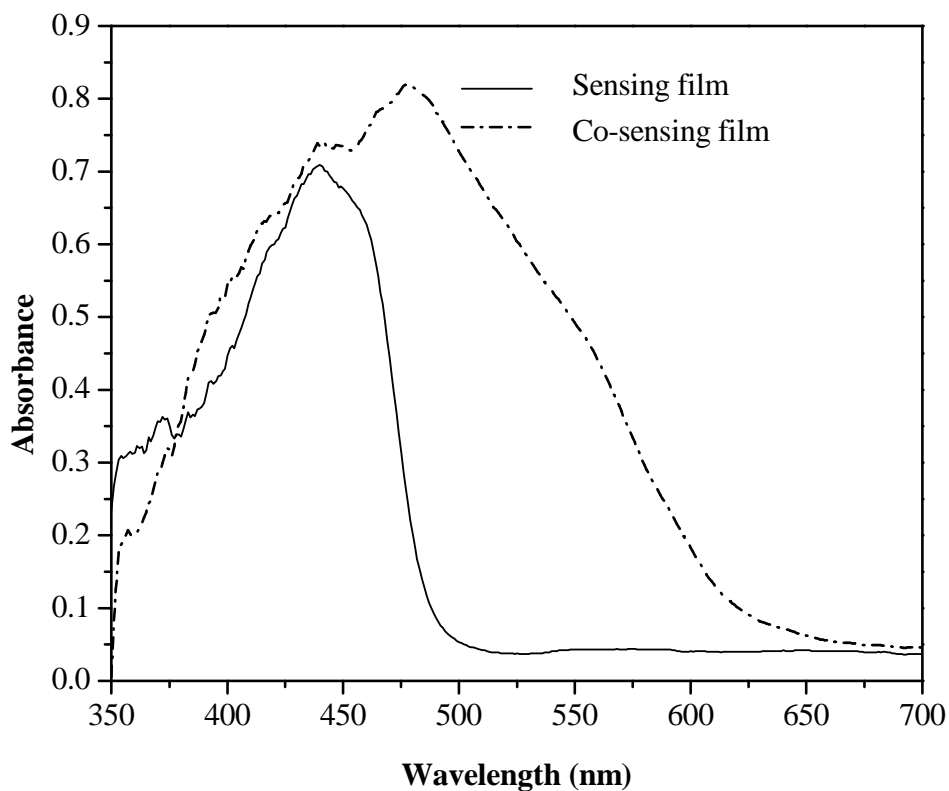
Nitroso-R salt reacted with Co(II) to form a reddish orange complex at pH 5.0 in aqueous solution with an absorption maximum at 383 nm, whereas NRS itself exhibited an absorption maximum at 372 nm as shown in Figure 4.2. The spectra of NRS immobilized in chitosan film and the film in Co(II) solution at pH 5.0 are shown





**Figure 4.2** Absorption spectra of NRS solution and NRS-Co(II) complex in solution pH 5.0.

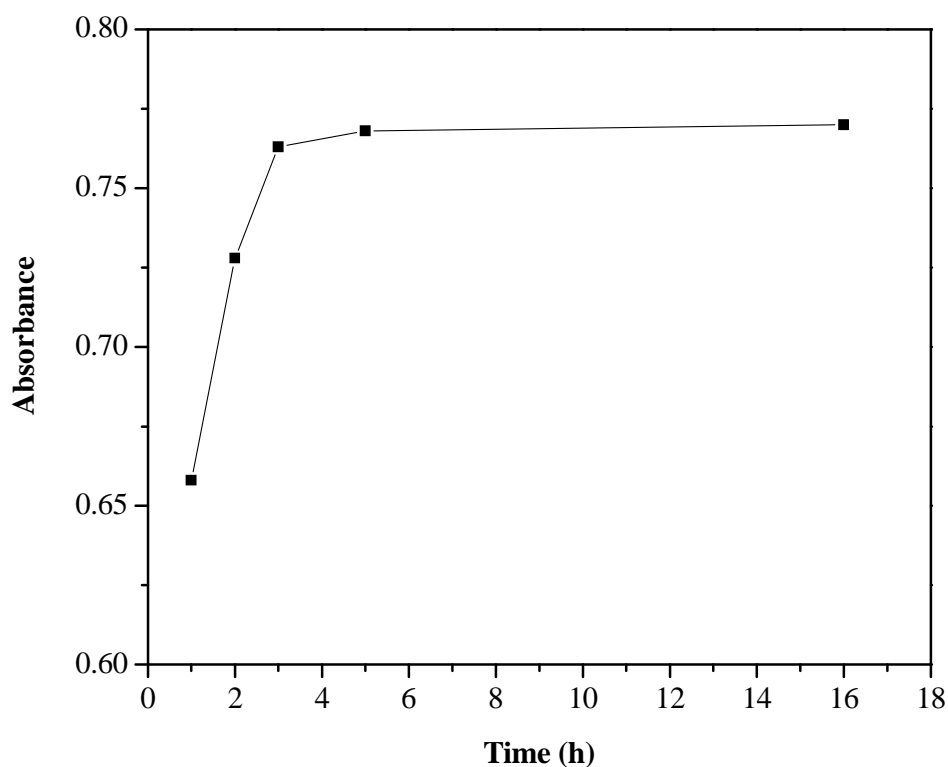
in Figure 4.3. The absorption maximum of the sensing film occurred at 435 nm and the sensing film immersed in Co(II) solution had absorption maxima at 435 and 478 nm. However, for quantitative measurement of Co(II) with the sensing film, the absorbance at 525 nm was used because of the minimum background absorption.



**Figure 4.3** Absorption spectra of the sensing film and the sensing film soaked in Co(II) solution at pH 5.0.

#### 4.3.1 Study of immobilization time

The time for immobilization of NRS was varied to obtain the maximum amount of NRS immobilized on a chitosan film. The NRS concentration used for this study 0.10% (w/v) and the absorbance of NRS on chitosan film at 435 nm was monitored. Figure 4.4 shows that the amount of immobilized NRS increased with an increasing soaking time of the chitosan film in NRS solution. The absorbance of the NSR immobilized chitosan film was relatively stable after 3 h.

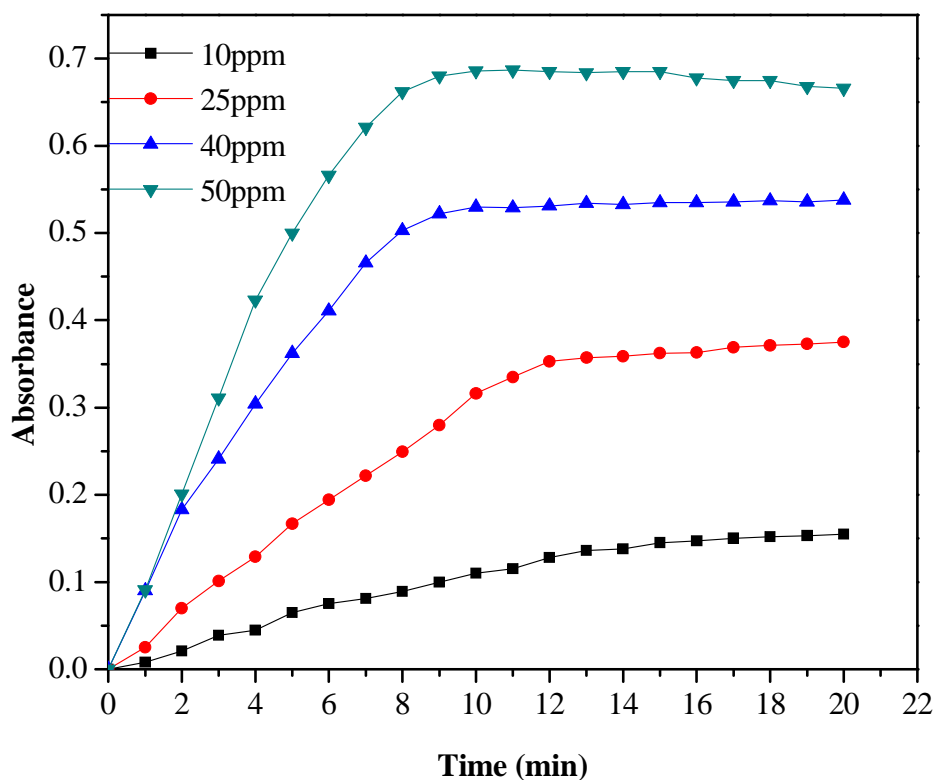


**Figure 4.4** The influence of time on the immobilization of NRS.

Therefore, a submerged time of 3 h was chosen for dye immobilization on chitosan film.

#### **4.3.2 Response of the sensing film to Co(II)**

The response of the sensing films immersed in solution with various concentrations of Co(II) were monitored spectrometrically at 525 nm. More rapid responses were obtained with higher concentration of Co(II) as shown in Figure 4.5. The absorbance approached a plateau in ~8 min for 40 and 50 ppm Co(II) solution and the signal was relatively stable after that. It took ~11 min for solution with lower



**Figure 4.5** Time dependence of the response signal at 525 nm of the sensing film with Co(II) at different concentrations.

Co(II) concentrations. The response time of the sensing film to Co(II) was controlled by diffusion process and the rate of complex formation. Therefore, the time for absorbance measurement was chosen to be 15 min so that a slight difference in measurement time is not critical.

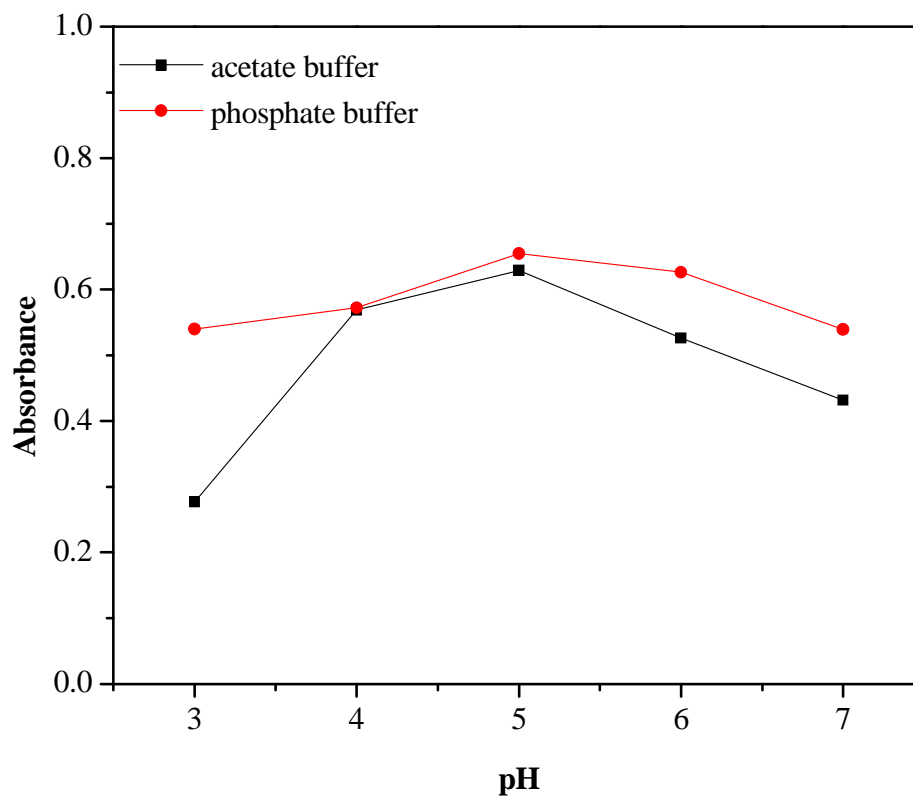
### 4.3.3 Effect of pH

The effect of pH over the range of 3-7 on the response signal of the sensing film to Co(II) was studied with a solution containing 50 ppm of Co(II) and two buffer systems, acetate and phosphate buffer, were used to control the pH of the solutions. The results are shown in Figure 4.6. Response signals in phosphate system were higher than those in acetate system and the sensing film was unstable in acetate buffer because chitocan film could dissolve in the buffer that contained acetic acid. Therefore, phosphate system was used to control pH for optimization of condition.

In phosphate buffer, maximum response was obtained at pH 5.0. At  $\text{pH} \geq 6.0$  response signals decreased because NRS leached out from the sensing film. Cobalt precipitation also occurred in a basic solution. Lower response signals were also obtained at  $\text{pH} \leq 4.0$  because the formation of Co(II)-NRS was not favorable in acid solution as also reported by Wünsch (1978). The optimum pH for the formation of Co(II)-NRS complex in solution was in a range of 5-8 (Ondrejcin, 1964; Forster and Zeitlin, 1966; Dewey and Marston, 1971).

Maximum response was also obtained at pH 5.0 in acetate buffer and similar behavior was observed at  $\text{pH} \geq 6$  as in the phosphate buffer. At pH 3.0, the signal decreased about a factor 2 compared to that at pH 5.0 because the sensing film deformed and the edges of the film started to peel off.

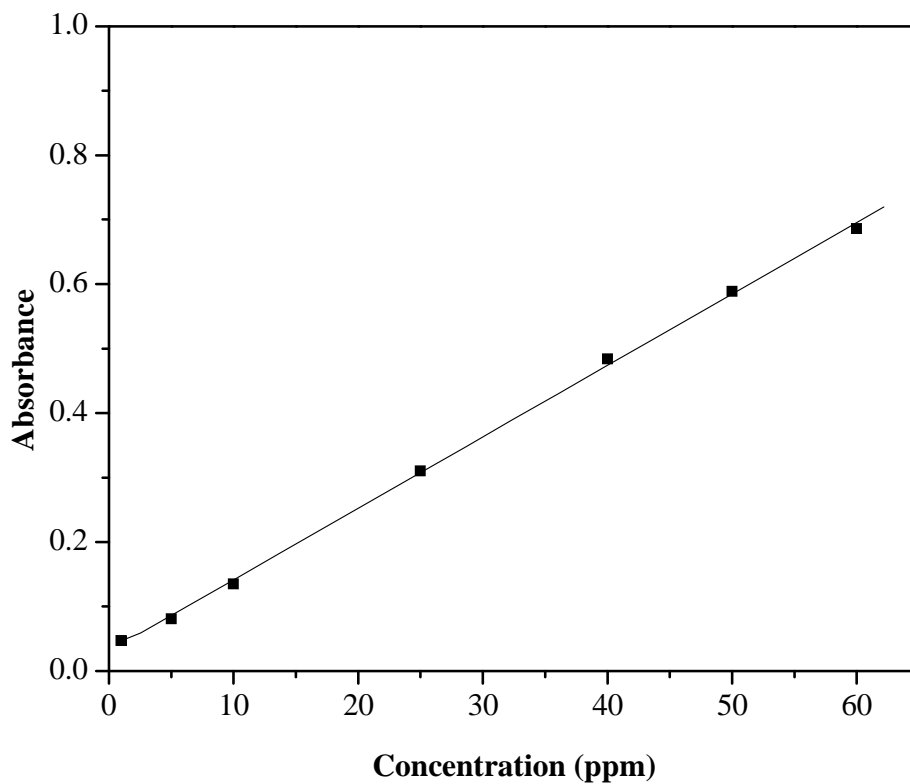
All the response signals in acetate buffer were lower than those in phosphate buffer. This could be resulted from the stability of cobalt complex in the two buffer solutions. That is cobalt formed more stable complex with acetate anion than with phosphate anion; consequently, there are less cobalt ions to form complex with NRS.



**Figure 4.6** The effect of pH on the response signal of the sensing film to 50 ppm of Co(II).

#### 4.3.4 Calibration

Calibration curves for Co(II) are shown in Figure 4.7, and 4.8. The calibration curve in Figure 4.7 was obtained with a solution volume of 3.0 mL. The linear correlation equation is  $y = 0.011x + 0.031$  and the linear correlation coefficient ( $R^2$ ) is 0.999 for Co(II) concentration range of 1-60 ppm. Limit of detection is 0.82 ppm and it was calculated from the following equation,



**Figure 4.7** Calibration curve for Co(II) obtained with 3.0 mL solution.

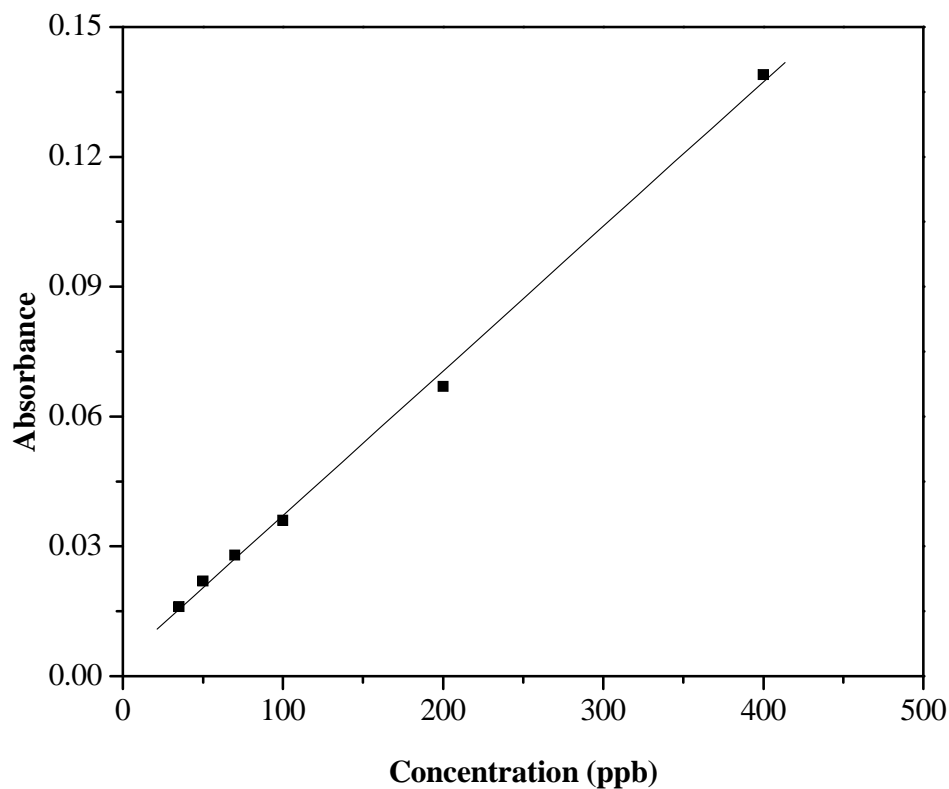
$$DL = \frac{3SD_{blank}}{m}, \text{ where}$$

DL is limit of detection

$SD_{blank}$  is a standard deviation of the blank

$m$  is the slope of the calibration curve

The calibration curve with lower Co(II) concentration was obtained with a 300 mL solution as shown in Figure 4.8. A linear fit equation is



**Figure 4.8** Calibration curve for Co(II) obtained with 300 mL solution.

$y = 0.335x + 0.004$  and the correlation coefficient equals to 0.998. The limit of detection is 12 ppb.

Lower limit of detection was obtained with a larger volume of solution because for a given amount of NRS in the sensing film and the same concentration of Co(II) in solution, a larger volume of solution contained more absolute amount of Co(II) which resulted in higher signal response.



### 4.3.5 Interference study

Some metal ions were examined for their reactivity to the sensing film by measuring the sensing film in a solution containing 50 ppm of the tested metal ion at pH 5.0 and measuring absorbance at 525 nm. The results are listed in Table 4.4.

**Table 4.4** Response of sensing film to some metal ions.

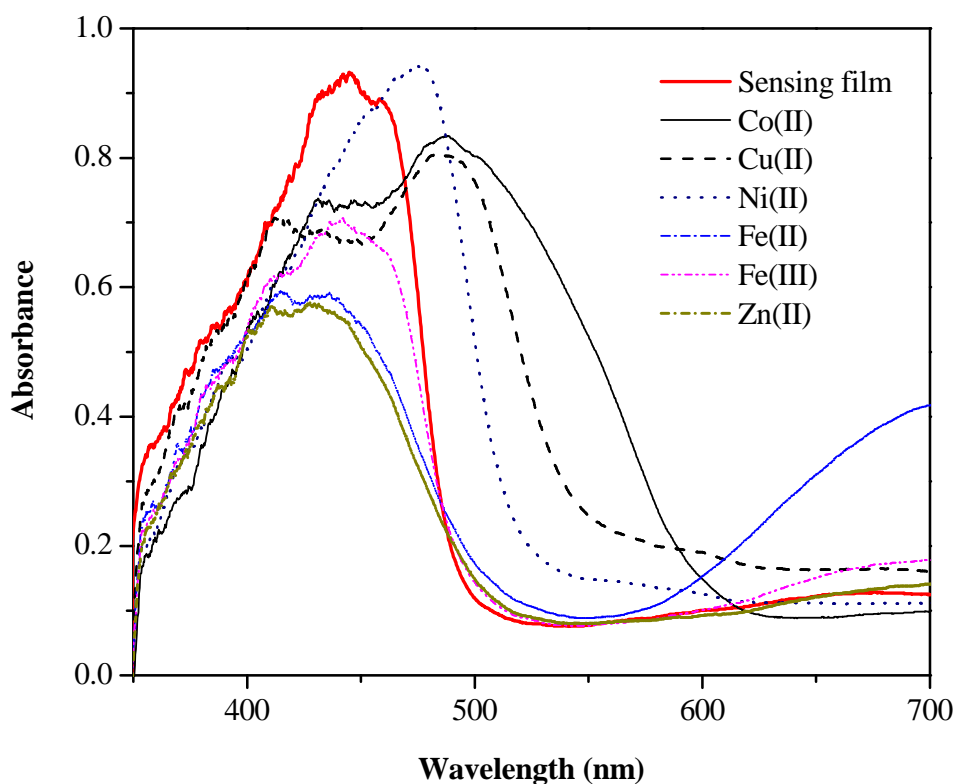
<b>Ion</b>	<b>Relative response<sup>a</sup></b>
Al(III), Ca(II)	0.006
Fe(III), Zn(II), K(I)	0.014
Fe(II)	0.039
Cu(II)	0.574
Ni(II)	0.188
Co(II)	1.000

<sup>a</sup>Relative response was calculated from the absorbance of other metal ion-sensing film divided by the absorbance of Co-sensing film.

The sensing film exhibited the highest response to Co(II). The reactivity of the sensing film to Cu(II) and Ni(II) were about a factor of 2 and 5 less than that of Co(II). The relative responses of Ca(II) and Al(III) were extremely low. K(I), Zn(II), and Fe(III) produced the response about a factor of 70 than that of Co(II). Fe(II) had a response of a factor of 25 lower than that of Co(II). Therefore, the major interferences for this sensing film were Cu(II) and Ni(II). However, the binding of Cu(II) and Ni(II) can be reduced by washing the sensing film with 1.0 M HCl and HNO<sub>3</sub>, respectively.

Co(II)-NRS complex was stable in strong acid solution (Wünsch, 1978; Przeszlakowski and Kocjan, 1982); consequently, removal of Co(II) from the sensing film was difficult and regeneration of the sensing film was not accomplished.

NRS form complex with some metal ions such as Ni(II), Cu(II), and Fe(II) (Pyzyska et al., 1994) and the spectra of NRS-metal ion complexes in chitosan films are shown in Figure 4.9.

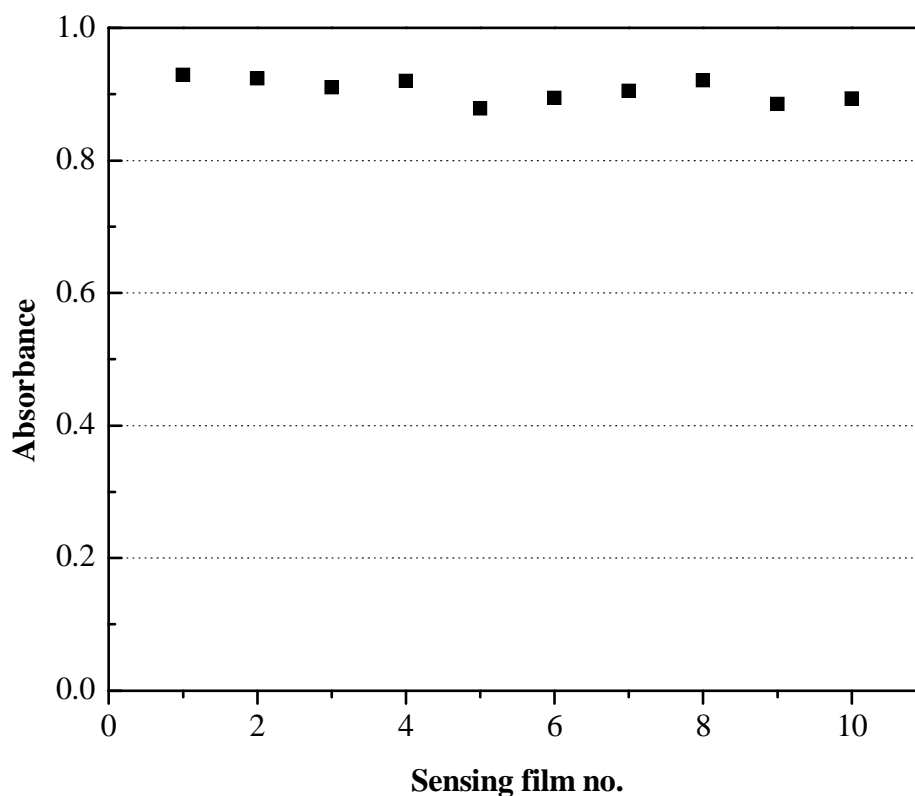


**Figure 4.9** Absorption spectra of sensing film and the sensing films with some interference ions.

From the spectra, it is evident that measuring the absorbance of the sensing film response to Co(II) at 525 nm provided relatively low contribution from the interference signals.

#### 4.3.6 Reproducibility and lifetime

The reproducibility of the preparation of the sensing film was evaluated by measuring the absorbance at 435 nm of ten sensing films and the results are shown in Figure 4.10 and from the data, the calculated relative standard deviation (RSD) was 1.96%.



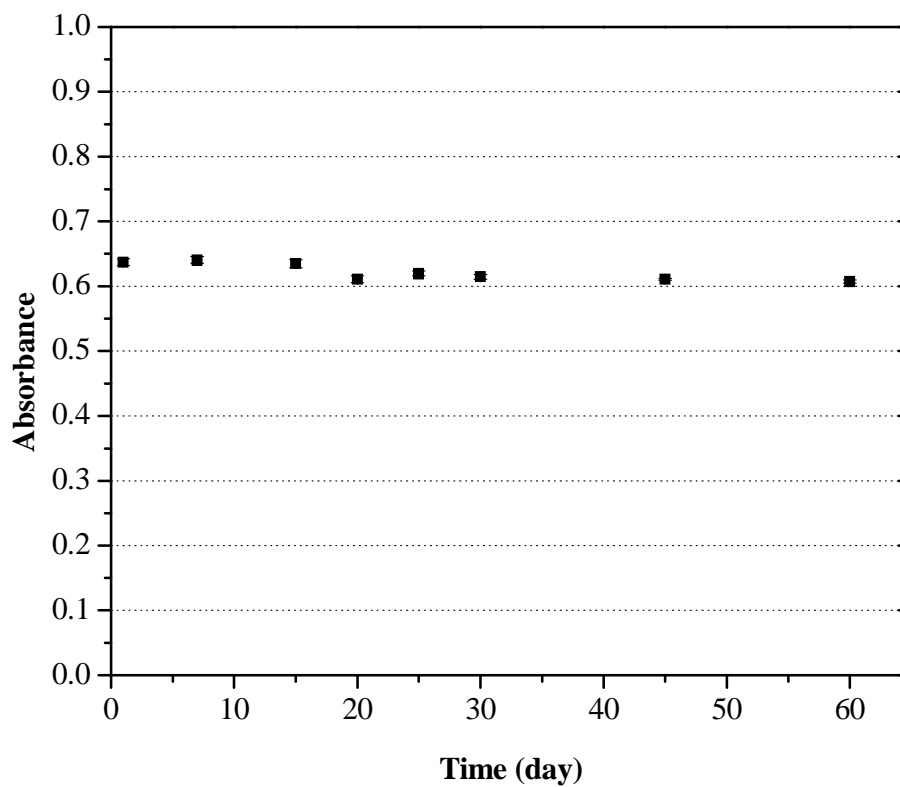
**Figure 4.10** Absorbance of the sensing film at 435 nm.

The reproducibility of the response of individual sensing film to Co(II) was assessed by measuring the signal of five separate sensing films with 10 and 60 ppm Co(II) standard solutions under optimum condition. The results are summarized in Table 4.5.

**Table 4.5** Absorbance of the sensing films with 10 and 60 ppm Co(II) solutions at 525 nm.

<b>Sensing film no.</b>	<b>10 ppm Co(II)</b>	<b>Sensing film no.</b>	<b>60 ppm Co(II)</b>
1	0.133	6	0.687
2	0.134	7	0.681
3	0.139	8	0.687
4	0.135	9	0.685
5	0.134	10	0.688

The RSD for the measurement with 10 ppm Co(II) solution was 1.74% and that with 60 ppm Co(II) solution was 0.41%. Lifetime of the sensing films was examined over a period of 60 days and the results are shown in Figure 4.11. The sensing films exhibited good stability for up to two months. No significant change in the absorbance was observed as the calculated RSD was 2.12%.



**Figure 4.11** Stability of sensing films over a period of 60 days.

#### 4.3.7 Water sample analysis

The applicability of the sensing film was tested with water samples collected from a water reservoir and a wastewater drainpipe located in SUT and the results are shown in Table 4.6. The results obtained with the proposed method agreed well with those obtained with AAS method, as evaluated by *t*-test (shown in appendix B).

**Table 4.6** Determination of Co(II) in water samples.<sup>a</sup>

Sample	Co(II) added (ppm)	Proposed method		AAS method	
		Co(II) found (ppm)	Recovery (%)	Co(II) found (ppm)	Recovery (%)
	0	ND <sup>b</sup>	-	ND	-
SUT water	2.00	2.11±0.01	105.5	2.12±0.01	106.0
reservoir	4.00	4.14±0.02	103.5	4.18±0.02	104.5
	8.00	8.14±0.04	101.8	8.02±0.04	100.3
	0	ND	-	ND	-
wastewater	2.00	2.09±0.01	104.5	2.10±0.01	105.0
	4.00	4.09±0.03	102.2	4.20±0.02	105.0
	8.00	8.13±0.03	101.6	8.02±0.04	100.3

<sup>a</sup>The reported values are means of five replicate measurements and the standard deviations.

<sup>b</sup>ND= not detected.

## **CHAPTER V**

### **CONCLUSIONS**

This research was directed towards the development of an optical chemical sensor for the determination of Co(II) in aqueous solution. The optical chemical sensor was constructed by immobilizing an organic dye that forms complex with Co(II) into an appropriate polymer matrix. High molecular weight chitosan was used as a supporting matrix and was made into a film by a casting technique. Optically transparent chitosan film was modified by crosslinking with epichlorohydrin (ECH) to enhance the stability of the film in aqueous solution.

From five organic dyes, 5-Me-TAR, PAN, PAR, murexide, and NRS that reacted with Co(II) in a solution at pH 5.0, only NRS was successfully immobilized in the modified chitosan film.

In the presence of Co(II), the color of the sensing film changed from yellow to reddish orange as a result of complex formation which enabled spectrometric determination of Co(II). Absorbance measurement of the complex was made at 525 nm because of the minimum background absorption and less signal contribution from the interferences. The optimum pH of the solution for the maximum response of the sensing film was 5.0. At lower pH, lower response was obtained because of unfavorable condition for complex formation, whereas, in basic solution leaching of NRS from chitosan film and precipitation of Co(II) occurred. The developed sensing film exhibited good sensitivity, high reproducibility, and good long-term stability.

The sensing film can be used for 60 days after preparation without significant change in the response signal. The detection limits for Co(II) were 0.82 ppm with 3.0 mL solution and 12 ppb with 300 mL solution. The detection limits of the method are adequate for the determination of Co(II) at environmental relevant level. The proposed method performed satisfactorily on the analysis of water samples.

The use of the sensing film provides an alternative method for the determination of Co(II) in aqueous sample with a simple measurement procedure and no requirement of expensive instrument. The proposed method also consumed less reagents and produced less waste than traditional molecular absorption spectrometry.



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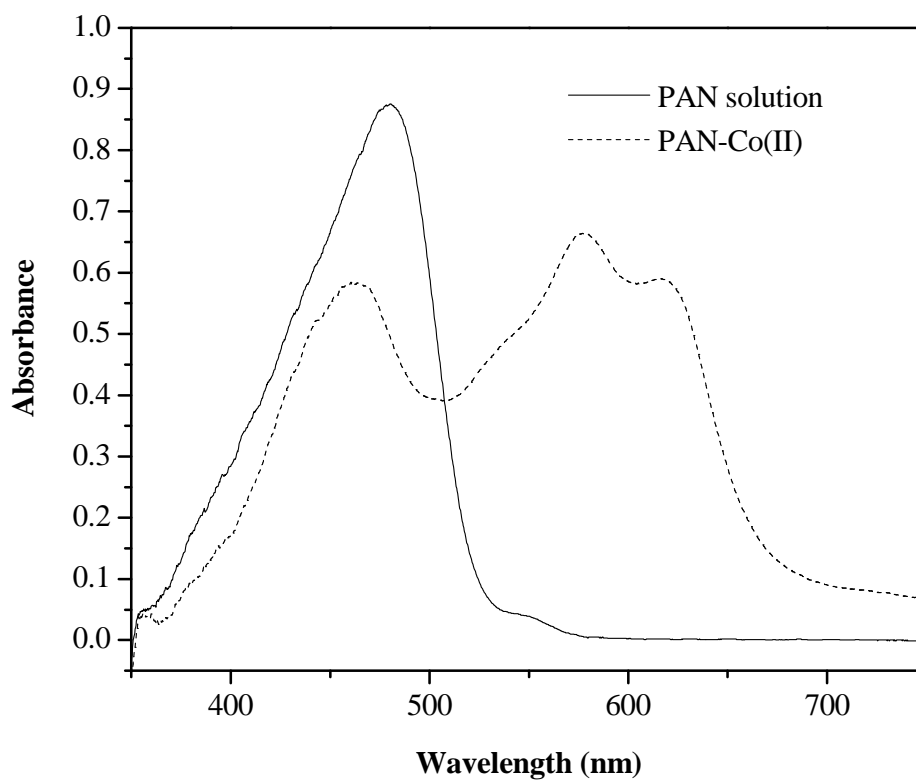
## **APPENDICES**



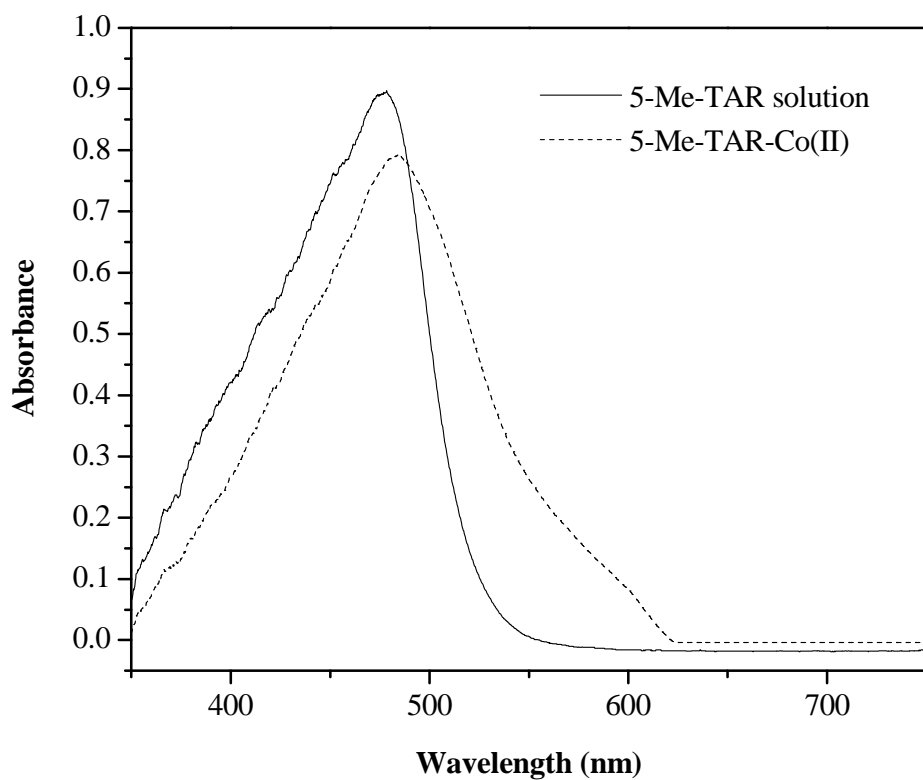
**APPENDIX A**

**ABSORPTION SPECTRA OF SOME ORGANIC DYES**

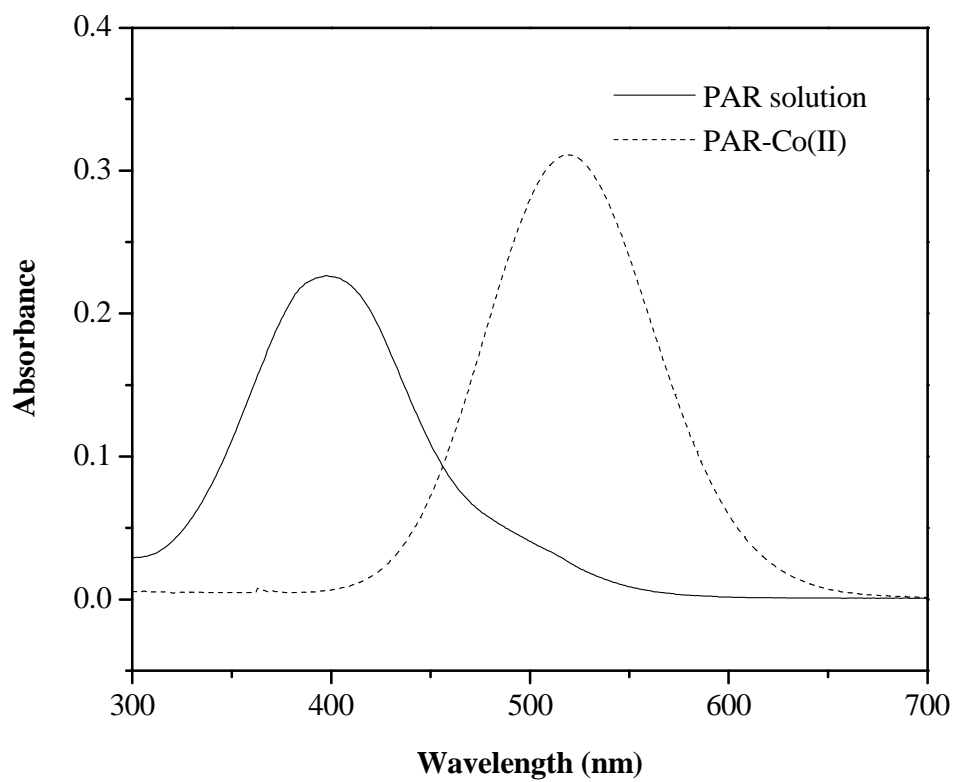
**AND THEIR COMPLEXES WITH COBALT(II) ION**



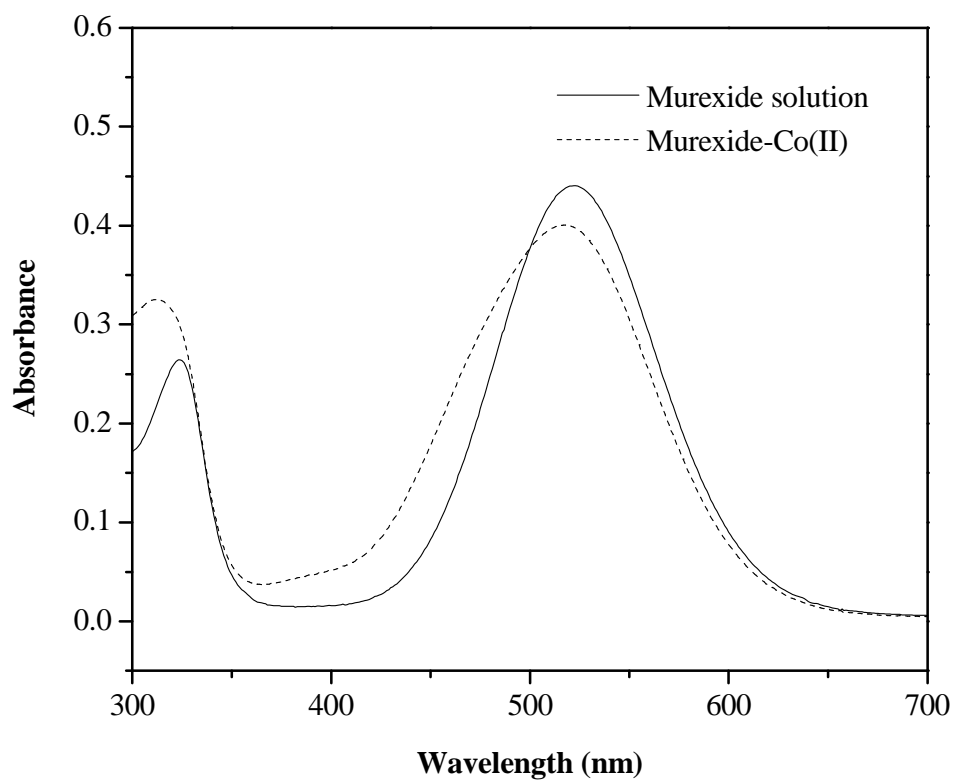
**Figure A.1** Absorption spectra of 1-(2-pyridylazo)-2-naphthol and its complex with Co(II) in solution at pH 5.0.



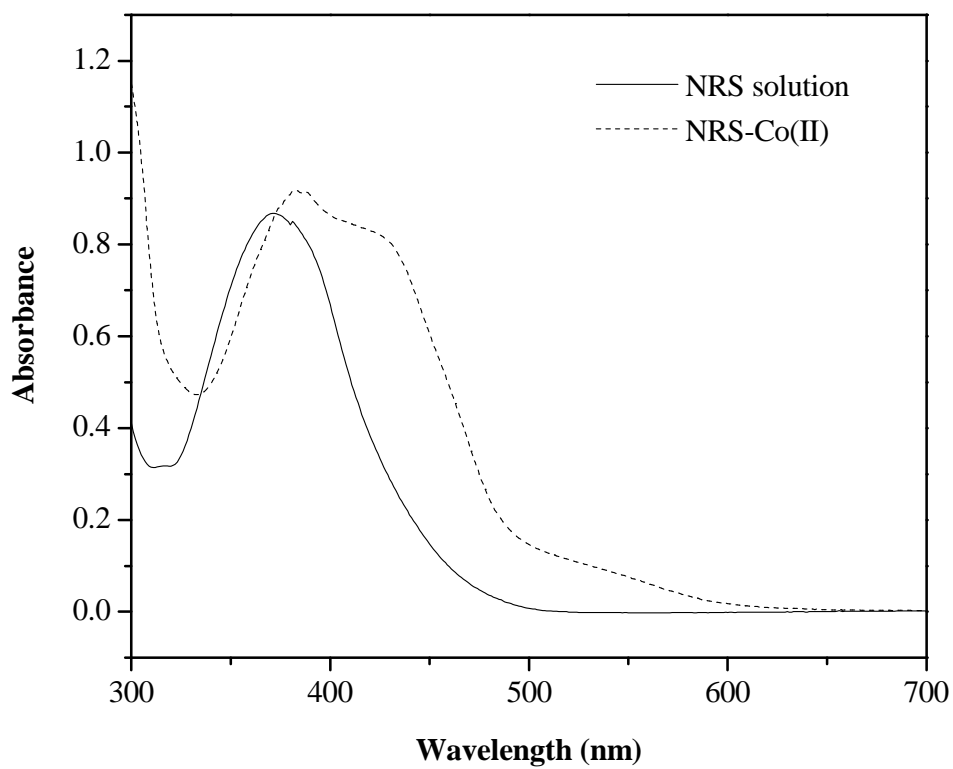
**Figure A.2** Absorption spectra of 5-Methyl-4-(2-thiazolylazo)resorcinol and its complex with Co(II) in solution at pH 5.0.



**Figure A.3** Absorption spectra of pyridylazo resorcinol and its complex with Co(II) in solution at pH 5.0.



**Figure A.4** Absorption spectra of murexide and its complex with Co(II) in solution at pH 5.0.



**Figure A.5** Absorption spectra of nitroso-R salt and its complex with Co(II) in solution at pH 5.0.

**APPENDIX B**

**STATISITICAL EVALUATION OF DATA FROM**

**WATER SAMPLE ANALYSIS**

## Paired *t*-test

Paired *t*-test was used to compare the difference in the results obtained from the measurements on several different samples by two analytical methods. A *t*-statistic was calculated with the following equation

$$t_{cal} = \frac{\bar{d}}{SD} \sqrt{N}$$

where,  $\bar{d}$  is the average difference equals to  $\frac{\sum |d|}{N}$

SD is standard deviation of the differences

N is number of the differences of the pairs of results

**Table B.1** Comparison of two analytical methods for the determination of Co(II)

Water sample	AAS method (ppm)	Proposed method (ppm)	$ d $	Calculated t-statistic
SUT reservoir				
(1)	2.11	2.12	0.01	$t_{cal} = \frac{\bar{d}}{SD} \sqrt{N}$ $= \frac{0.06}{0.06} \sqrt{3}$ $= 1.73$
(2)	4.14	4.18	0.04	
(3)	8.14	8.02	0.12	
	$\bar{d}$		0.06	
	SD		0.06	
Wastewater				
(1)	2.09	2.10	0.01	$t_{cal} = \frac{\bar{d}}{SD} \sqrt{N}$ $= \frac{0.08}{0.06} \sqrt{3}$ $= 2.31$
(2)	4.09	4.20	0.11	
(3)	8.13	8.02	0.11	
	$\bar{d}$		0.08	
	SD		0.06	



The values of the calculated t-statistic of SUT reservoir and wastewater are 1.73 and 2.31, respectively. The critical value of t-statistic at 95% confidence levels and 2 degree of freedom is 4.30 (Skoog et al., 2004). Since  $t_{\text{cal}} < t_{\text{critical}}$ , the results obtains from the two methods are not significantly different.

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2. "Simple optical chemical sensor for detection of cobalt(II) ion". The 2nd SUT Graduate Conference, 21-22 January 2009, Surasammanakhan, Nakhon Ratchasima. Suranaree University of Technology, Thailand.