การเตรียมฟิล์มรับรู้เคมีเชิงแสงด้วยวิธีการตรึงสีย้อมสำหรับการหาปริมาณไอออนเหล็ก(III)

นางสาวกัลยาณี กาจสันเทียะ

PREPARATION OF OPTICAL SENSING FILM

BASED ON DYE IMMOBILIZATION FOR THE

DETERMINATION OF IRON(III) ION

Kanlayanee Kajsanthia

A Thesis Submitted in Partial Fulfillment of the Requirements for the

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PREPARATION OF OPTICAL SENSING FILM BASED ON DYE

IMMOBILIZATION FOR THE DETERMINATION OF

IRON(III) ION

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for Master's Degree.

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กัลยาณี กาจสันเทียะ : การเตรียมแผ่นฟิล์มรับรู้เคมีเชิงแสงด้วยวิธีการตรึงสีย้อมสำหรับ การหาปริมาณไอออนเหล็ก(III) (PREPARATION OF OPTICAL SENSING FILM BASED ON DYE IMMOBILIZATION FOR THE DETERMINATION OF IRON(III) ION) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.สัญชัย ประยูรโภคราช, 80 หน้า.

แผ่นฟิล์มรับรู้เคมีเชิงแสงอย่างง่ายบนพื้นฐานของการตรึง 1,1′- ไดเอทิล 2,2′- ไซยาไนน์ ไอโอไดด์ หรือ ซูโดไซยาไนน์ ไอโอไดด์ ในแผ่นแนฟฟิออนได้ถูกพัฒนาขึ้นเพื่อหาปริมาณไอออน เหล็กในสารละลายในน้ำ แผ่นฟิล์มรับรู้ทำขึ้นจากการกระจายสารละลายของ 1% น้ำหนักต่อ ปริมาตรซูโดไซยาไนน์ ไอโอไดด์ในแนฟฟิออนลงบนแผ่นฟิล์มโปร่งแสง แผ่นฟิล์มที่ได้เกิดขึ้น หลังจากการระเหยไปของตัวทำละลาย

ในการศึกษาด้วยสเปกโทรโฟโตเมทรีการดูดกลืนแสงเชิงโมเลกุล การลดลงของความ ดูดกลืน รังสีเกิดขึ้นที่ 518 นาโนเมตร เมื่อแผ่นฟิล์มรับรู้แช่อยู่ในสารละลายเหล็ก(III) การ ตอบสนองเหมาะสมที่สุดของแผ่นฟิล์มรับรู้เกิดขึ้นในสารละลายบัฟเฟอร์ พีเอช 4.0 เวลาการ ตอบสนองมีค่า 1.0 นาที เส้นตรงสำหรับการเทียบมาตรฐานเกิดขึ้นในพิสัยความเข้มข้นเหล็ก(III) 1-30 ส่วนในล้านส่วนและขีดจำกัดการตรวจวัดมีค่า 0.71 ส่วนในล้านส่วนในสารละลายปริมาตร 2.0 มิลลิลิตร สารรบกวนหลักสำหรับการวิเคราะห์เหล็ก(III) คือ แคดเมียม(II) แผ่นฟิล์มรับรู้ สามารถทำให้คืนสภาพได้ 30 ครั้ง โดยการจุ่มแผ่นฟิล์มในสารละลายกรดไฮโดรคลอริกความ เข้มข้น 1 มิลลิโมลาร์ เป็นเวลา 30 วินาที แผ่นฟิล์มรับรู้มีความเสถียรดีในเวลาสองเดือนและมีความ เที่ยงสูง การประยุกต์แผ่นฟิล์มรับรู้สำหรับการหาปริมาณเหล็ก(III) ในตัวอย่างน้ำและในเม็ด ไวตามินได้บรรลุผลสำเร็จอย่างดี

สาขาวิชาเคมี   ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2555   ลายมือชื่ออาจารย์ที่ปรึกษา_____

KANLAYANEE KAJSANTHIA : PREPARATION OF OPTICAL SENSING FILM BASED ON DYE IMMOBILIZATION FOR THE DETERMINATION OF IRON(III) ION. THESIS ADVISOR : ASST. PROF. SANCHAI PRAYOONPOKARACH, Ph.D. 80 PP.

IRON(III) / NAFION / OPTICAL SENSING FILM / PSEUDOCYANINE IODIDE

A simple optical chemical sensing film based on immobilizing of 1,1′- diethyl 2,2′-cyanine iodide (pseudocyanine iodide) in a Nafion film was developed for the determination of Fe(III) in aqueous solutions. The sensing film was made by spreading a solution of 1% w/v pseudocyanine iodide in 5% w/v Nafion onto a transparent polyester film and the sensing film was obtained after the evaporation of a solvent.

Applied in a molecular absorption spectrophotometry study, a decrease of the absorbance occurred at 518 nm when the sensing film was immersed in Fe(III) solution. An optimum response of the sensing film for Fe(III) was obtained in phosphate buffer solution of pH 4.0. The response time was 1 min. A linear calibration curve over an Fe(III) concentration range of 1-30 ppm was obtained and the limit of detection was 0.71 ppm with 2.0 mL solution. The major interference for the quantitative analysis of Fe(III) is Cd(II). The sensing film could be regenerated up to 30 times by dipping in 1 mM HCl solution for 30 s. The sensing film exhibited good stability for two months and high reproducibility. Application of the sensing film for the determination of Fe(III) in water samples and vitamin tablets was successfully achieved.

School of Chemistry   Student's Signature_____

Academic Year 2012   Advisor's Signature_____

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LIST OF ABBREVIATIONS

^{o}C	degree celsius
μL	microliter
AAS	atomic absorption spectrometry
cm	centimeter
cm^{-1}	wavenumber
conc.	concentrated
DOP	dioctyl phthalate
EDTA	ethylenediaminetetraacetic acid disodium salt
g	gram
h	hour
kg	kilogram
L	liter
LOD	limit of detection
M	molar
MW	molecular weight
mg	milligram
min	minute
mL	milliliter
mm	millimeter
NaTPB	sodium tetraphenylborate
nm	nanometer

LIST OF ABBREVIATIONS (Continued)

NRS   Nitroso-R salt

ppb   part per billion

ppm   part per million

PVC   poly(vinyl chloride)

RSD   relative standard deviation

s   second

THF   tetrahydrofuran

UV-vis   ultraviolet-visible

v/v   volume by volume

w/v   weight by volume

w/w   weight by weight
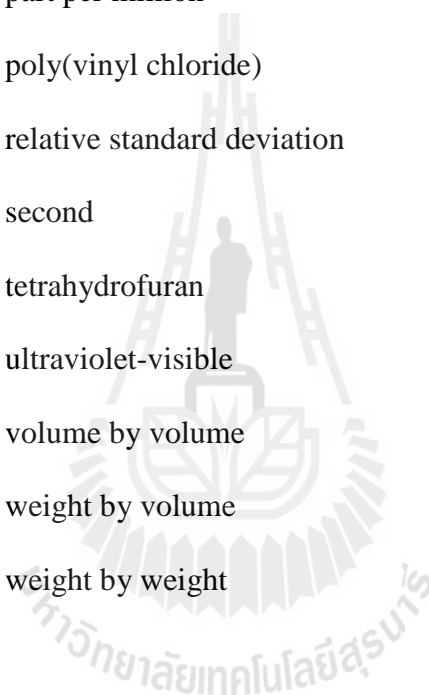
CHAPTER I

INTRODUCTION

Heavy metals have many benefits to our lives in terms of their applications in industry, food, and nutrition. From these applications, heavy metals can be released into the environment if they are not treated properly. Iron is one of the most important metals and appears in many real and synthetic samples. It is used in the steel industry and cosmetic dermatology. It can also be found in food and herbal products. Iron is an essential element to humans as it plays an important role in the formation of hemoglobin and is necessary for immune activity. Besides its beneficial functions, iron can affect human health when it is overloaded. Hemochromatosis, headache and irritability are the consequences of iron overload. The World Health Organization (WHO, 2006) has recommended the concentration limits for iron in drinking water to be < 0.3 mg/L, in groundwater to be < 0.5 mg/L, in food to be < 15 mg/kg, and in wine, liquor and nonalcoholic beverage to be < 15 mg/L.

There are several techniques for the determination of iron such as atomic absorption spectroscopy (AAS) (Bag et al., 1998; Paleologos et al., 2002), flow injection spectrometry (Asan et al., 2010; Kruanetr et al., 2007), and high performance liquid chromatography (HPLC) (Makino et al., 2011). These methods require relatively expensive and sophisticated equipments, a skilled operator and a sample pretreatment. In addition, a bulky bench top instrument limits its use to the laboratory. This is a disadvantage for field sample analysis. Therefore, alternative techniques, for example chemical sensors have been developed to compensate for the disadvantages

of the mentioned techniques.

1.1 Chemical sensors

Sensors can be divided into two classes. The first class is physical sensors which are used for measuring parameters such as temperature and pressure. The second one is chemical sensors which are used for measuring particular chemicals.

A chemical sensor is a device which responds to a particular analyte in a selective way through a chemical interaction and can be used for the qualitative or quantitative determination of the analyte. Chemical sensors can be classified based on the transducer used to transform the chemical information; these are, for example, optical, electrochemical, mass-sensitive, and heat-sensitive chemical sensors (Baldini et al., 2006).

In this thesis, an optical chemical sensor for the determination of Fe(III) is of interest. In general, optical chemical sensors contain two major parts which are a receptor part and transducer part as shown in Figure 1.1. When the receptor part responds to an analyte, a chemical signal such as absorbance, reflectance or fluorescence is produced and transformed into electrical signal by the transducer.

The receptor part of the sensor is based on two major components which are a supporting matrix and a sensing reagent. Polymers and organic dyes are commonly used as the supporting matrix and the sensing reagent, respectively.

1.2 Research objectives

The aims of this research are as follows;
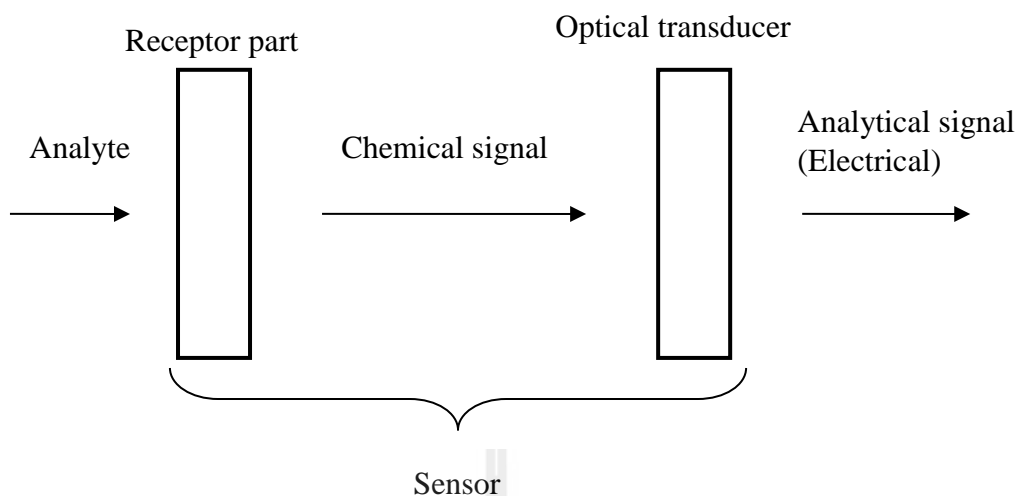
Figure 1.1 A simplified diagram of components and function of an optical chemical sensor.

1. to construct optical chemical sensing films for the determination of Fe(III) ions based on different types of sensing reagents immobilized in various supporting matrices,

2. to study parameters that affect the response of the sensing films, and

3. to determine Fe(III) in real samples using the developed sensing film.

1.3 Scope and limitations of the study

1. Nafion, PVC, and chitosan were investigated as a supporting matrix for organic dye immobilization.

2. A UV-vis spectrophotometer and luminescence spectrophotometer were used to obtain some information of dye-metal complex in solution, sensing film and sensing film in metal ion solutions.

3. Responses of the developed sensing film to metal ions was studied by a UV-vis spectrophotometer.

4. Performance characteristics of the sensing film such as selectivity, response time, working range, detection limit, reversibility, reproducibility, and lifetime were determined.

CHAPTER II

LITERATURE REVIEWS

Optical chemical sensors usually contain two basic components which are a receptor and a transducer part. The receptor part contains two main components which are a supporting matrix and a sensing component. The supporting matrix is used to immobilize the sensing component. Compatibility with the sensing reagent, transparency, and homogeneity are important characteristics of the supporting matrix. Polymers such as poly(vinyl chloride) or PVC, chitosan, and Nafion are usually used as the supporting matrix. The second component is the sensing reagent which interacts with the analyte to generate chemical information that is transformed into an electrical signal by the transducer part. Organic dyes are commonly used as the sensing reagent in optical chemical sensors for heavy metal ions.

To prepare a sensing film, the sensing reagent has to be immobilized onto the supporting matrix. Retention of the sensing reagent relies on interaction of the two components, physical or chemical immobilization. Physical immobilization depends on electrostatic interaction or entrapment between the sensing reagent and the supporting matrix. It is a simple method, but the sensing reagent can leach from the supporting matrix. Leaching affects sensitivity and a lifetime of the sensing film. In chemical immobilization, the sensing reagent is covalently bonded with the supporting matrix. The method reduces the leaching problem. However, chemical modification of the sensing reagent and the supporting matrix might be needed and this has to be done

properly so that the activity of the sensing reagent is not affected.

2.1 Sensing reagent

A sensing reagent in the optical chemical sensor for the determination of heavy metal ions is one of the most important components. Organic indicator dyes that interact selectively with investigated metal ions are commonly used as the sensing reagent. Types of organic dyes such as azo, nitro, nitroso, and the others can be categorized according to their chemical properties and structures. In this thesis, several organic dyes were investigated.

2.1.1 Azo dyes

Azo dyes are compounds with an -N=N- functional group. They account for 60-70% of all dyes. Yellow, orange, and red dyes are more commercially supplied than any other colored dyes. Some azo dyes have been reported as sensing reagents for determination of many metal ions such as pyridylazo resorcinol (PAR) for Co(II) (Yusof and Ahmed, 2002) and for Hg(II) (Ensafi and Fouldagar, 2006), 1,3-di(2-methoxyphenyl)triazene for Hg(II) (Gholivand et al., 2010), pyridylazo naphthol (PAN) for Co(II) (Paleologos et al., 2002), 4-decyloxy-2-(2-pyridylazo)-1-naphthol (DPAN) (Sands et al., 2002) and hydroxyl methylphenylazo napthalenesulfonic acid (calmagite) (Hashemi et al., 2008) for Cu(II), 2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol for Ni(II) (Amini et al., 2004), 2-[2-(5-methylbenzothiazolyl)azo]-5-dimethylaminobenzoic acid for Ni(II) (Fan et al., 1998), 2-(2-quinolylazo)-5-diethylaminoaniline (Hu et al., 2003) as a chromogenic reagent for Ni(II), 1-(2-thiazolylazo)-2-naphthol for Co(II), Ni(II), and Cu(II) (Niazi and Yazdanipour, 2008), and 2-(2-thiazolylazo)-5-dimethylaminobenzoic acid for Ni(II)

(Furukawa, 1982) and Pd(II) (Bhalotra and Puri, 2000).

2.1.2   Nitro and nitroso dyes

Nitro and nitroso dyes are compounds bearing the functional group -NO_2 and -NO, respectively. A well-known and widely used dye in this class for metal ions determination is disodium-1-nitroso-2-naphthol-3,6-disulphonate or nitroso-R salt. Metal ions that were reported to complex with nitroso-R salt are, for example, Co(II) (Sombatsri et al., 2012; Issa et al., 1980), Cu(II) (Jianzhong and Zhujun, 1995), Ni(II) (Ghasemi et al., 2004; Issa et al., 1980), Fe(II) and Fe(III) (Ivanov et al., 2006; Kruanetr et al., 2007), and Cu(II) (Kruanetr et al., 2008).

2.1.3 Other types of dyes

Other colorimetric reagent used as a sensing reagent are 5,10,15,20-teatraphenyeporphyrin for Hg(II) determination (Chan et al., 2001), bis(7-methoxybenzofuran-2-il)ketoxime (BFK) (Oter et al., 2007a), 2-(2′-hydroxy-phenyl)-4(3H)-quinazolinone (HPQ) (Zhang et al., 2007), 4-(2-furylmethlene)-2-phenyl-5-oxazolone (PFO) (Ozturk et al., 2007b), ferrozine (Stookey, 1970), coumarin (Yao et al., 2009), 2,6-bis(benzoxazolyl)pyridine (Feng et al., 2006), 1-(2-thiazolylazo)-2-naphthol (Teixeira et al., 2002) for Fe(III) determination, 4-hydroxy benzaldehyde-4-bromophenyl hydrazone as a color reagent for Ni(II) (Rekha et al., 2007), dimethylglyoxime for Ni(II) (Ershova and Ivanov, 2000), and murexide for Ca(II) Grudpan et al., 1998).

1-1′-diethyl-2,2′-cyaninde iodide is a cationic dye which is a strong light absorber. Brito de Barros and Ilharco (2001) studied the absorption and emission of 1-1′-diethyl-2,2′-cyaninde iodide in cellulose acetate. Vieira Ferreira et al. (1996) studied emission of 1-1′-diethyl-2,2′-cyaninde iodide adsorbed onto microcrystalline

cellulose. Botelho do Rego et al. (1997) reported the spectroscopic study of 1-1′-diethyl-2,2′-cyaninde iodide adsorbed onto microcrystalline cellulose. All of their works are to characterize dye properties. There is no report of 1-1′-diethyl-2,2′-cyaninde iodide used as sensing reagent for heavy metal determination in optical chemical sensors. In this research, the dye was used as the sensing reagent for Fe(III).

2.2 Supporting matrix

Polymers are widely used as a supporting matrix for dye immobilization in optical chemical sensors. The polymer has the function to entrap the sensing reagent in place so that, when the sensor is brought into contact with the analyte solution, the probe reaction occurs on the sensors. Various requirements have to be fulfilled for polymers to be reusable in making an optical chemical sensor. The polymer has to be compatible with the sensing reagent, permeable to the analyte, and chemically and physically stable. It should also be optically transparent in the investigated spectral range. Toxicity and biocompatibility of the polymer should be concerned in some applications. Many polymers such as cellulose, agorose, PVC, chitosan, and Nafion have been used as the supporting matrix in optical chemical sensors and some polymers are discussed here.

2.2.1 Cellulose

Cellulose is a polysaccharide consisting of a linear chain of several $\beta(1\rightarrow4)$ linked D-glucose units as shown in Figure 2.1. The hydroxyl groups (-OH) of cellulose can be partially or fully reacted with various reagents that can be modified for immobilization of the sensing reagent. Ensafi and Isfahani (2011) immobilized 2-amino-cyclopentene-1-dithiocarboxylic acid (ACDA) on triacetylcellulose membrane
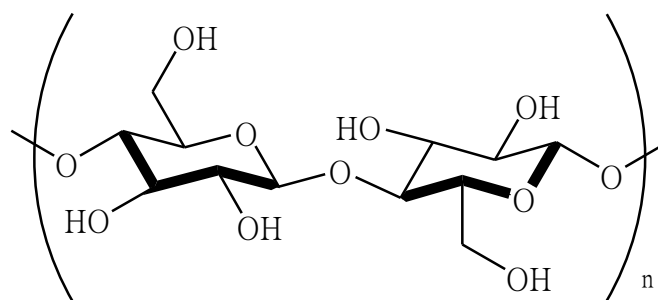
Figure 2.1 Chemical structure of cellulose.

for Cd(II) determination in a solution pH 6.8. The sensors can be used for at least 45

membrane for Co(II) determination. Ensafi and Fouladgar (2005) developed a Co(II)

sensor based on pyrogallol red immobilized onto cellulose membrane. Rastegarzadeh

and Fatahinia (2011) used methyltrioctylammonium chloride ion pairs to assist the

immobilization of thorin and zincon immobilized onto cellulose membrane because

the dye is soluble in water and is not compatible with triacetylcellulose membrane.

Cellulose can be swollen in water and can be hydrolyzed in acidic and basic solutions.

Therefore, its applications are limited.

2.2.2 Agarose

Agarose is a polysaccharide consisting of β-D-galactose and 1,4-linked 3,6-

anhydro-α-L-galactose units as shown in Figure 2.2. It is widely used in gel

electrophoresis. An agarose membrane can be easily formed and handled. Hashemi et

al. (2008) immobilized calmagite on a transparent agarose film and used the film for

determination of Cu(II). No evidence of dye leaching was reported. Alizadeh et al.

(2010) immobilized 2-[(2- sulfanylphenyl)ethanimidoyl]phenol on an agarose

membrane. Hashemi and Abolghasemi (2006) immobilized congo red on epoxy-

activated agarose. Kriz and Mosbach (1995) reported amperometric morphine sensor
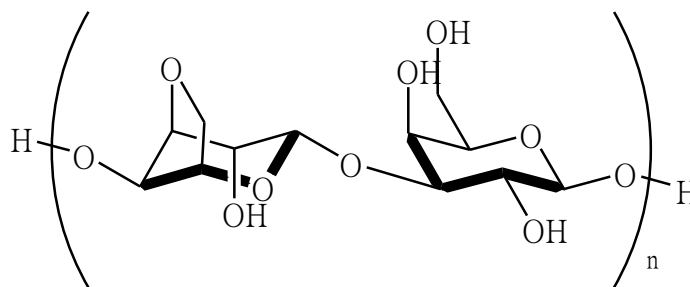
Figure 2.2 Chemical structure of agarose.

based on an agarose immobilised molecularly imprinted polymer. Agarose is relatively expensive and uncommonly used in optical sensor.

2.2.3 Poly(vinyl chloride)

Poly(vinyl chloride), Figure 2.3, is useful as a supporting matrix because it is flexible and optically transparent. A method for preparing a PVC film is relatively simple. Many researchers reported the used of PVC as the supporting matrix for many sensing reagents such as entrapped bis(7-methoxybenzofuran-2-il)ketoxime (Oter et al., 2007a), 2-(2′-hydroxy-phenyl)-4(3H)-quinazolinone (Zhang et al., 2007), 4-(2-furylmethlene)-2-phenyl-5-oxazolone (Ozturk et al., 2007), benzofuran derivative (Oter et al., 2007b), and 1,3-di(2-methoxylphenyl)triazene (Gholivand et al., 2010).

2.2.4 Chitosan

Chitosan, Figure 2.4, is a polysaccharide composed of β-(1-4)-linked D-glucosamine. Active groups of chitosan are amine and hydroxyl groups which can form hydrogen bonds with water. Therefore, a chitosan film can be swollen in an aqueous solution and stability of the film is reduced. To lower the swelling effect, the chitosan film can be modified by crosslinking hydroxyl and amino groups of chitosan using glutaraldehyde (Monteiro et al., 1999; Beppu et al., 2007; Vieira and Beppu,
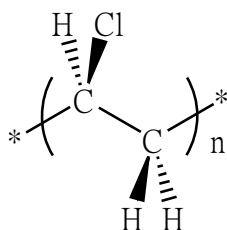
Figure 2.3 Chemical structure of poly(vinyl chloride).
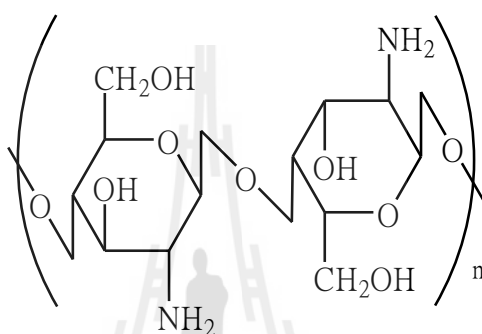
Figure 2.4 Chemical structure chitosan.

2006), epichlorohydrin (Wan et al., 2003; Vieira and Beppu, 2006), and glyoxal (Patel and Amiji, 1996; Yang et al., 2005). Sombatsri et al. (2012) developed an optical chemical sensor for Co(II) based on disodium-1-nitroso-2-naphthol-3,6-disulfonate immobilized in chitosan film crosslinked with epichlorohydrin. Wang et al. (2002) immobilized tyrosinase in chitosan as a biosensor for the detection of phenols. Dubas et al. (2006) reported an optical alcohol sensor based on dye-chitosan polyelectrolyte multilayers.

2.2.5 Nafion

Nafion, Figure 2.5, is a sulfonated tetrafluoroethylene consisting of a poly tetrafluoro ethylene backbone and perfluorovinyl ether pendent side chain with sulfonic acid group built into its structure. Nafion is used in the optical chemical
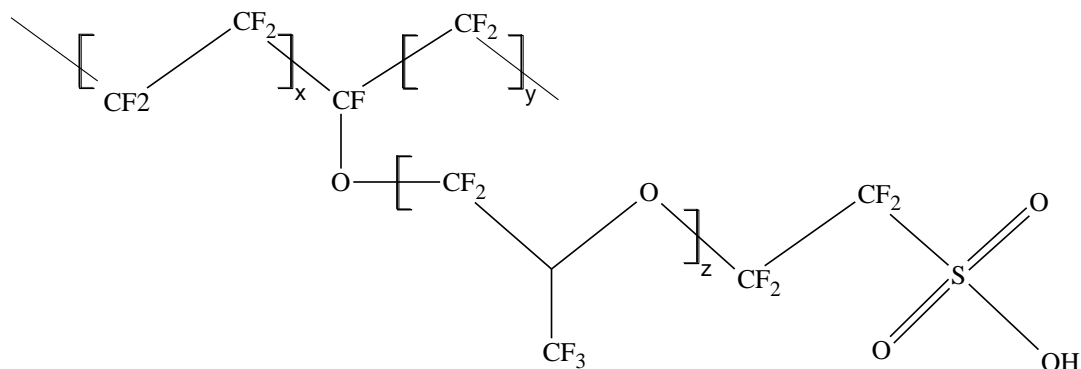
Figure 2.5 Chemical structure of Nafion

because it is hydrophobic and resistant to most solvents, oxidants, and bases. Amini et al. (2004) used 2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol (Br-PADAP) immobilized in Nafion for the determination of Ni(II). Sands et al. (2002) used 4-decyloxy-2-(2-pyridylazo)-1-naphthol in Nafion for Cu(II) determination. Atta et al. (2011) used gold nanoparticles/Nafion carbon paste modified sensor electrode for the determination of morphine. Coo and Martinez (2004) reported a Nafion immobilized with *p*-amino-*p'*-methoxydiphenylamine for the determination of selenium in water samples.

Optical chemical sensors reported for the determination of Fe(III) are summerized in Table 2.1. From the literatures, the sensors have been developed based on different sensing reagents and supporting matrices. The sensing reagent is the most important component as it has to be selective to the analyte and it directs the supporting matrix to be used because the two components have to be compatible. The search for the new selective sensing reagents for Fe(III) has led to the continuous development of the sensors.

Table 2.1 Some optical chemical sensors for the determination of Fe(III).

Sensing reagent	Supporting matrix	Mode of signal measurement	Response time (min)	LOD (ppm)	Major interference	Sample	Reference
2,4-dinitroresorcinol	amberlite^{TM} (XAD-7)	absorbance	10	0.044	Ni(II)	sea water	Malcik and Caglar (1997)
thiocyanate	exchange resin	absorbance	- (flow system)	0.078	-	natural water	Lopes da Consceicao et al. (1997)
pyoverdin	controlled pore glass	fluorescence	2	0.003	-	natural water and wines	Pulido-Tofiño et al. (2001)
4-(2-furylmethylene)-2-phenyl-5-oxazolone	PVC	fluorescence	2	0.212	-	-	Ozturk et al. (2007)
bis(7-methoxybenzofuran-2-il)ketoxime	PVC	fluorescence	30 s	0.033	Fe(II)	-	Oter et al. (2007a)

Table 2.1 (continued)

Sensing reagent	Supporting matrix	Mode of signal measurement	Response time (min)	LOD (ppm)	Major interference	Sample	Reference
2-(2′-hydroxyphenyl)-4(3H)-quinazolinone	PVC	fluorescence	1	0.004	-	Multi-vitamin tablets	Zhang et al., (2007)
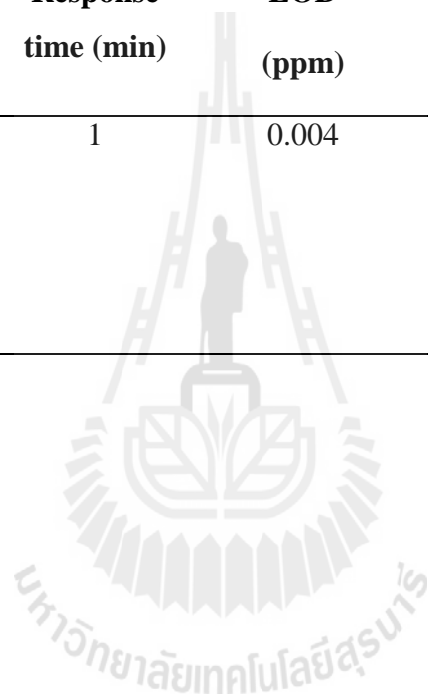
In this thesis, some dyes were investigated as a complexing agent for Fe(III) in aqueous solution and only those that provided positive results were used to immobilize in the supporting matrix such as PVC, chitosan, and Nafion. The sensing films were studied spectrometrically for their response to Fe(III).

CHAPTER III

EXPERIMENTAL

3.1 Chemicals and materials

1. Acetic acid (99.9%, J.T.Baker)

2. Cadmium nitrate tetrahydrate (98%, Sigma-Aldrich)

3. Calcium nitrate tetrahydrate (99.997%, Sigma-Aldrich)

4. Chitosan highly viscous (MW > 600,000, Fluka)

5. Chemicals for preparation of phosphate buffer: phosphoric acid (85.0%, UNIVAR), disodium hydrogen phosphate (99.0%, Qrect^{TM}), sodium dihydrogen phosphate (99.0%, Qrect^{TM}), and deionized water

6. Chemicals for preparation of universal buffer: citric acid (99.9%, Sigma-Aldrich), disodium hydrogen phosphate (99.0%, Qrect^{TM}), and deionized water

7. Cobalt(II) nitrate hexahydrate (98.0%, Sigma-Aldrich)

8. Copper(II) nitrate hexahydrate (99.99%, Merck)

9. Chromium(III) nitrate nonahydrate (99%, Sigma-Aldrich)

10. Dioctyl phthalate (99.0%, Aldrich)

11. Ethylenediaminetetraacetic acid disodium salt (EDTA) (99.0%, Sigma-Aldrich)

12. Glutaraldehyde solution (25% w/w, Unilab)

13. Hydrochloric acid (37% w/w, Fluka)

Table 4.1 Absorption study of some dye solutions with Fe(III).

14. Hydroxylamine hydrochloride (99.0%, Sigma-Aldrich)

15. Indicator dyes: 1,1'-diethyl-2,2'-cyanine iodide (97.0%, Aldrich), 5-methyl-4-(2-thiazolylazo)resorcinol (95.0%, Aldrich), 5,5'-nitrilodibarbituric acid monoammonium salt or murexide (AR grade, Carlo Erba), Sudan III (99.0%, Sigma-Aldrich), disodium-1-nitroso-2-naphthol-3,6-disulfonate or nitroso-R salt (AR grade, Fluka), and acrifavine (AR grade, Fluka).

16. Iron(III) nitrate (98.0%, Ajax Finechem Pty Ltd.)

17. Magnesium(II) nitrate (98.0%, Ajax Finechem Pty Ltd.)

18. Nafion® 117 solution (5.0% in low aliphatic alcohol, Fluka)

19. Nickel(II) nitrate hexahydrate (98.0%, Sigma-Aldrich)

20. Poly(vinyl chloride) high molecular weight (Fluka)

21. Potassium nitrate (99.0%, Sigma-Aldrich)

22. Sodium hydroxide (97%, Sigma-Aldrich)

23. Sodium nitrate (99.0%, Sigma-Aldrich)

24. Sodium tetraphenylborate (99.5%, Sigma-Aldrich)

25. Tetrahydrofuran (99.8%, Carlo Erba)

26. Zinc nitrate hexahydrate (98%, Sigma-Aldrich)

3.2 Apparatus and instruments

1. Apparatus for membrane preparation; glasswares, glass slides, polyester transparent films, magnetic stirrers, magnetic bars, micropipettes, pipettes, polypropylene (PP) bottles, and hot air oven.

2. CHEM4-Vis-fiber spectrometer (USB 4000, Ocean optics)

3.Flame-atomic absorption spectrometer (AAnalyst 100, Perkin-Elmer)

3.3 Preparation of aqueous solutions

1. Various dyes with a concentration of 1×10^{-3} M were prepared by dissolving appropriate amount of a dye in a suitable solvent such as ethanol and water to produce a 50 mL solution.

2. An acetic acid solution with a concentration of 1% (v/v) was prepared by diluting 1 mL of glacial acetic acid in distilled water and the volume was made up to 100 mL.

3. Epichlorohydrin solution with a concentration of 0.02 M 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.

4. Citric acid solution with a concentration of 0.1 M was prepared by dissolving 4.80 g of citric acid in distilled water and the solution volume was adjusted to 250 mL in a volumetric flask.

5. Phosphoric acid solutions with concentrations of 0.1 and 0.5 M were prepared by dissolving 6.17 and 30.83 mL 85.0% phosphoric acid in distilled water and the solution volume was adjusted to 1000 mL in a volumetric flask.

6. Disodium hydrogen phosphate solutions with concentrations of 0.1 and 0.5 M were prepared by dissolving 7.10 and 35.50 g of disodium hydrogen phosphate in distilled water and the solution volume was adjusted to 500 mL in a volumetric flask.

7. Sodium dihydrogen phosphate solutions with concentrations of 0.1 and 0.5 M were prepared by dissolving 6.00 and 30.00 g of sodium dihydrogen

phosphate in distilled water and the solution volume was adjusted to 500 mL in a volumetric flask.

8. Universal buffer solutions with pH 3.0, 5.0, and 7.0 were prepared by mixing 0.1 M citric acid and 0.2 M disodium hydrogen phosphate solutions and the pH of the solutions was adjusted by varying appropriate volume of both solutions. A pH meter was used to measure the pH of the solutions.

9. Phosphate buffer solutions with pH 3.0, 5.0, and 7.0 were prepared by mixing appropriate volumes of 0.5 M phosphoric acid 0.5 M and sodium dihydrogen phosphate solutions and pH of the solutions were adjusted by varying appropriate volume of both solutions. A pH meter was used to measure the pH of the solutions.

10. Phosphate buffer solutions with pH 2.0-8.0 were prepared by mixing appropriate volumes of 0.5 M phosphoric acid 0.5 M and sodium dihydrogen phosphate solutions and the pH of the solutions was adjusted by varying appropriate volume of both solutions. A pH meter was used to measure the pH of the solutions.

11. Solutions of cadmium(II), calcium(II), chromium(II), cobalt(II), copper(II), iron(III), nickel(II), sodium(I), and zinc(II) were prepared by diluting appropriate volume of 1000 ppm stock solution of the metal ion and the solution volume was adjusted with distilled water and buffer solution. A solution of Fe(II) was prepared by reducing Fe(III) solution with 0.5 mL 10% hydroxylamine hydrochloride and the solution volume was adjusted with distilled water and buffer solution.

3.4 Selection of indicator dyes for Fe(III)

A 1.0×10^{-3} M dye solution was mixed with 1.0×10^{-3} M Fe(III) solution to obtain a mole ratio of 1:1 of dye:Fe(III). The color change of the solution was recorded after 30-60 min of mixing. An absorption spectrum of the solution mixture was recorded using the UV-vis spectrometer in the wavelength range of 350-700 nm and compared to that of the dye solution.

3.5 Selection of buffer solution for dye-Fe(III) complex

Two buffer systems, universal and phosphate buffer, were used to control the pH of the solutions to investigate their influence on the formation of dye-Fe(III) complex in the pH range 3.0-7.0. Solutions of 1.0×10^{-3} M 1,1'-diethyl-2,2'-cyanine iodide with or without 20 ppm Fe(III) were prepared at pH 3.0, 5.0, 7.0. The absorbance of the solutions were measured at 522 nm. The blank signals were obtained from the dye solutions at the studied pHs. For the study with sensing films, a sensing film was placed in a cuvette containing universal or phospohate buffer at pH 3.0, 5.0, 7.0 and the absorbance was recorded as a blank and then change the solution in the cuvette to Fe(III) solution at the given pHs. The response signals were obtained by subtraction between the absorbance of the sensing film in buffer and that of the sensing film in Fe(III) solution.

3.6 Preparation of sensing films

3.6.1 Preparation of PVC sensing films

PVC sensing films were prepared by dissolving appropriate amount of PVC, dioctyl phthalate, sodium tetraphenylborate, and 1,1'-diethyl-2,2'-cyanine iodide in 5

mL of tetrahydrofuran (THF). The mixture was stirred for 5 min at room temperature. An aliquot of the solution mixture was cast onto a micro glass slide which was cut into a size 0.9 cm × 4.0 cm and THF was evaporated overnight at room temperature.

3.6.2 Preparation of chitosan sensing films

Chitosan sensing films were prepared using the method from a literature (Sombatsri et al., 2012). High molecular weight chitosan, 1.00 g, was dissolved in 100 mL 1% (v/v) acetic acid solution. The obtained solution was stirred at room temperature for 3 h and filtered through a glass frit. The filtrate was sonicated for 30 min to remove air bubbles. The chitosan solution was mixed with an appropriate amount of 1 mM 1,1'-diethyl-2,2'-cyanine iodide and 0.25% glutaraldehyde solution. 200 μL of the final mixture was cast into a polyester transparent film which was cut into a size 0.9 cm × 4.0 cm and dried in a hot air oven at 60 °C for 24 h. The sensing films were kept in a cabinet to avoid direct exposure to the room light. There was another procedure used to make chitosan sensing film: 200 μL of chitosan solution was spread into a size 0.9 cm × 4.0 cm polyester transparent film and dried in a hot air oven at 60 °C for 24 h. A chitosan film was immersed in a solution of 0.25% (w/w) glutaraldehyde without agitation at room temperature for 2 h and washed with deionized water to remove unreacted glutaraldehyde (Baroni et al., 2008). The obtained films were then immersed into the dye solution.

3.6.3 Preparation of Nafion sensing films

Nafion sensing films were prepared by dissolving 5-20 mg 1,1'-diethyl-2,2'-cyanine iodide in 2 mL of 5% Nafion solution with 60 min sonication. The appropriate volume of the cocktail solution was spread into a size of 1.0 cm × 1.0 cm on a polyester transparent film and dried for 24 h in a desiccator.

3.7 Characterization of the sensing films

3.7.1 Response of the sensing films to Fe(III)

A 2 mL buffer solution was put in a cuvette and a sensing film was dipped in the cuvette with stirring provided. The decrease in absorbance signal was monitored until the signal was stable. This usually took about 2 min, then the solution in the cuvette was replaced by 2.0 mL of Fe(III) solution. The absorbance of the sensing film was measured in the wavelength range of 350-700 nm at various times.

For the fluorescence measurement, the sensing film was placed in diagonal position in a quartz cell containing 2 mL Fe(III) solution. Excitation and emission scans were recorded in a wavelength range of 200-800 nm to obtain excitation and emission wavelengths that provided the maximum fluorescence signal. To obtain the signal related to Fe(III) concentration, fluorescence signal was measured with the sensing film in the buffer and in the Fe(III) solutions at the selected excitation and emission wavelengths.

3.7.2 Effect of pH on the response of Nafion sensing film to Fe(III)

Influence of pH of the solution on the response of the sensing film to Fe(III) was investigated over the pH range of 2.0-8.0 at fixed Fe(III) concentration. The solution of 20 ppm Fe(III) was used and prepared from dilution of 5 mL of 100 ppm Fe(III) in 25 mL volumetric flask with phosphate buffer. Absorbance at 522 nm was measured with the sensing film dipped in the solution with or without Fe(III) at a given pH.

3.7.3 Stability of Nafion sensing films

Film swelling and dye leaching were used to evaluate the short term stability of the Nafion sensing films. The sensing films were soaked in 10 mL of water, 0.1 M

HCl and phosphate buffer pH 4.0 in 25 mL beaker for 30 min to 1 h to observe the swelling of the films. Leaching of the dye from the Nafion sensing film was studied by dipping a sensing film in the cuvette containing 2 mL buffer. The film was placed in a parallel position to a light path. The absorbance of the solution at 522 nm was measured every 30 min for 8 h.

3.7.4 Reproducibility of the sensing film

The reproducibility of the method used to prepare the sensing films was evaluated by measuring the absorbance at 518 nm of sensing films in phosphate buffer pH 4.0. The sensing films were prepared from different batches of solutions.

The reproducibility of the response of the sensing film was studied by measuring the absorbance at 518 nm of sensing films in 20 ppm Fe(III) solution. The data was used to calculate a relative standard deviation (RSD).

3.7.5 Regeneration of sensing films

The regeneration of the sensing film was studied by using 0.001 and 0.1 M HCl solution and 0.001 and 0.1 M EDTA solution as regenerating reagents. In this study, the sensing film was dipped in 20 ppm Fe(III) solution until the signal reached the steady state which took 1 min. Then the sensing film was submerged in a 10 mL regenerating reagent in 25 mL beaker for 30 s to 5 min. The sensing film was washed with distilled water to remove the remained reagent on the sensing film. The sensing film was dipped again in the buffer solution until the absorbance reached the study state.

3.7.6 Lifetime of sensing films

The lifetime of the sensing film was studied over the period of 2 months. Absorbance of a sensing film in 20 ppm Fe(III) was measured once a week. Average

signals of each week were used to calculate the relative standard deviation (RSD) which was used to evaluate the deviation of the response signal of the sensing film to Fe(III).

3.7.7 Stoichiometric determination of 1,1'-diethyl-2,2'-cyanine iodide-Fe(III) complex

The mole-ratio method and continuous variation method were used to determine the stoichiometry of 1,1'-diethyl-2,2'-cyanine iodide-Fe(III) complex in solution. In the mole-ratio method, solutions of 1,1'-diethyl-2,2'-cyanine iodide-Fe(III) complex were prepared by holding concentration of 1,1'-diethyl-2,2'-cyanine iodide constant at 8.95×10^{-5} M and varying the amount of Fe(III) solutions to obtain ligand-to-metal ratio in a range of 0-4.0. Fe(III) concentration used for solution preparation was 8.95×10^{-5} M. The pH of the solutions was controlled by using 1.0 M phosphate buffer pH 4.0. The final volume of the solutions was 10 mL. After mixing, the solutions were left for 1.30 h before the absorbance measurement.

For continuous variation method, solutions of 1,1'-diethyl-2,2'-cyanine iodide-Fe(III) complex were prepared by mixing aliquots of equimolar solutions of the dye and Fe(III) where the number of moles of the two species were varied but the sum was constant. After mixing, the solution volume was made up to 10 mL using 1.0 M phosphate buffer pH 4.0. The solutions were left for 1.30 h before the absorbance measurement.

3.7.8 Calibration study

Calibration experiments were carried out over Fe(III) concentration range of 1.0-50 ppm. The absorbance was recorded until the steady-state signal was obtained. The absorbance signals at the steady state were plotted against Fe(III)

concentrations to obtain a calibration curve. A calibration curve obtained from using sensing films with no regeneration and that obtained from using one sensing film with regeneration, and one sensing film for each Fe(III) concentration were compared.

3.7.9 Interference study

The response of the sensing films to foreign ions was evaluated at the optimum condition for Fe(III) by dipping the sensing film in a solution of Fe(III) or in a solution of Fe(III) mixed with the studied foreign ion at a molar ratio of 1:1. The concentration of Fe(III) and the studied ion were 3.58×10^{-4} M. The ions investigated were Cu(II), Fe(II), Ni(II), K(I), Co(II), Mg(II), Cr(II), Cd(II), Ca(II), Na(I), and Zn(II). The absorption spectra were recorded by the UV-vis spectrophotometer in the wavelength range 350-700 nm.

3.7.10 Analysis of water samples

Water samples were collected from a reservoir located in Suranaree University of Technology (SUT), Nakhon Ratchasima, Thailand. The water samples were filtered and stored in polypropylene containers at 4.0 °C before the analysis. For the measurement with the sensing film, the pH of the sample solution was adjusted to 4.0 with a buffer solution. For comparison purpose, determination of Fe(III) with the atomic absorption spectrophotometer (AAS) was done according to a reference method (Greenburg et al., 1992). A 100 mL water sample was placed in a 600 mL beaker and 5 mL conc. HNO_3 was added. The solution was boiled gently and evaporated on a hot plate until the volume of the solution was reduced to 20 mL. If precipitation occurred, conc. HNO_3 was added and the solution was heated until a clear solution was obtained. The digested solution was cooled down to room temperature and the volume of the solution was adjusted to 100 mL with

deionized water. Parameters for setting the spectrophotometer are air:acetylene ratio of 2:5, iron hollow cathode lamp current of 30 mA, and absorption wavelength of 248.2 nm.

3.7.11 Analysis of vitamin tablets

A method from a literature (Zhang et al., 2007) was used for sample preparation. A vitamin tablet sample (Hemovit, 49.6 mg Fe/tablet) was ground into powder. A 409.5 mg of powder was then dissolved in 2.0 mL aquaregia and heated to dryness to oxidize Fe(II) to Fe(III). After being cooled, it was transferred into a volumetric flask and diluted to 1000 mL with 1.0 M phosphate buffer pH 4.0.
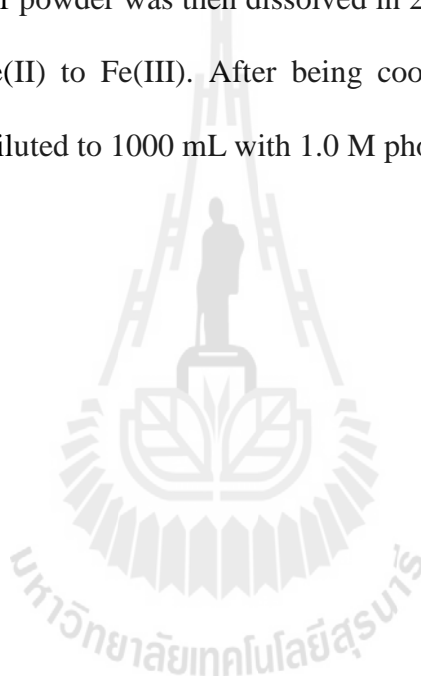
Table 4.1 Absorption study of some dye solutions with Fe(III).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Selection of indicator dye for Fe(III)

Various organic dyes were studied as a complexing agent for Fe(III) ion in an aqueous solution. A solution of 1×10^{-3} M of organic dye was mixed with 1.0×10^{-3} M Fe(III) solution in a buffer pH 4.0. Absorption spectra were recorded during the time interval of 30 min to 1 h. The results are shown in Table 4.1.

1,1'-diethyl-2,2'-cyanine iodide, Figure 4.1, reacted with Fe(III) in solution at pH 4.0 resulting in apparent change of the color of the organic dye solution. Absorption spectra of the dye solution and the dye solution mixed with Fe(III) were recorded by a UV-vis spectrophotometer and are shown in Figure 4.2. The absorption peak at 492 and 522 nm remained the same, but the absorbance decreased when the dye solution was mixed with Fe(III). The decrease in the absorbance signal was later shown to be linearly correlated to the concentration of Fe(III).

No color change of the solutions of sudan III, acriflavine and murexide was observed. For disodium-2-nitroso-2-naphthol-3,6-disulfonate (NRS), the color of the solution changed from yellow to yellow-green when mixed with Fe(III) and the absorption peak was shifted from 372 to 386 nm. Although there is change in absorption spectrum, NRS was not further investigated because it was reported that the dye could complex with other metal ions such as Co(II) (Sombatsri et al., 2012), Cu(II) (Jianzhong and Zhujun, 1995), Ni(II) (Issa et al., 1980; Ghasemi et al.,

Table 4.1 Absorption study of some dye solutions with Fe(III).

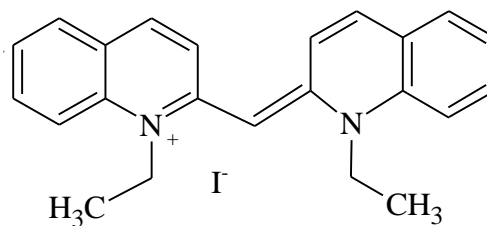| Organic dye	Color		Absorption peak (nm)	
	Dye solution	Fe(III) and dye mixture	Dye solution	Fe(III) and dye mixture
1,1'-diethyl-2,2'-cyanine iodide	red	reddish-orange	370, 492, 522	370, 492, 522
sudan III	reddish-brown	reddish-brown	500	500
acriflavine	yellow	yellow	449	449
murexide	purple	purple	505	505
NRS	yellow	yellow-green	372	386

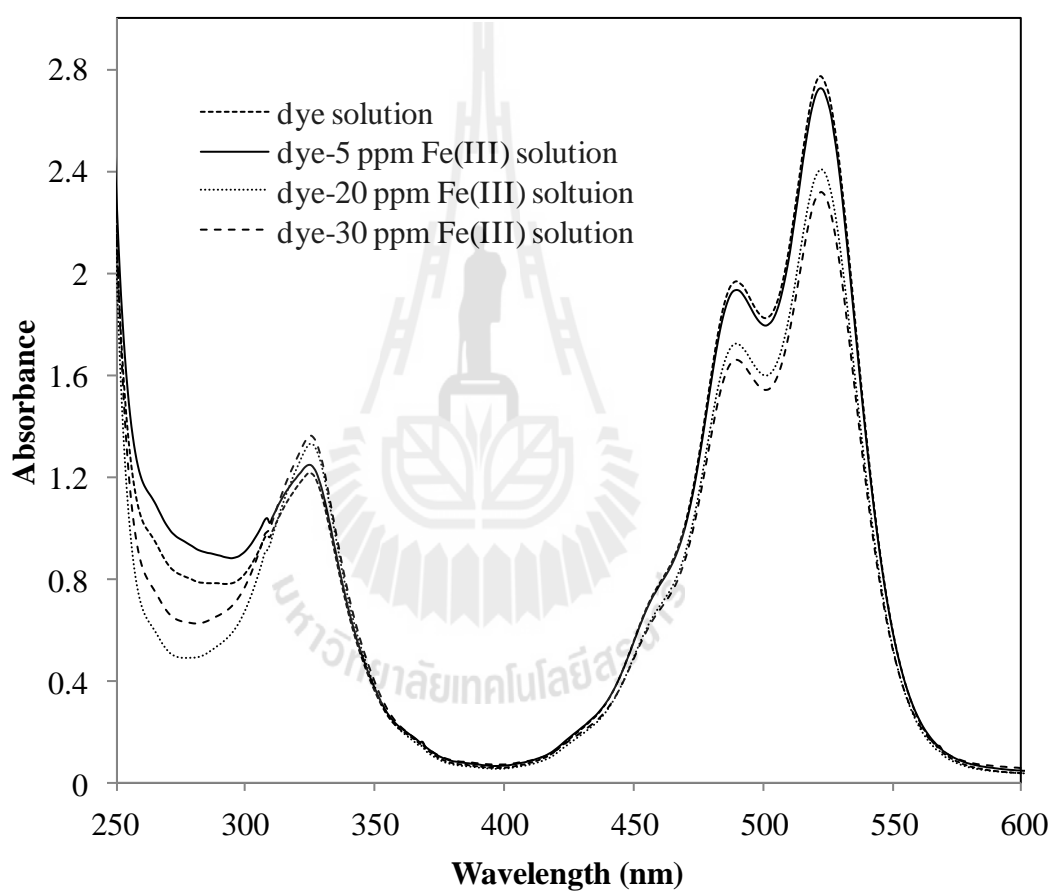**Figure 4.1** Structure of 1,1'-diethyl-2,2'-cyanine iodide.

Figure 4.2 Absorption spectra of the solution of 1,1'-diethyl-2,2'-cyanine iodide and

the solutions of the dye mixed with Fe(III).

2004), Fe(II), and Fe(III) (Ivanov et al., 2006; Kruanetr et al., 2008). Thus, these ions

could become interferences for determination of Fe(III).

Fluorescence measurement of the solution of 1,1'-diethyl-2,2'-cyanine iodide and the solutions of the dye mixed with Fe(III) were investigated. The fluorescence spectra are shown in Figure 4.3. The dye has excitation and emission peaks at 540 and 640 nm, respectively. When the dye solution was mixed with the Fe(III) solution, the fluorescence signal decreased with increasing concentration of Fe(III).

Quenching of the fluorescence provides an evidence of the Fe(III)-dye complex formation which is in agreement with the absorption study. The dye exhibited strong fluorescence signal even at relatively low concentration, 1.0×10^{-5} M. Overloading signal was observed at slightly higher dye concentration.
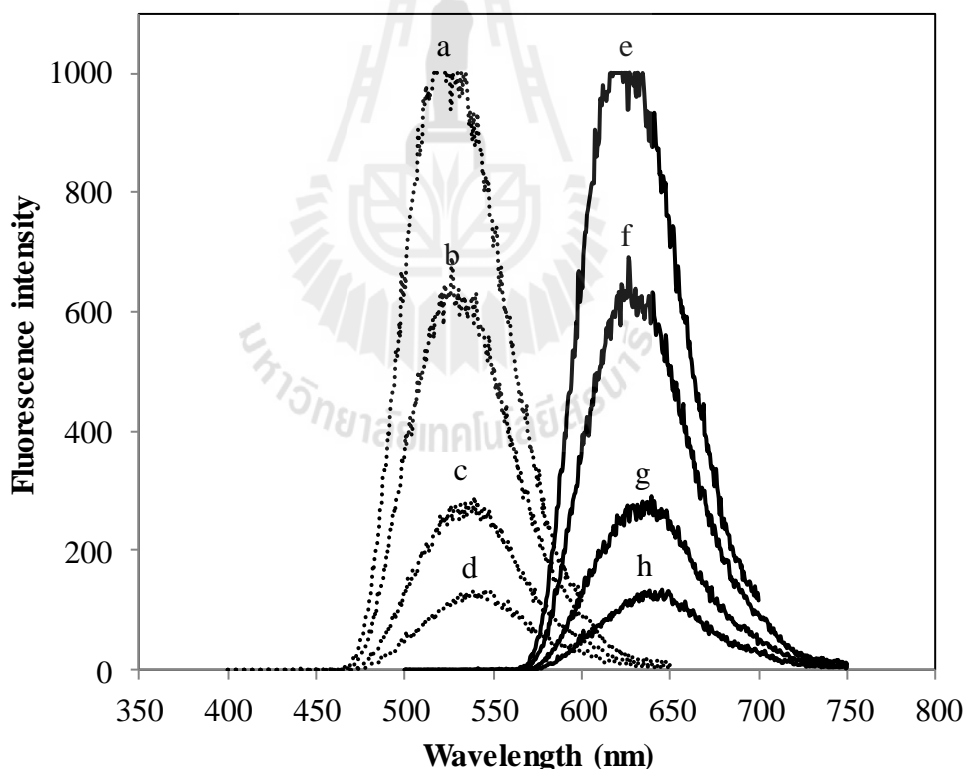

Figure 4.3 Excitation (dashed line) and emission (solid line) spectra of the dye solution (a, e), and the dye in solutions of 10 ppm (b, f), 30 ppm (c, g), and 50 ppm (d, h) Fe(III), respectively. The dye concentration was fixed at 10 μM.

This led to small amount of dye immobilized into polymer membrane. However, with small amount of the dye in the film, the change in the fluorescence signal in the presence of Fe(III) was not significant. Therefore, a sensing system based on fluorescence measurement was not further investigated.

4.2 Supporting matrix for 1,1'-diethyl-2,2'-cyanine iodide

PVC, chitosan, and Nafion were investigated as supporting matrices for immobilization of 1,1'-diethyl-2,2'-cyanine iodide. PVC was studied as a supporting matrix for dye immobilization because it is hydrophobic and optically transparent. It also has good mechanical and film forming properties. Additives such as plasticizer and ionic additive were also incorporated into the film to improve the film properties. A plasticizer, dioctyl phthalate (DOP), was incorporated into a PVC film to make it soft and flexible. Sodium tetraphenylborate (NaTPB) was used as an ionic additive to provide an anion sites for charge balancing when Fe(III) is extracted into the sensing film.

Compositions for the preparation of PVC sensing films are shown in Table 4.2. The results show that the dye was not well incorporated into the films for all studied compositions, although the dye was retained in film no. 4 and 8. Therefore, PVC was not a suitable supporting matrix for the preparation of optical sensing film for this work.

Chitosan was investigated as a supporting matrix because it is natural polymer, optically transparent, nontoxic, hydrophilic, and resistant to many chemicals. Chitosan has functional groups including hydroxyl and amine entities on the polymer chain that can be modified and used to couple with ligands under mild conditions.

Table 4.2 PVC sensing films prepared from different contents of dye, NaTPB, and DOP.

Film number	Composition*			Observation
	Dye (mg)	NaTPB (mg)	DOP (mL)	
1	8	10	0.6	leaching, inhomogenous
2	8	10	1.2	leaching, inhomogenous
3	8	20	0.6	leaching, inhomogenous
4	8	20	1.2	no leaching, inhomogenous
5	16	10	0.6	leaching, inhomogenous
6	16	10	1.2	leaching, inhomogenous
7	16	20	0.6	leaching, inhomogenous
8	6	20	1.2	no leaching, inhomogenous

*All of components were dissolved in 5.00 mL THF.

Adsorption of the dye on chitosan could be attributed to the interaction between the hydroxyl (-OH) group of chitosan and the N-atom of the dye molecule. A chitosan film without chemical modification swells in an aqueous solution. Therefore, to reduce the film swelling, crosslinking of polymer chains using glutaraldehyde was made. Glutaraldehyde linked the polymer chains by reacting with amino groups of chitosan. This led to lower number of amino groups to interact with water molecules (Vieira and Beppu, 2007). Two types of crosslinking procedures were made in the study, which were homogenous and heterogeneous crosslinking processes.

In homogeneous method, crosslinking was done by mixing all reagents together before casting a film. In heterogeneous method, crosslinking was made by soaking a chitosan film in crosslinking solution and later on the film was immersed in the dye solution. The results are shown in Table 4.3.

The results in Table 4.3 showed that the dye leached from the sensing film as a consequence of weak interaction between the dye and chitosan. Therefore, chitosan was not a suitable supporting matrix for the preparation of optical sensing film for this work.

Table 4.3 Chitosan sensing film prepared with different conditions.

| Film number | Crosslinking method | Composition | | | Observation |
		Chitosan (g)	Dye (mM)	Glutaraldehyde (%)	
1	homogeneous	0.25	5	0.25	swelling, leaching, homogenous
2	homogeneous	0.25	10	0.25	leaching, homogenous
3	homogeneous	0.50	5	0.50	leaching, homogenous
4	homogeneous	0.50	10	0.50	leaching, homogenous
5	heterogenous	0.25	5	0.25	leaching, homogenous
6	heterogenous	0.25	10	0.50	leaching, homogenous
7	heterogenous	0.50	5	0.25	leaching, homogenous
8	heterogenous	0.50	10	0.50	leaching, homogenous

Nafion was chosen as a supporting matrix because it is hydrophobic; resistant to most solvents, oxidants and bases; and permeable for many cations. 1,1'-diethyl-2,2'-cyanine iodide could dissolve in a solution of Nafion and the solution could be cast to prepare sensing films. Dye leaching from the sensing film into the solution was not observed because of cationic charge of the dye interacted electrostatically with sulfonated groups of Nafion.

1,1'-diethyl-2,2'-cyanine iodide reacts with Fe(III) ion forming a colored complex. In the presence of Fe(III) ions, the color of the dye in a buffer solution at pH 4.0 changed from red to reddish orange and the absorbance at 522 nm decreased. Similar color change was also observed in the Nafion film, but the absorption maximum shifted to 518 nm. Nitrogen on the dye is probably responsible for the binding of Fe(III).

4.3 Stoichiometric determination of 1,1'-diethyl-2,2'-cyanine iodide-Fe(III) complex

The mole-ratio method and the continuous variation method were used to determine the stoichiometric of the dye and Fe(III) in the complex in solution. Figure 4.4 shows results obtained from the study using the mole ratio method. There are two parts of the plot that have different slopes and the intersection of the two parts suggests that the Fe(III)-to-dye mole ratio is 1:1. Moreover, the stoichiometric ratio of Fe(III)-to- dye obtained from the method of continuous variation was also 1:1, as shown in Figure 4.5. An absorbance difference, ΔA, was calculated from the subtraction of the absorbance of the dye solution from the absorbance of the dye-Fe(III) solution.
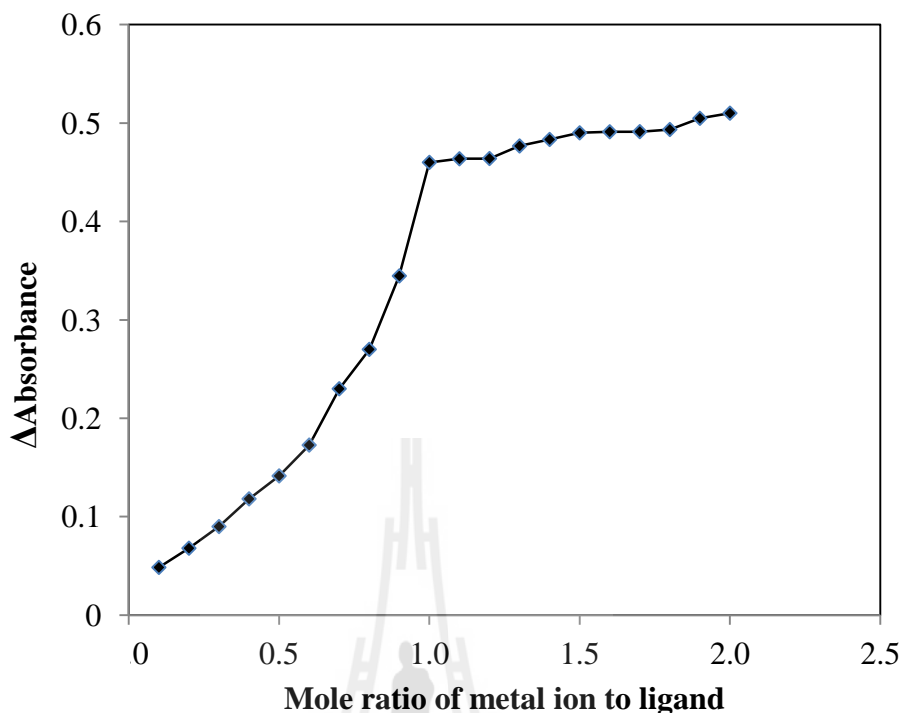
Figure 4.4 Stoichiometric study of 1,1'-diethyl-2,2'-cyanine iodide-Fe(III) complex in solution using the mole-ratio method.

4.4 Optical chemical sensor based on immobilized 1,1'-diethyl-2,2'-cyanine iodide in Nafion film

4.4.1 Immobilization of 1,1'-diethyl-2,2'-cyanine iodide onto Nafion film

The concentration of 1,1'-diethyl-2,2'-cyanine iodide was varied to obtain the optimum amount of the dye immobilized on a Nafion film. The dye was dissolved in Nafion solution and its concentration was varied from 0.5-2% w/v. The absorbance of the dye on the Nafion film was monitored at 518 nm. Figure 4.6 shows that the signal of sensing films increased with increasing dye concentration. Moreover, similar change in the signal was observed when the films immobilized with the dye
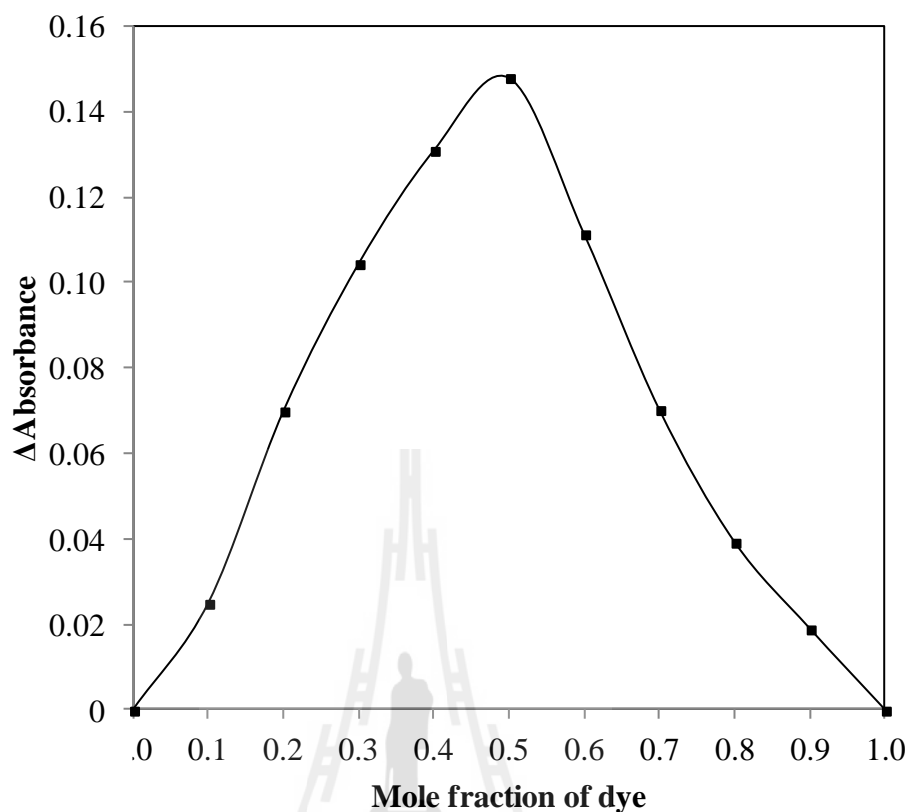
Figure 4.5 Study of 1,1'-diethyl-2,2'-cyanine iodide-Fe(III) complex in solution using the continuous variation method.

of 0.5, 1, and 2%w/v were soaked in 20 ppm Fe(III) solution as shown in Figure 4.7. An absorbance different, ΔA, was calculated by subtraction of the absorbance of the sensing film in buffer solution from the absorbance of the sensing film in Fe(III) solution. The absorbance was relatively constant after 1%w/v dye concentration because the limited solubility of the dye in the Nafion solution. In addition, with 2%w/v dye concentration the film was not homogeneous. Therefore, dye concentration of 1 %w/v was chosen for immobilization on Nafion film.
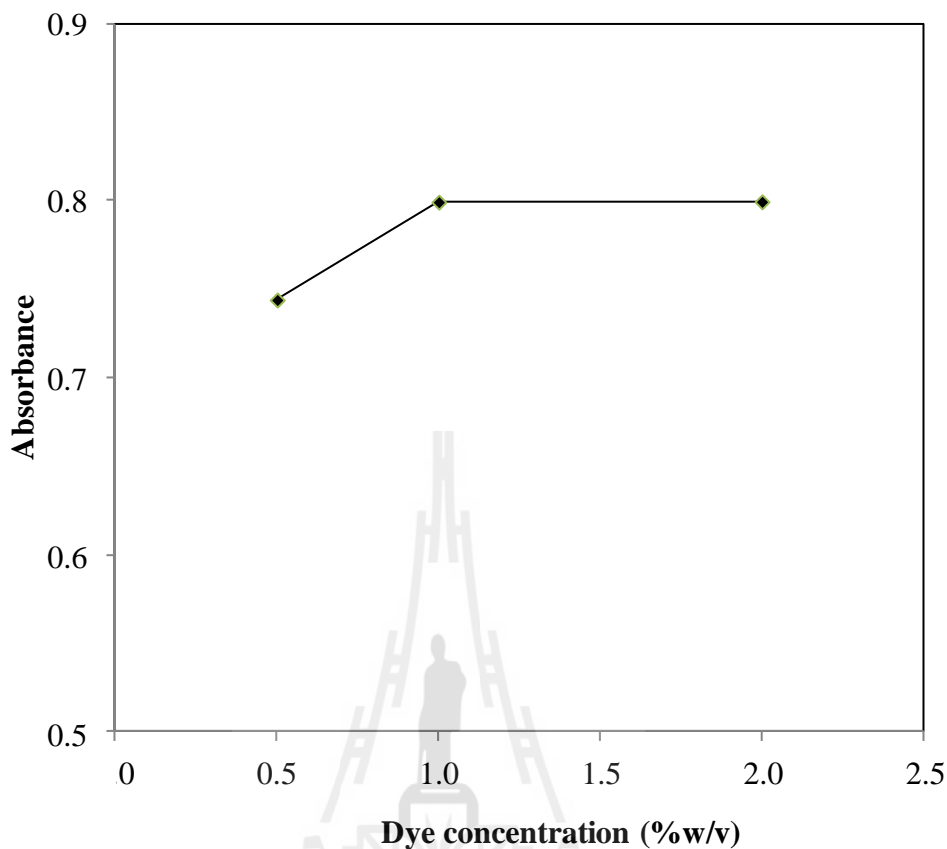
Figure 4.6 Study of the amount of dye immobilized in Nafion film. The absorbance of the films was measured in phosphate buffer pH 4.0.

4.4.2 Effect of pH on the response of the sensing film to Fe(III)

Influence of pH on the response of the sensing film to Fe(III) was studied using two buffer systems, universal buffer and phosphate buffer. The two systems were chosen because they have a wide pH range for buffer action. Figure 4.8 shows the response signals of the sensing films to Fe(III) in solutions containing 20 ppm of Fe(III) at pH 3.0, 5.0, and 7.0. All the response signals in the universal buffer were lower than those in the phosphate buffer. This could be resulted from the stability of Fe(III) in the two buffer solutions. In the universal buffer which were made from
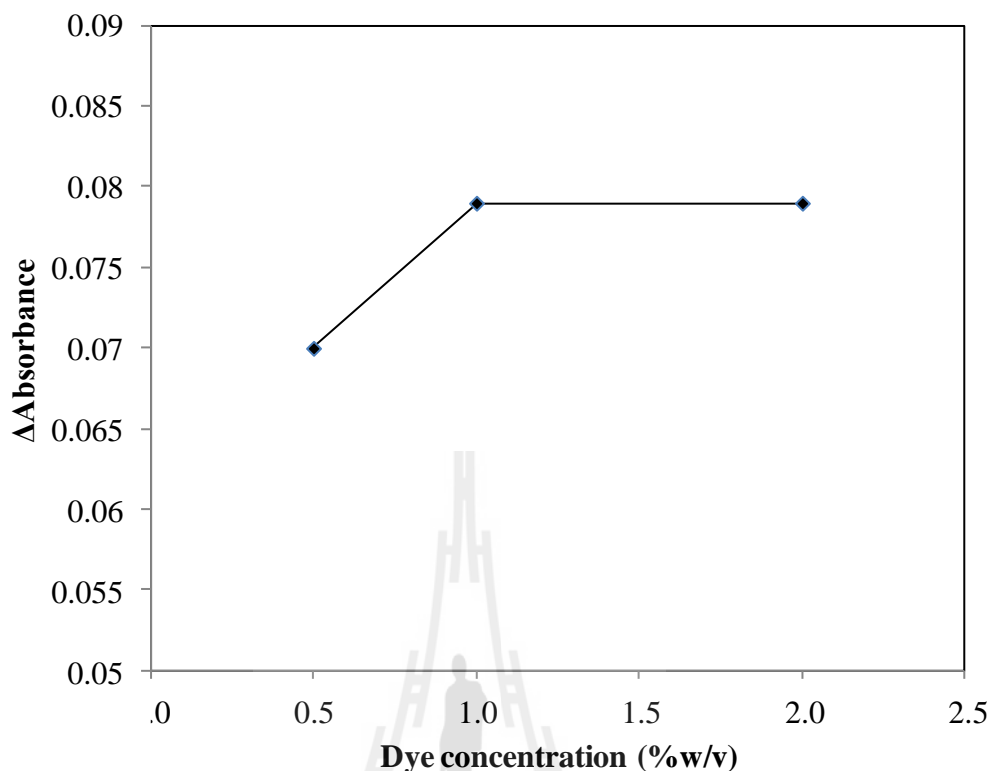
Figure 4.7 Influence of the amount of dye immobilized in Nafion film on the response to Fe(III). The absorbance of the films was measured in 20 ppm Fe(III) solution and the solution pH was maintained at 4.0 using phosphate buffer.

Na_2HPO_4 and citric acid, citric acid and citrate ion in the buffer formed complex with Fe(III) (Srinivasa et al., 2009; Seraghni et al., 2012) that could be more stable than the complex of Fe(III) and phosphate species. Consequently, less Fe(III) ions formed complex with the dye. Phosphate buffer was further used to control the pH of studied solutions.

Effect of the pH on the response of the sensing film to Fe(III) was investigated over a pH range of 2.0-8.0 with 20 ppm Fe(III) in phosphate buffer. Maximum response was obtained at pH 4.0 as shown in Figure 4.9. At pH > 4.0 response signals
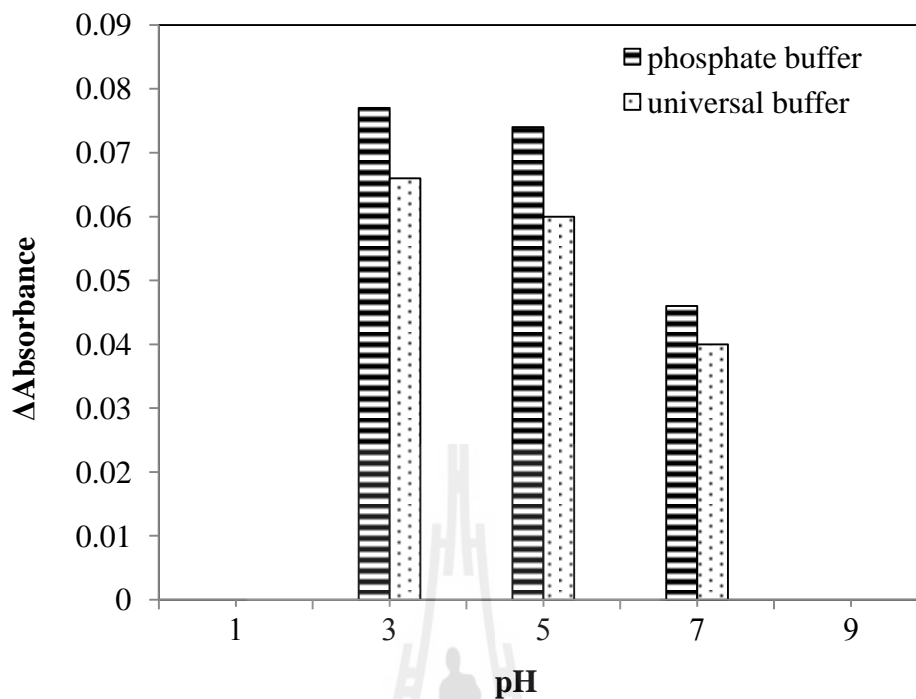
Figure 4.8 The effect of buffer solution on the response of the sensing film to 20 ppm Fe(III).

decreased because Fe(III)-precipitates could be formed by a hydrolysis reaction of Fe(III) in an aqueous solution at higher pH. Lower response signals were also obtained at pH < 4.0 due to the protonation of the sensing reagent. Therefore, further study solutions were buffered at pH 4.0.

4.4.3 Time dependence of the response of the sensing film to Fe(III)

Before making measurement of the sensing film in Fe(III) solution, the sensing film has to be soaked in phosphate buffer at pH 4.0 to obtain a steady background signal as shown in Figure 4.10. The absorbance of the sensing film was relatively stable after 2 min. Therefore, a submerged time of 2 min was chosen for conditioning the sensing film before placing the film in Fe(III) solution.
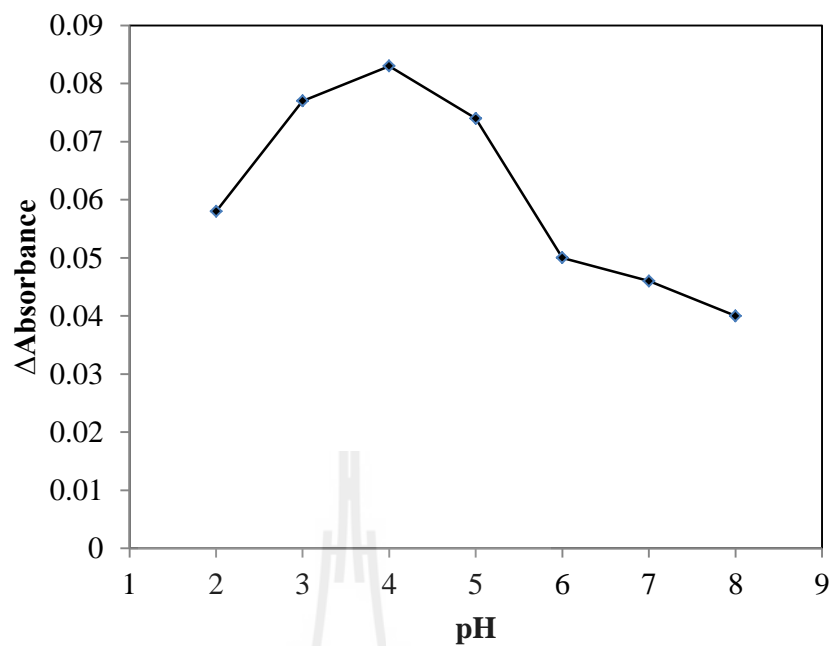
Figure 4.9 The effect of pH on the response signal of the sensing film to 20 ppm Fe(III).
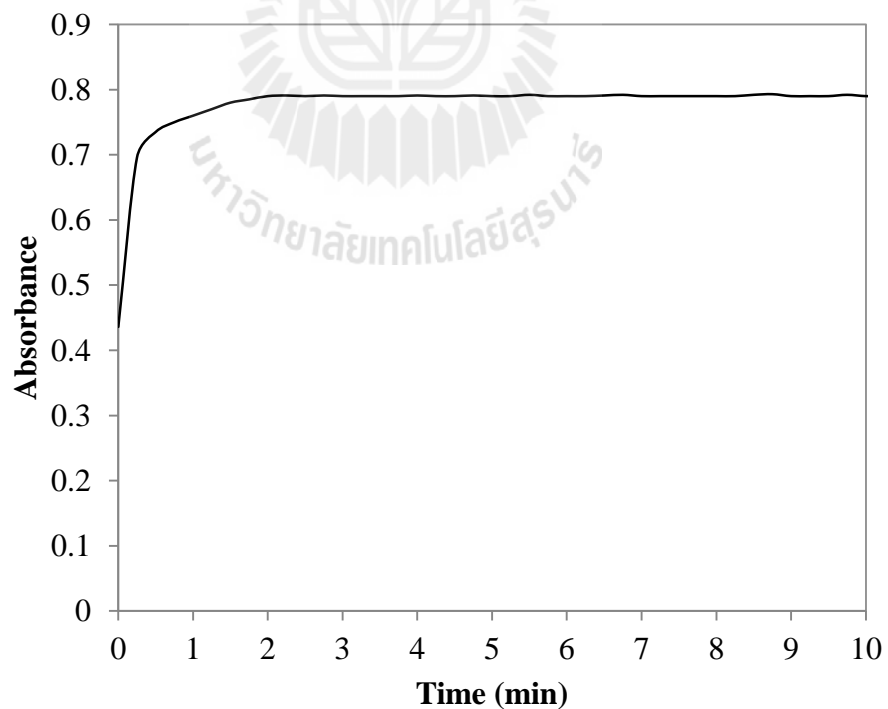

Figure 4.10 Background absorbance of the sensing film in buffer pH 4.0 monitored at 518 nm.

Figure 4.11 shows kinetic response of the sensing film submerged in solutions with different Fe(III) concentrations. The signal approached a plateau in less than 1 min for 20 ppm Fe(III) solution and the signal was relatively stable after that. It took ~1 min for the signal to reach the plateau for the solutions with lower Fe(III) concentrations. The response time of the sensing film to Fe(III) was controlled by the rate of diffusion, the signal reaches the steady state faster for higher Fe(III) concentration. Therefore, the time for absorbance measurement was chosen to be 1 min.

4.4.4   Calibration

Calibration curve for Fe(III) is shown in Figure 4.12. The calibration curve was obtained with a solution volume of 2.0 mL.
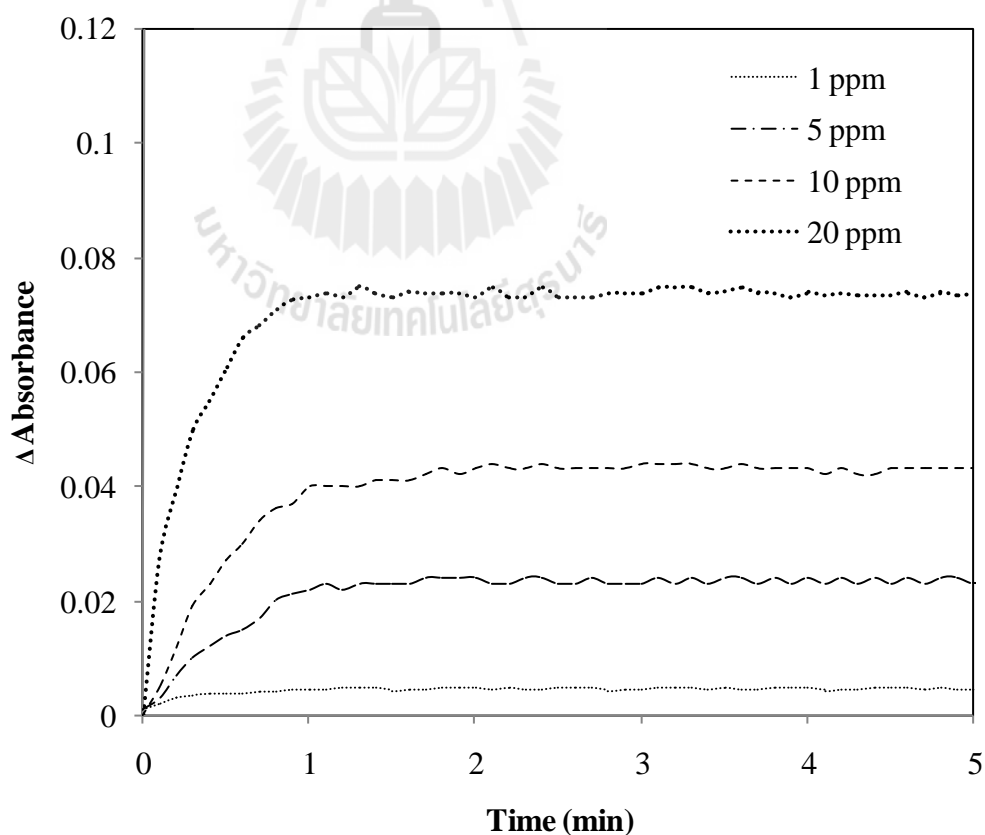

Figure 4.11 Time profiles of the response of the sensing films with various Fe(III) concentrations.
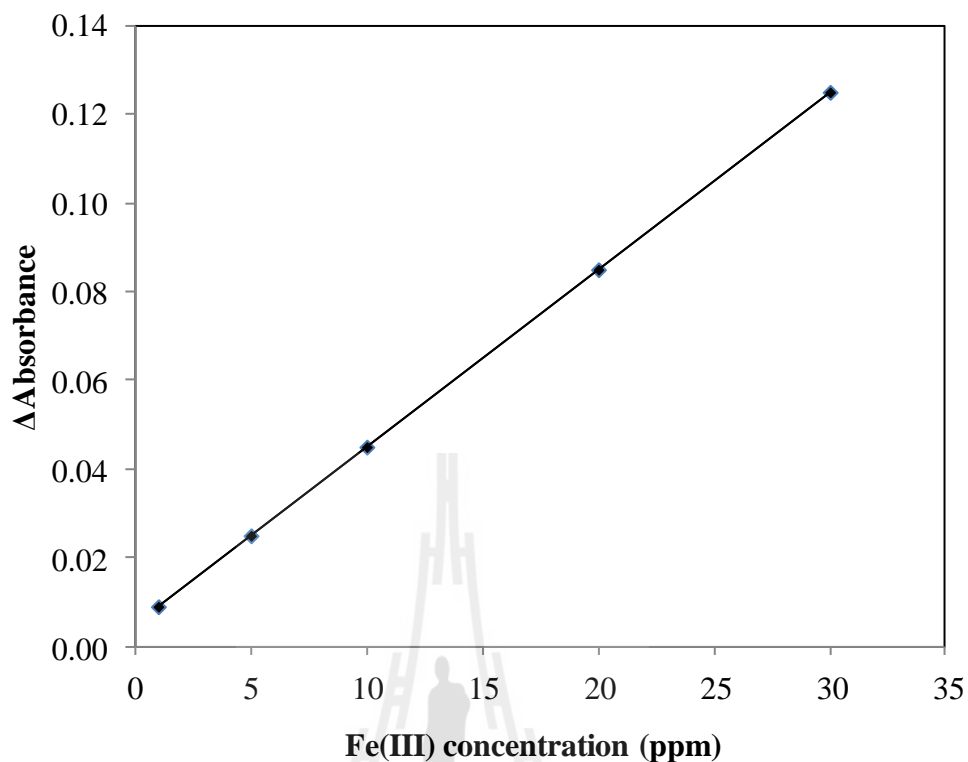
Figure 4.12 Calibration curve for Fe(III).

The linear correlation equation is $y = 0.0043x + 0.007$ and the linear correlation coefficient (R^2) is 0.9990 for Fe(III) concentration range of 1-30 ppm. Limit of detection is 0.71 ppm and calculated from the following equation,

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

where DL is a limit of detection, SD_{blank} is a standard deviation of the blank signal, and m is the slope of the calibration curve.

Calibration curves at longer response time were also obtained and the results are shown in Table 4.4. The graphs are shown in Appendix B. It is clear that measurement time of 1 min was adequate and provided relatively equivalent sensitivity as those at longer measurement time.

Table 4.4 Calibration equations obtained at various response times.

Response time (min)	Equation	R^2
1	0.0040x + 0.0050	0.999
2	0.0036x + 0.0030	0.999
3	0.0036x + 0.0030	0.999
5	0.0036x + 0.0028	0.999

Nonlinearity was observed when the concentration of Fe(III) was more than 30 ppm. The signal of the sensing film in 40 ppm Fe(III) solution was about 22% lower than that calculated from the linear equation. The lower signal at 40 ppm Fe(III) could be resulted from the limited amount of dye immobilized on the film.

4.4.5 Interference study

The effect of interference ions on the determination of Fe(III) was investigated at the optimum condition for Fe(III) by measuring the absorbance difference at 518 nm of the sensing film in a solution of Fe(III) or in a solution of Fe(III) mixed with a studied foreign ion at the molar ratio of 1:1. Fe(III) concentration and the foreign ion concentration were 3.58×10^{-4} M. The results are summarized in Table 4.5. % Error is defined as follows,

$$\% \text{Error} = \frac{(\Delta A_{\text{Fe(III), ion}} - \Delta A_{\text{Fe(III)}})}{\Delta A_{\text{Fe(III)}}} \times 100$$

where $\Delta A_{\text{Fe(III), ion}}$ is the absorbance difference of the sensing film in the solution of Fe(III) mixed with a foreign ion and $\Delta A_{\text{Fe(III)}}$ is the absorbance difference of the sensing film in the solution of Fe(III) only. The ion ratio that exhibits an error of less than ±5% is considered as tolerated for the analysis. Therefore, Cd(II) is considered as

Table 4.5 Interference study at 1:1 molar ratio of foreign ion:Fe(III).

Ion	% Error
Cu(II), Fe(II)	0.13
Ni(II), K(I)	0.06
Co(II)	0.10
Mg(II)	0.03
Cr(II)	0.48
Cd(II)	5.34
Ca(II), Na(I)	0.12
Zn(II)	4.80

the major interference for this sensing film.

4.4.6 Reproducibility, regeneration and lifetime

The reproducibility of the procedure for the preparation of the sensing film was evaluated by measuring the absorbance at 518 nm of five sensing films which were prepared from different batches. Besides, the response of individual sensing film to Fe(III) was assessed by measuring the signal of five sensing films with 20 ppm Fe(III) solution under the optimum condition. The results are summarized in Table 4.6. The calculated relative standard deviations of the absorbance of the films in phosphate buffer and ΔA were 0.27 and 4.68%, respectively. This indicates that the preparation procedure is reproducible.

To reuse a sensing film, a regeneration process was investigated by dipping a sensing film in an appropriate solution to remove Fe(III) out of the sensing film.

Table 4.6 Absorbance of sensing films in phosphate buffer and 20 ppm Fe(III) solution at 518 nm.

	Absorbance		
Sensing film no.	Film in phosphate buffer	Film in 20 ppm Fe(III)	Δ Absorbance
1	0.800	0.720	0.080
2	0.802	0.729	0.073
3	0.805	0.725	0.080
4	0.800	0.727	0.073
5	0.800	0.725	0.075

Solutions of EDTA and HCl were investigated as regenerating reagents. The sensing film after soaking in Fe(III) solution at the optimum condition to obtain a stable signal was dipped into a regenerating solution for 30 s. As shown in Figure 4.13, the absorbance decreases rapidly within 30 s. When the film was placed in the regenerating reagent and the color of the sensing film disappeared. The disappearance of the dye color was from the reaction of the dye and hydrogen ions. When the film was submerged in the buffer, the color of the film was recovered and the absorbance of the film returned to its initial value. The recovery of the absorbance signal of the sensing film suggests that the regenerating reagents effectively removed Fe(III) ions from the sensing films.

The time for soaking the sensing film in the regenerating solutions was also studied and the results are shown in Figure 4.14. The signal was obtained from the film in buffer after the film was immersed in 20 ppm Fe(III) solution for 15-120 s and then in the regenerating reagent at a given time. All of the studied regenerating solutions could
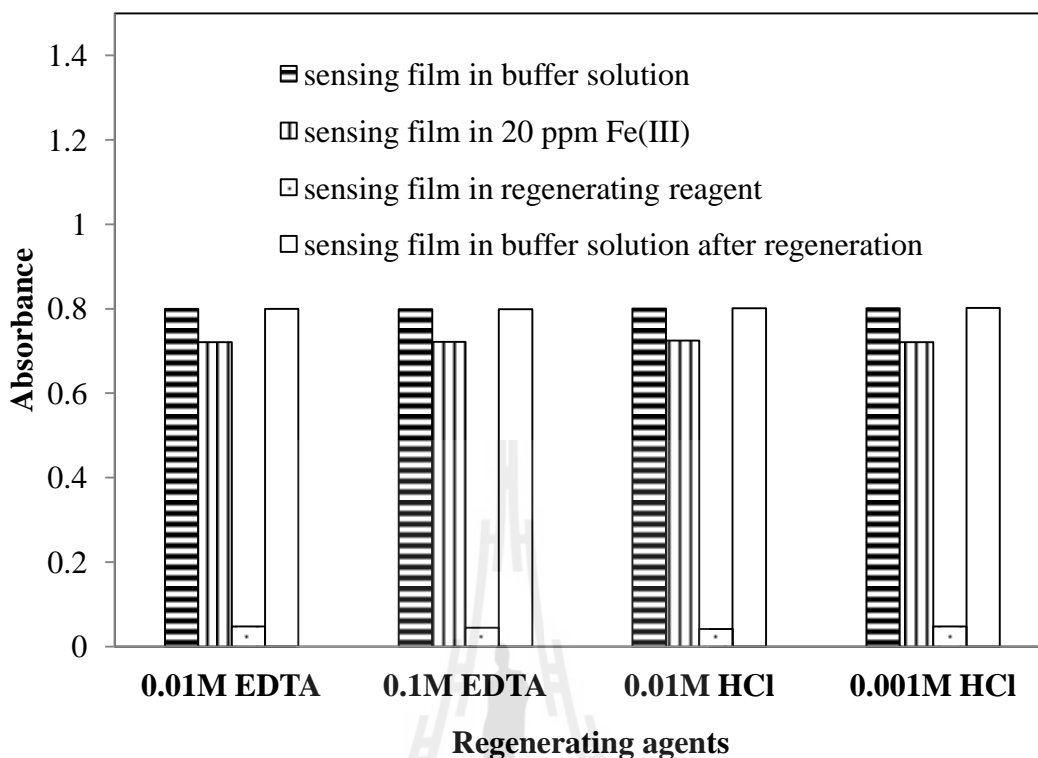
Figure 4.13 Regeneraton of the sensing film in various regenerating reagents.

strip Fe(III) out of the sensing film. For further studies, 0.001 HCl was chosen as the regenerating and the regeneration time was chosen at 30 s.

Number of time for regenerating the sensing film was studied by using one sensing film and the results are shown in Figure 4.15. The calculated RSDs were 3.96 % for the film in buffer solution and 4.82% for the film in 20 ppm Fe(III) solution. It is clear that one sensing film can be repeatedly used for at least 30 times. Calibration using one sensing film was also conducted and the linear relationship was also obtained (Appendix C).

Lifetime of the sensing films was examined over a period of 60 days and the results are shown in Figure 4.17. The sensing film exhibited good stability over two
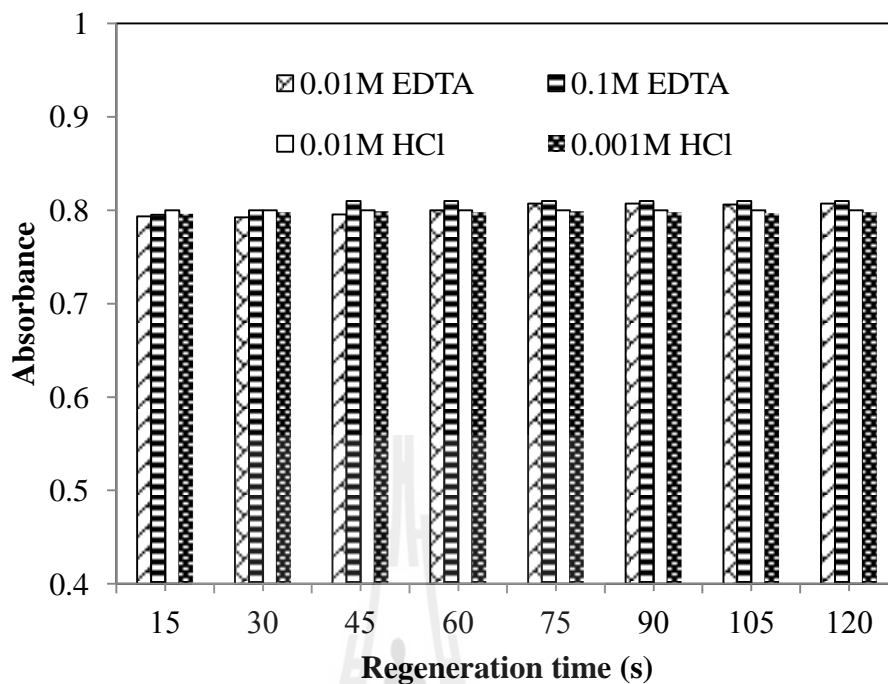
Figure 4.14 Regeneration time of the sensing film using various regenerating reagent.

months with the calculated RSD of 3.12% based on the tested with 20 ppm Fe(III) solution.

4.4.7 Real sample analysis

To evaluate the applicability of the sensing film, analyses of real samples were performed with water samples collected from a water reservoir located at SUT and tap water and vitamin tablets. The results are summarized in Table 4.7. The results obtained with the proposed method agreed well with those obtained with the AAS method, as evaluated by *t*-test (shown in Appendix D).
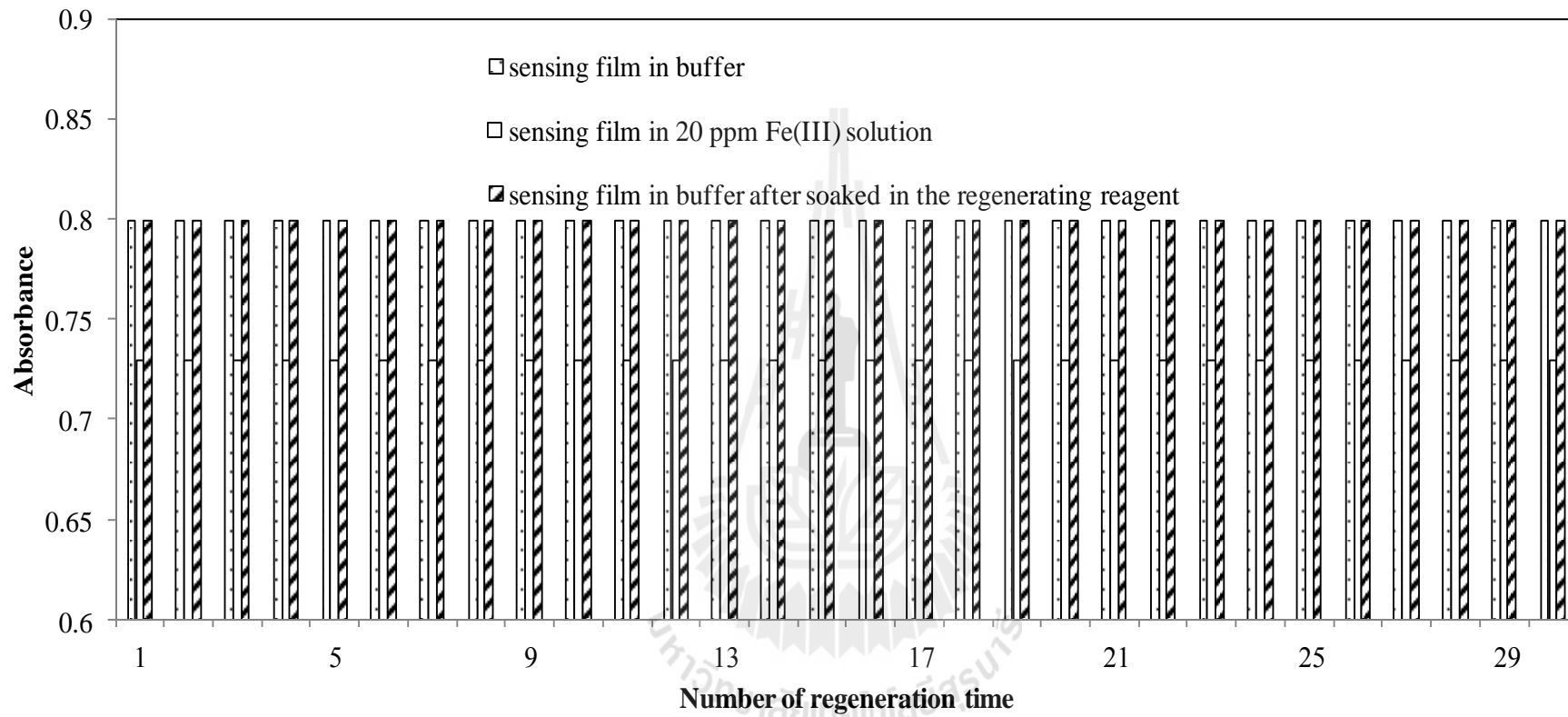
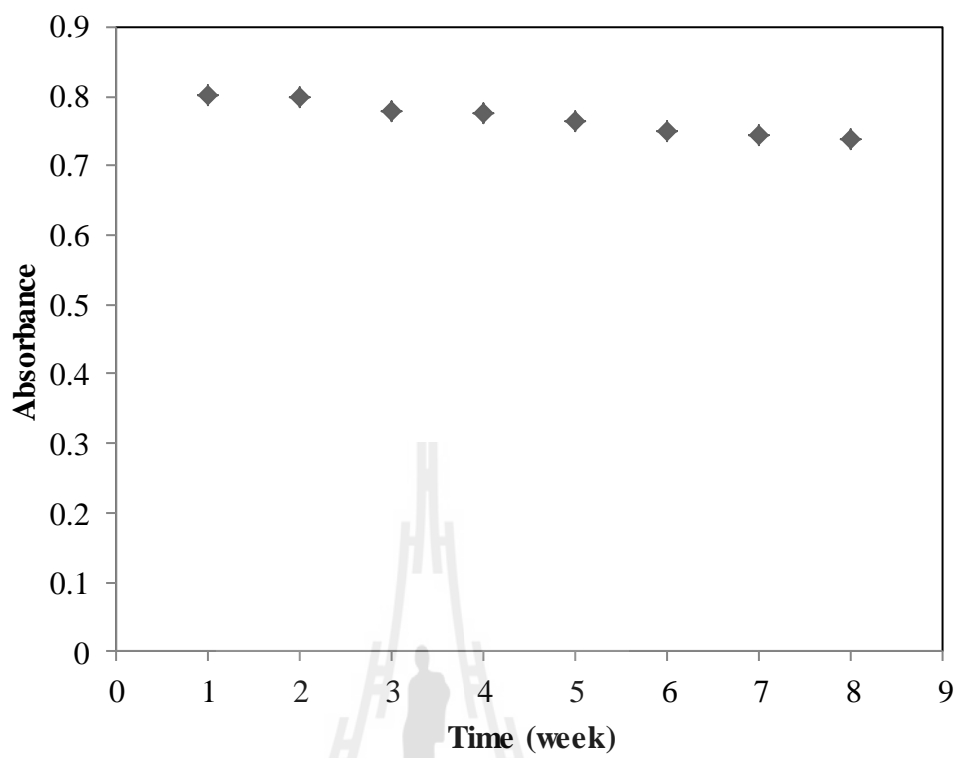**Figure 4.15** Regeneration study of one sensing film.

Figure 4.16 Stability of the sensing films over a period of 60 days.

Table 4.7 Determination of Fe(III) in water samples and in vitamin tablets.[a]

Sample	Fe(III) added (ppm)	Proposed method		AAS method	
		Fe(III) found (ppm) ± SD	Recovery (%)	Fe(III) found (ppm) ± SD	Recovery (%)
	0	ND[b]	-	ND	-
SUT water	5.00	4.98±0.01	99.6	5.51±0.10	110.2
reservoir	10.00	10.07±0.02	100.7	10.76±0.02	107.6
	15.00	15.05±0.05	100.3	15.55±0.10	103.7
	0	ND	-	ND	-
Tap water	5.00	5.26±0.01	105.2	5.27±0.02	105.4
	10.00	10.06±0.02	100.6	10.57±0.02	105.7
	15.00	15.55±0.10	103.7	15.71±0.02	104.7
Vitamin tablet[c]	-	49.06±0.81	-	49.10±0.86	-

[a]The reported values are means of 3 triplicate measurements.

[b]ND = not detected.

[c]The iron content in the vitamin tablet was 49.6 mg/tablet.

CHAPTER V

CONCLUSIONS

This research focused on the development of an optical chemical sensing film as an alternative method for the determination of Fe(III) in aqueous solutions. An optical chemical sensor contains two main components, which are receptor and transducer part. The receptor part, which is composed of a sensing reagent, and a supporting matrix was constructed in this work.

The optical chemical sensing film was produced by immobilizing an Fe(III) selective organic dye, 1,1′-diethyl-2,2′-cyanine iodide, into Nafion film. Nafion is a good supporting matrix for the selected dye because it is compatible with the dye, hydrophobic, resistant to acid and basic solutions, and permeable to Fe(III). A solution of Nafion mixed with the dye could be made easily into a film by a casting technique.

In the presence of Fe(III), the color of the sensing film changed from red to reddish-orange as a result of complex formation which caused a decrease in the absorbance at 518 nm. The decrease in the absorbance signal was linearly correlated to the concentration of Fe(III) in the range of 1-30 ppm. The optimum pH of the solution for the maximum response of the sensing film was 4.0. At higher pH, response signals decreased because Fe(III)-precipitates could be formed by a hydrolysis reaction. Lower response signals were also obtained at lower pH due to the protonation of the sensing reagent. The stoichiometric ratio of Fe(III)-to-dye obtained

from the method of mole ratio and continuous variation was 1:1.

The developed sensing film exhibited high sensitivity, reproducibility, and good short-term and long-term stability. One sensing film can be regenerated and reused at least 30 times. A batch of sensing film once prepared could be used over 2 months. The detection limit for Fe(III) was 0.71 ppm, which is well suited for analysis of various samples. The main interference is Cd(II), which should be removed from the aqueous solution before the determination of Fe(III). The developed method was applied well for the determination of Fe(III) in real samples.

The developed sensing film offers an alternative tool for the determination of Fe(III) in aqueous samples. Measurement with the sensing film requires lower amounts of reagents and produces less amount of waste compared to that with traditional molecular absorption spectrophotometry. The sensing film is user-friendly, so that is personnel with limited skills could make a measurement without excessive manipulation.

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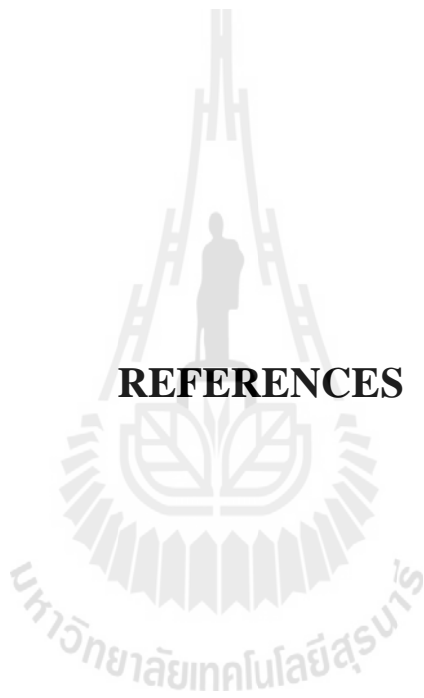
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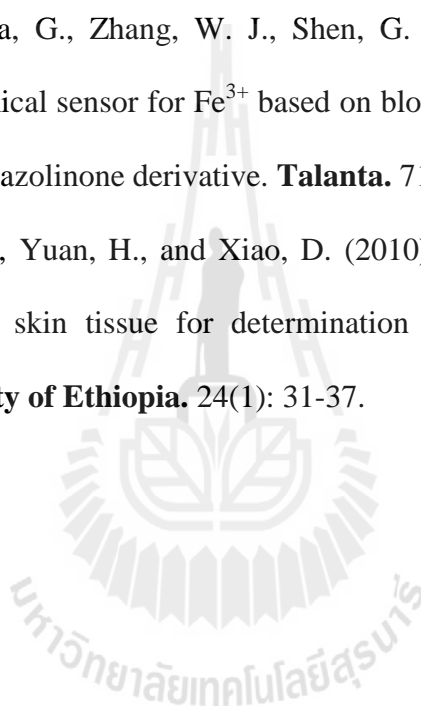
APPENDICES
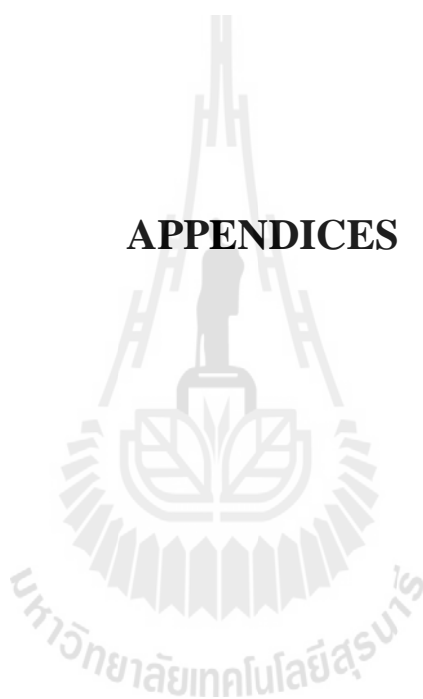
APPENDIX A

ABSORPTION SPECTRA OF SOME ORGANIC DYES

AND DYES WITH IRON(III) SOLUTIONS
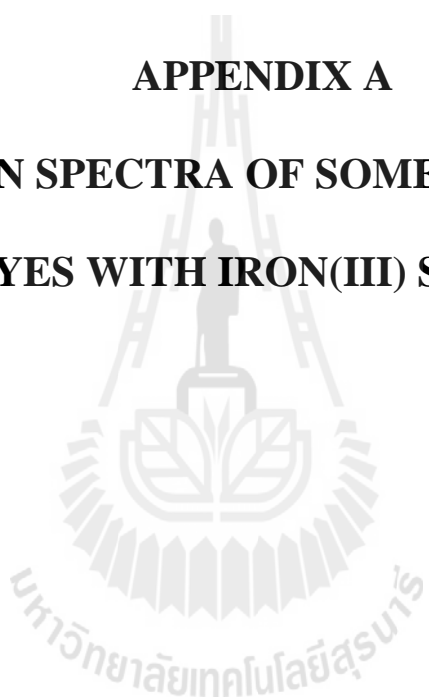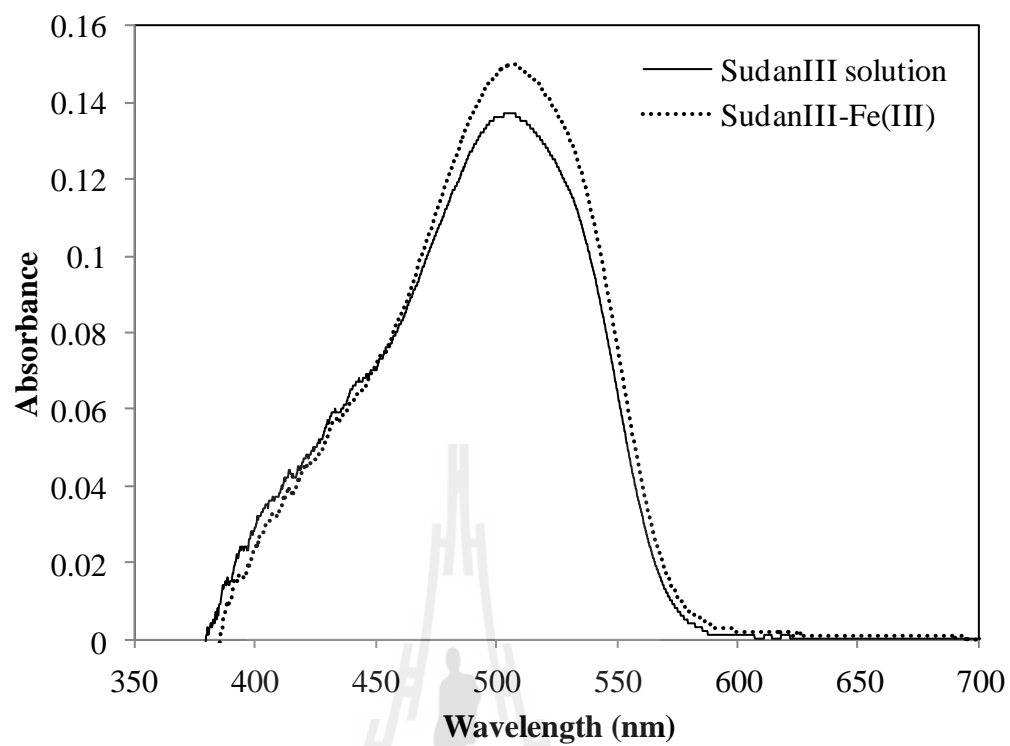
Figure A.1 Absorption spectra of sudan III solution and sudan III with Fe(III) solution.
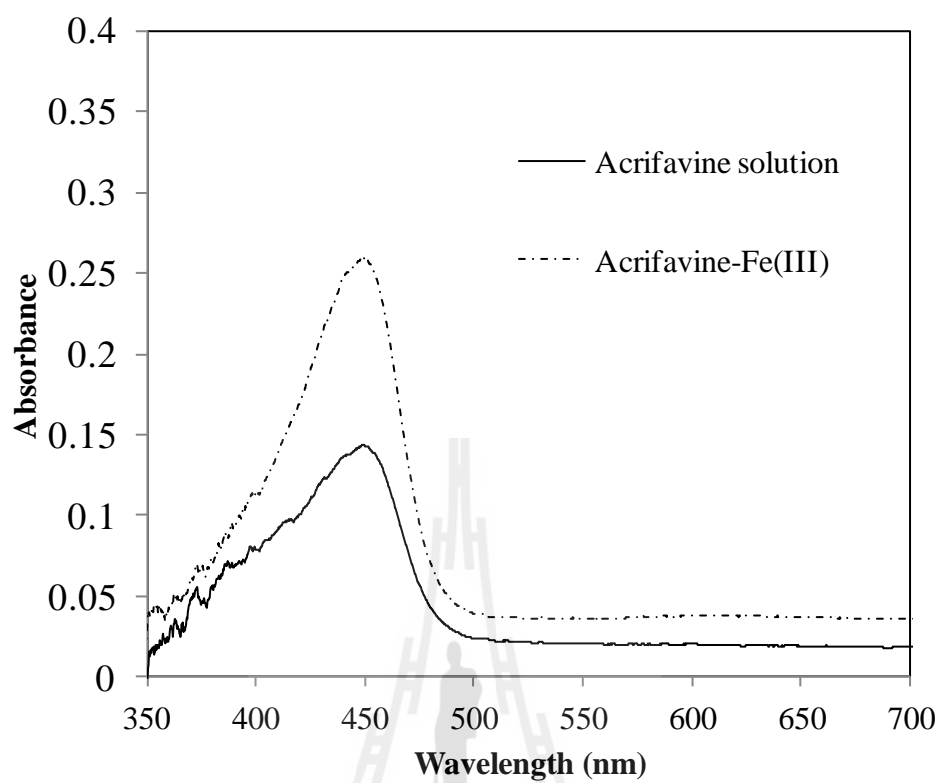
Figure A.2 Absorption spectra of acriflavine solution and acriflavine with Fe(III) solution.
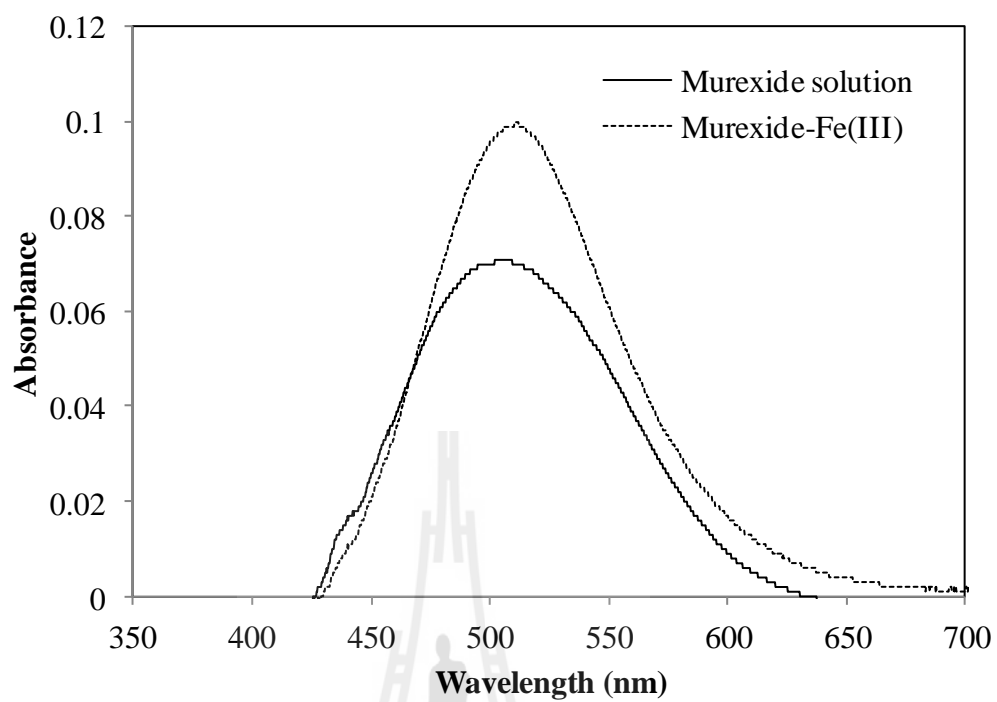
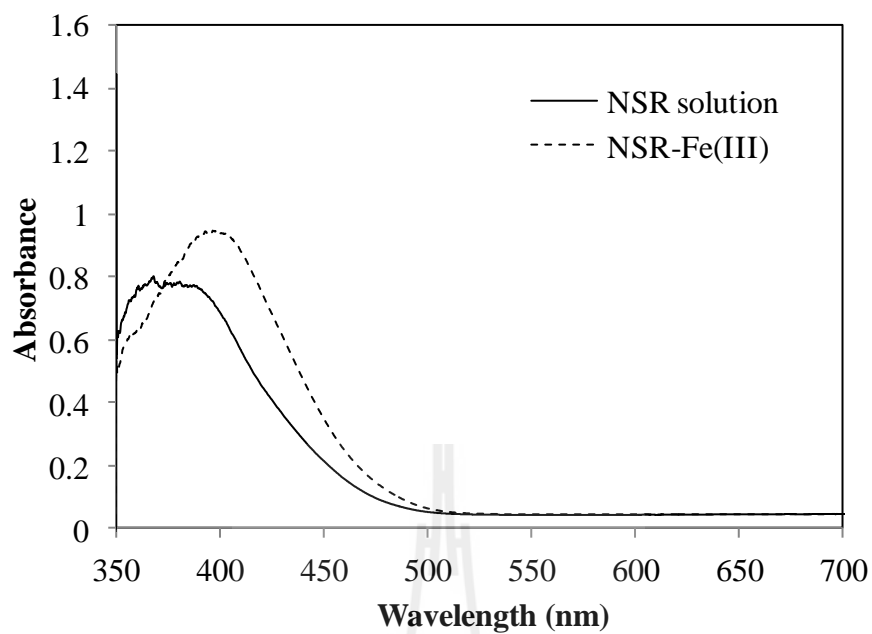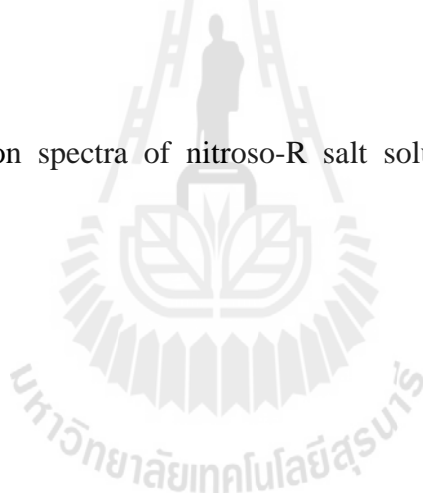**Figure A.3** Absorption spectra of murexide solution and murexide with Fe(III) solution.

Figure A.4 Absorption spectra of nitroso-R salt solution and nitroso-R salt with Fe(III) solution.

APPENDIX B

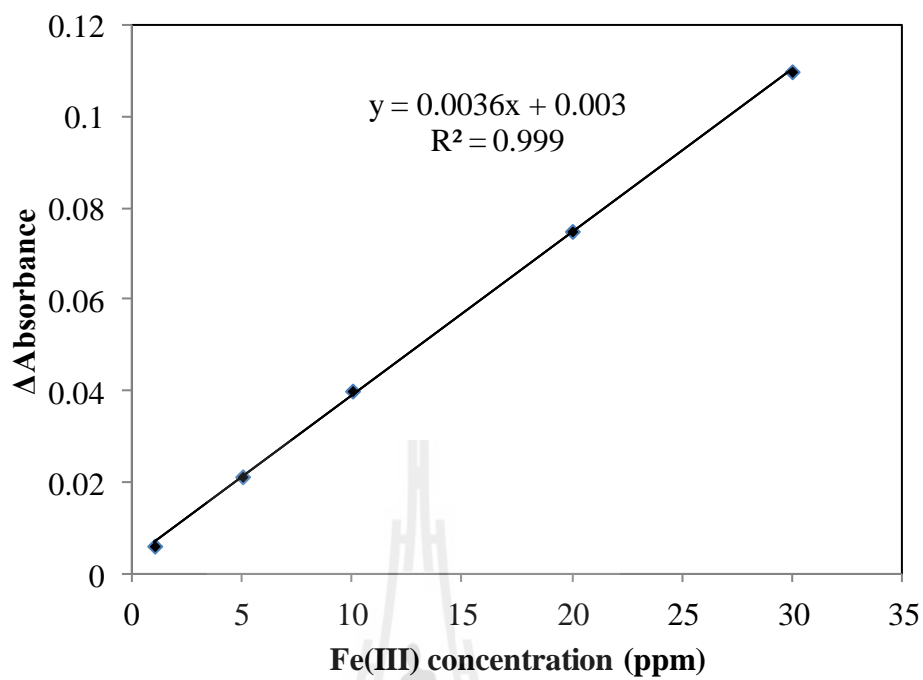**CALIBRATION CURVES OBTAINED FROM VARIOUS**

RESPONSE TIMES

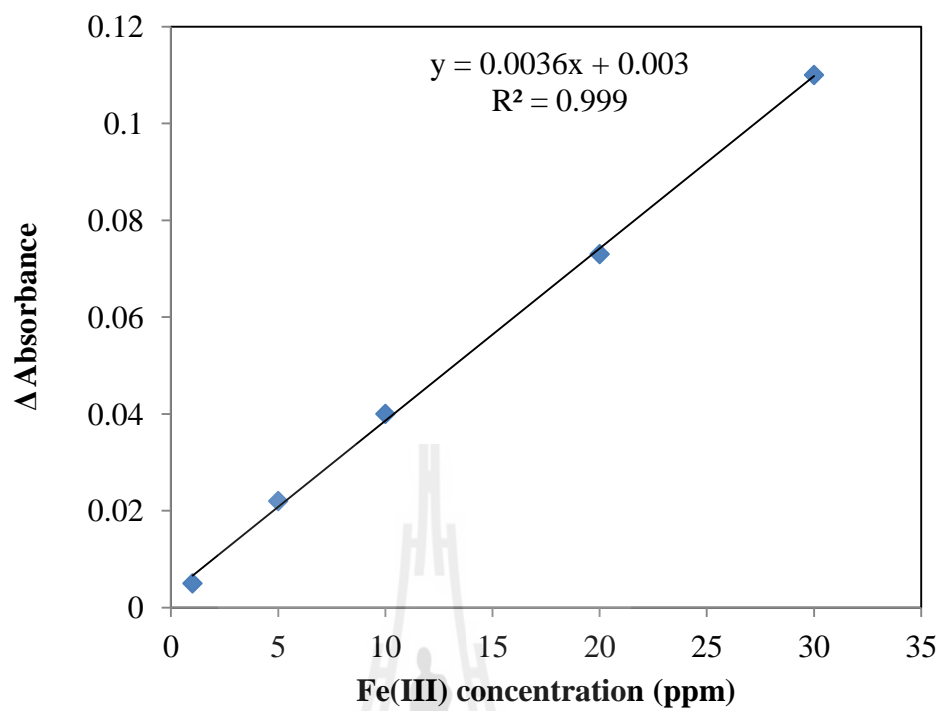**Figure B.1** Calibration curve of the sensing film obtained from 2 min of the response time.

Figure B.2 Calibration curve of the sensing film obtained from 3 min of the response time.
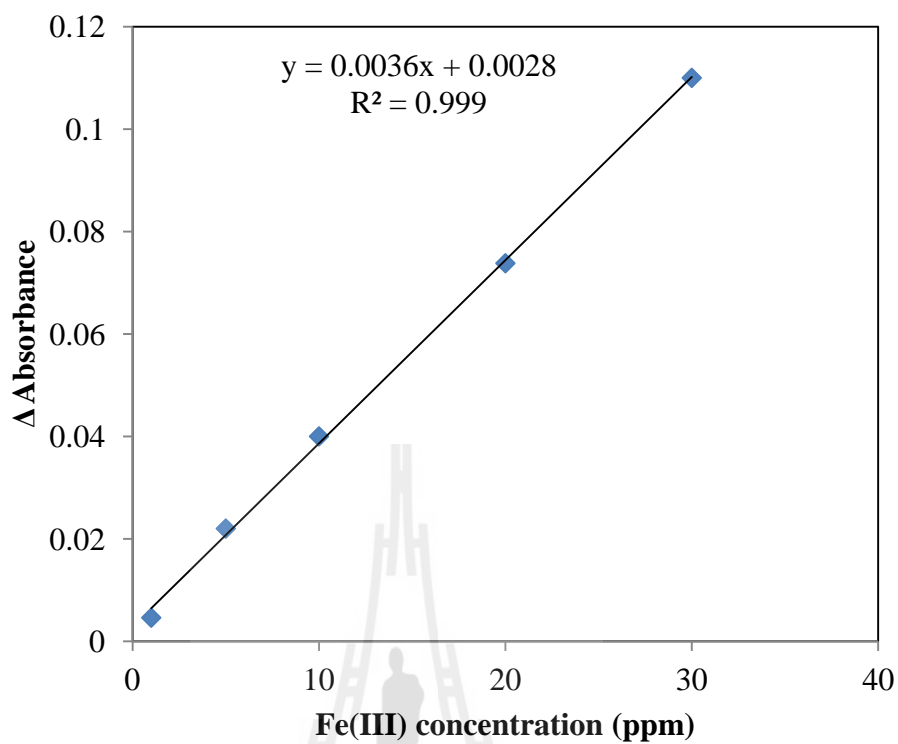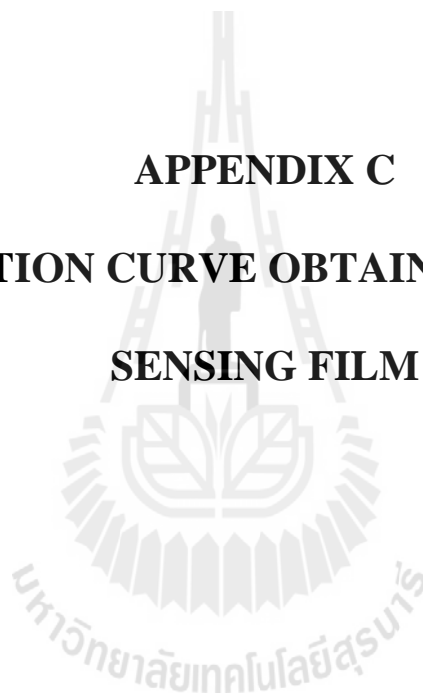
Figure B.3 Calibration curve of the sensing film obtained from 5 min of the response

time.

APPENDIX C

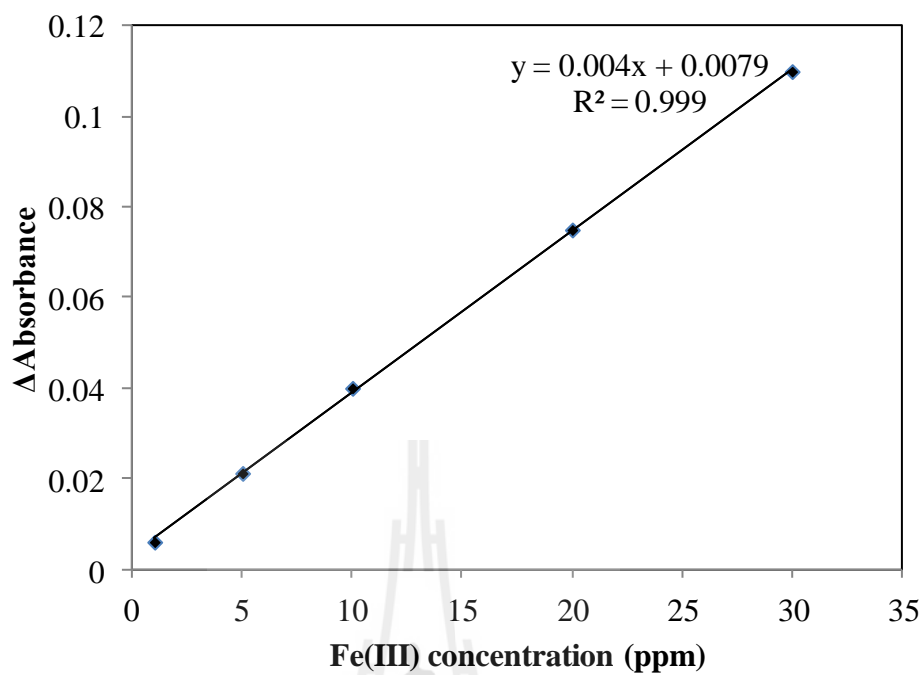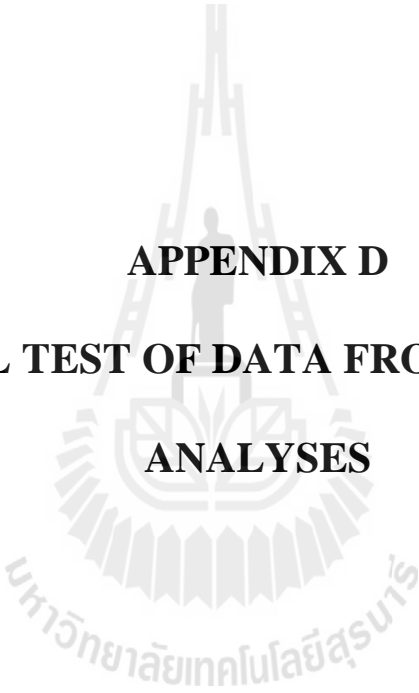# CALIBRATION CURVE OBTAINED FROM ONE

SENSING FILM

Figure C.1 Calibration curve obtained from one sensing film.

APPENDIX D

STATISTICAL TEST OF DATA FROM REAL SAMPLE

ANALYSES

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, and N is number of the differences of the pairs of results.
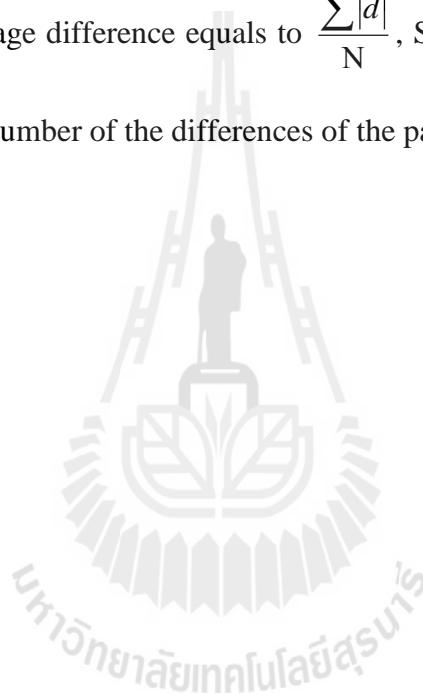
Table D.1 Comparison of two analytical methods for the determination of Fe(III).

Sample	AAS method (ppm)	Proposed method (ppm)	|d|	$\bar{d}$	SD	Calculated t-statistic
SUT reservoir						$t_{cal} = \dfrac{\bar{d}}{SD}\sqrt{N}$
1	4.98	5.51	0.53			
2	10.07	10.76	0.69	0.57	0.10	$= \dfrac{0.57}{0.10}\sqrt{3}$
3	15.05	15.55	0.50			$= 3.29$
Tap water						$t_{cal} = \dfrac{\bar{d}}{SD}\sqrt{N}$
1	5.26	5.27	0.01			
2	10.10	10.57	0.49	0.22	0.24	$= \dfrac{0.13}{0.14}\sqrt{3}$
3	15.55	15.72	0.17			$= 1.58$
Vitamin tablet						$t_{cal} = \dfrac{\bar{d}}{SD}\sqrt{N}$
1	48.60	48.10	0.05			
2	50.00	49.60	0.04	0.36	0.55	$= \dfrac{0.36}{0.55}\sqrt{3}$
3	48.60	49.60	1.00			$= 1.33$

3   The values of the calculated t-statistic of SUT reservoir, tap water and vitamin tablet are 3.29, 1.58 and 1.3, respectively. The critical value of t-statistic at 95% confidence levels and 2 degree of freedom is 4.30 (Skoog et al., 2004). Since $t_{cal} < t_{critical}$, the results obtained from the two methods are not significantly different.

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Kajsanthia, K., Wittayakun, J., and Prayoonpokarach, S. Preparation of optical sensing film based on dye immobilization for the determination of iron(III) ion. Presented at The 6th Science and Technology Conference for Youths, BITEC : Bangkok International Trade and Exhibition Centre, Bangkok, Thailand, 2010.

Kajsanthia, K., Wittayakun, J., and Prayoonpokarach, S. An optical sensing film for Fe(III) determination based on 1,1′- diethyl 2,2′-cyanine iodide immobilized in nafion film. Proceeding of ICACBE 2012 : International Conference on

Applied Chemistry and Biological Engineering, Geroldswil Swiss Quality Hotel (Zurich), Switzerland : World Academy of Science, Engineering and Technology. (2012) 67: 243-246.

Sombatsri, S., Wittayakun, J., Sanai, K., **Kajsanthia, K**., and Prayoonpokarach, S. (2012) An optical sensing film for the determination of Co(II) based on disodium-1-nitroso-2-naphthol-3,6-disulfonate immobilized in chitosan film. Sensors and Actuators B: Chemical. 166: 772-776.