

การตรวจวัดไอออนโลหะหนักด้วยตาเปล่าหลังเกิดสารเชิงซ้อน
กับไซลิโนลออกเรนจ์โดยวิธีคอลัมน์

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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ปีการศึกษา 2555

บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์นี้จะต้องถูกจัดเก็บและเผยแพร่ในคลังปัญญาจุฬาฯ (CUIR)

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NAKED-EYE DETECTION OF HEAVY METAL IONS AFTER FORMING
COMPLEXES WITH XYLENOL ORANGE BY COLUMN METHOD

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry

Department of Chemistry

Faculty of Science

Chulalongkorn University

Academic Year 2012

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พินิตา มงคลวุฒิมิกุล : การตรวจวัดไอออนโลหะหนักด้วยตาเปล่าหลังเกิดสารเชิงซ้อนกับ
ไซลีนอลออเรนจ์โดยวิธีคอลัมน์. (NAKED-EYE DETECTION OF HEAVY METAL IONS
AFTER FORMING COMPLEXES WITH XYLENOL ORANGE BY COLUMN
METHOD) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร.วัลภา เอื้องไมตรีภรณ์, 59 หน้า.

ได้ทดสอบการตรวจวัดไอออนโลหะหนักเบื้องต้น (แคดเมียม(II) โคบอลต์(II) คอปเปอร์(II)
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ออเรนจ์เท่ากับ 0.01 มิลลิโมลาร์ อัตราการไหลผ่านคอลัมน์ขนาดเล็ก (เส้นผ่านศูนย์กลาง 4 มิลลิเมตร
ยาว 2 เซนติเมตร) ที่บรรจุซิลิกาเจล 25 มิลลิกรัม เท่ากับ 1 มิลลิลิตรต่อนาที ปริมาตรสารตัวอย่างสูงสุด
เท่ากับ 50 มิลลิลิตร สำหรับโคบอลต์(II) และ 100 มิลลิลิตร สำหรับนิกเกิล(II) และเหล็ก(III) ความเข้มข้น
ต่ำที่สุดที่มองเห็นด้วยตาเปล่าสำหรับโคบอลต์(II) นิกเกิล(II) และเหล็ก(III) เท่ากับ 0.6, 0.6 และ 0.06
มิลลิกรัมต่อลิตรตามลำดับ ใช้ช่วงความเข้มข้นทำงาน 0.1-60 มิลลิกรัมต่อลิตรสำหรับโคบอลต์(II)
นิกเกิล(II) และ 0.1-120 มิลลิกรัมต่อลิตรสำหรับเหล็ก(III) ในการเตรียมแถบเทียบสีมาตรฐานด้วย
ตาเปล่า ศึกษาการรบกวนของไอออนโซเดียม ไอออนโพแทสเซียม ไอออนแคลเซียม และไอออน
แมกนีเซียมพบว่าความเข้มข้นของไอออนโซเดียม ไอออนโพแทสเซียม ไอออนแคลเซียม และไอออน
แมกนีเซียมที่สูงไม่มีผลรบกวนต่อการตรวจวัดด้วยตาเปล่าของโคบอลต์(II) นิกเกิล(II) และเหล็ก(III)
วิธีการตรวจวัดด้วยตาเปล่าที่น่าเสนอสามารถประยุกต์สำหรับการตรวจวัดโคบอลต์(II) นิกเกิล(II) และ
เหล็ก(III) ที่ระดับต่ำตามแหล่งน้ำต่าง ๆ โดยปราศจากการใช้เครื่องมือพิเศษ

ภาควิชา.....เคมี..... ลายมือชื่อนิสิต.....
สาขาวิชา.....เคมี..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....
ปีการศึกษา.....2555.....

5372406723 : MAJOR CHEMISTRY

KEYWORDS : NAKED-EYE DETECTION / HEAVY METAL IONS / XYLENOL ORANGE

PANIDA MONGKHONWUTTIKUN : NAKED-EYE DETECTION OF HEAVY METAL IONS AFTER FORMING COMPLEXES WITH XYLENOL ORANGE BY COLUMN METHOD. ADVISOR : ASST.PROF.WANLAPA AEUNGMAITREPIROM, Ph.D. 59 pp.

Preliminary naked-eye detection of heavy metal ions (Cd(II), Co(II), Cu(II), Ni(II), Pb(II), Zn(II), Fe(III) and Cr(III)) complexed with xylenol orange adsorbed onto silica gel by batch and column methods was investigated. The color change of all metal complexes in comparison with control was observed at pH 5 in acetate buffer changing from orange to red for Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II), to purple for Co(II) and Cr(III) and to black for Fe(III). The colors of only Ni(II), Co(II) and Fe(III) complexes at low concentration were obviously observed. The maximum f(R) signals measured by DR-UV-Vis spectrophotometer of metal complexes adsorbed onto silica gel were found at 580 nm for Co(II), Ni(II) and 595 nm for Fe(III). The formation ratio of the complex of Co(II), Ni(II) and Fe(III) and xylenol orange in aqueous solution was found to be 1:1 by Job's plot method. The conditions of Co(II), Ni(II) and Fe(III) naked-eye detection by the column method were optimized. The concentration of xylenol orange was 0.01 mM. The flow rate of 1 mL/min passed through a mini-column (4 mm o.d., 2 cm length) packed with 25 mg of silica gel was found. The maximum sample volumes were 50 mL for Co(II) and 100 mL for Ni(II) and Fe(III). The lowest concentration of Co(II), Ni(II) and Fe(III) detected by naked-eye were 0.6, 0.6 and 0.06 mg/L, respectively. The working concentration ranges of 0.1-60 mg/L for Co(II) and Ni(II) 0.1-120 mg/L for Fe(III) were used to prepare the naked-eye color calibrations. Interfering ions as Na⁺, K⁺, Ca²⁺ and Mg²⁺ were also studied. The results showed that high concentration of Na⁺, K⁺, Ca²⁺ and Mg²⁺ did not interfere the naked-eye detection of Co(II), Ni(II) and Fe(III). The proposed naked-eye method can be applied for determination of Co(II), Ni(II) and Fe(III) in various water sources at trace level without special instruments.

Department : Chemistry Student's Signature

Field of Study : Chemistry Advisor's Signature

Academic Year : 2012

ACKNOWLEDGEMENTS

I would like to express the deepest appreciation to my thesis advisor, Assistant Professor Dr. Wanlapa Aeungmaitrepirom for invaluable guidance and inspiration. Furthermore, I would like to thank Assistant Professor Dr. Warinthron Chavasiri, Assistant Professor Dr. Suchada Chuanuwatanakul and Dr. Nisakorn Thongkon for their suggestions and comments as thesis examiners.

This thesis could not be completed without the help and support of many people. I would like to thank the Environmental Analysis Research Unit for the facilities. I wish to express my most sincere thanks to Dr. Wanwisa Janrungroatsakul for all help and encouragement. This thesis was partially financial supported by the Thailand Research Fund (RTA 5380003) and Ratchadaphiseksompoj Endowment Fund Chulalongkorn University (GRU54-019-23-004), Department of Chemistry, Faculty of Science, Chulalongkorn University

Finally, I would like to thank my parent, my friends and others for their support and entirely care all the way.

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LIST OF ABBREVIATIONS

mg/L	Milligram per liter
µg/L	Microgram per liter
M	Molar
mM	Millimolar
ppm	Part per million
nm	Nanometer
cm	Centimeter
min	Minute
o.d.	Outer diameter
°C	Degree celsius
Ka	Acid dissociation constant
LOD	Limit of detection

CHAPTER I

INTRODUCTION

1.1 Statement of the problem

At this moment, rapid economic and technological development leads to higher polluted environment. Heavy metal is considered as one of the environment problems affecting organisms. Moreover, it is mostly found in various contaminated water resources. This contaminated water will cause the long-term effects upon human healthy. Thus, heavy metal accumulation within the body may be harmful to body systems, for example respiratory and immunity systems [1] or it may cause disorder in brain and nervous systems [2]. Therefore, the determination of heavy metal is important.

Generally, spectroscopic techniques such as flame atomic absorption spectrometry [3], inductively coupled plasma-atomic emission spectrometry (ICP-AES) [4] and inductively coupled plasma-mass spectrometry (ICP-MS) [5] are used in detecting heavy metals but these techniques require both complicated and expensive tools and the specialists.

Naked-eye detection has been used as the simple method for determination of heavy metals without any special tools. This technique bases on the change of color of ligand, either in solution or solid phase which occurs as a result of the complex. The complex occurs whenever the ligand binds to metal ion [6-7]. However, recent researches showed a limit of naked-eye detection which is an inability to detect heavy metal ion at trace level according to the regulation of United States Environmental Protection Agency (US EPA) [8-11].

Solid phase extraction (SPE) is widely used to separate and preconcentrate the target analyte because the modification of the surface by chemical immobilization or physical adsorption is simple. The main solid supports used in SPE are silica gel [12], alumina [13], naphthalene [14], Amberlite resin [15], and activated carbon [16]. Among these, silica gel is mostly used because of high surface area, the ability of

no swell or shrinks whenever exposed to solution in water, its heat and chemical resistant ability, and low price. Moreover, silica gel is widely used in solid phase extraction, both in batch and column systems. Lots of researchers preferred to use the column system [12, 17-18] because of high volume use and high preconcentration factor. These two reasons can improve the limit of detection.

In this research, xylenol orange is selected as a ligand. It gains the ability to bind with various metal ions [19-20] because it contains oxygen and nitrogen donor atoms which can form the complex with metal ions. Structure of xylenol orange is shown in Figure 1.1. The determination of heavy metal ions after forming complexes with xylenol orange adsorbed onto silica gel in column system was studied by naked-eye detection.

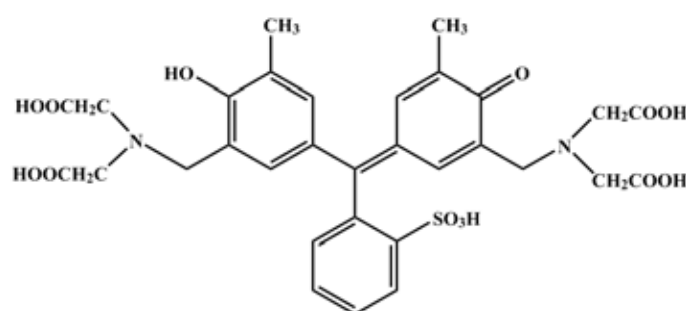


Figure 1.1 Structure of xylenol orange

1.2 Objective and scope of the research

To develop the naked-eye method for determination of metal ions after forming the complexes with xylenol orange in column system.

The naked-eye method was investigated in batch and column systems. In batch method, the preliminary naked-eye detection test of metal ions e.g. Cd(II), Co(II), Cu(II), Ni(II), Pb(II), Zn(II), Fe(III) and Cr(III) complexed with xylenol orange adsorbed onto silica gel were studied. In column method, parameters affected to naked-eye detection such as pH of the solution, concentration of xylenol orange, flow rate of solution, sample volume, naked-eye detection limit of heavy metal ions and interfering ions were studied. Finally, the proposed naked-eye method was

applied to determine some metal ions in water samples including Chulalongkorn University's pond water, Physics Building water, drinking water and tap water.

1.3 Benefit of the research

The lower limit of detection of the naked-eye method was obtained and this method can be used to determine metal ions at trace level in water resources.

CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Heavy metals in water

Heavy metals in water can enter and accumulate in human body which will be harmful for health both in short and long term. Therefore, the quality of water should be controlled to prevent danger from heavy metals in water. Pollution Control Department has regulated quality of drinking water, surface water, tap water, and waste water for Thailand [21] as shown in Table 2.1.

Table 2.1 Maximum acceptable concentration for heavy metals in water sources

Heavy metals	Maximum acceptable concentration (mg/L)			
	Drinking water	Surface water	Tap water	Wastewater
Cadmium	0.005	0.03	0.005	0.01
Chromium	0.05	0.75	0.05	0.05
Copper	1.0	2.0	0.1	1.0
Iron	0.3	-	0.5	-
Lead	0.05	0.2	0.05	0.05
Nickel	-	0.1	-	1.0
Zinc	5.0	5.0	1.0	5.0

Heavy metal in water is an indicator for quality control of water sources. Therefore, an analysis of heavy metals in water is important. This can determine heavy metals accumulated in water.

The amount of heavy metals enters to the environment from human activities and natural of processes. The concentration of heavy metal over the regulation limits leads to dangerous level for human health via drinking, eating and breathing. Human health effects of cobalt, iron and nickel are shown in Table 2.2.

Table 2.2 Heavy metal on human health effect

Metal	Symptoms	reference
Cobalt	<u>Inhalation</u> : reducing pulmonary function, asthma, interstitial lung disease and dyspnea in primarily respiratory system <u>Oral</u> : increasing in erythrocyte numbers or polocythemia <u>Dermal</u> : allergic dermatitis <u>Others</u> : thyroid and lung cancer	[22]
Iron	<u>Inhalation</u> : dizziness, irritation of the respiratory tract <u>Oral</u> : vomiting, nausea, abdominal pain and diarrhea <u>Dermal</u> : skin pigmentation lethargy <u>Others</u> : loss of weight and body hair, liver disease, parkinson's disease	[23-24]
Nickel	<u>Inhalation</u> : chronic bronchitis, emphysema, respiratory system disease and heart attack <u>Oral</u> : vomiting, diarrhea and oral cancer <u>Dermal</u> : allergic contact dermatitis <u>Others</u> : reducing body weight, liver damage, Anorexia, kidney dysfunction and lung cancer	[25]

2.2 Determination of heavy metals in water

Spectroscopic technique is widely used for the determination of heavy metals in water. The detection limits of flame atomic absorption spectrometry (FAAS) and inductively coupled plasma-atomic emission spectrometry (ICP-AES) are shown in Table 2.3 [26-27].

Table 2.3 Detection limits of FAAS and ICP-AES

Heavy metals	Detection limits ($\mu\text{g/L}$)	
	FAAS	ICP-AES
Cadmium	1	0.07
Chromium	4	0.08
Cobalt	9	0.2
Copper	2	0.04
Iron	6	0.09
Lead	8	1
Nickel	3	0.2
Zinc	1	0.1

These techniques give quite low detection limits and can be measured the concentration of analyte from low to high level (ppb to ppm) but tools are very expensive and require high maintenance. Utility operators of these tools must also have skills.

2.3 Naked-eye detection technique

Lots of researches reported the methods for heavy metal detections using FAAS and ICP-AES. Another technique required no special equipment for analysis is “Naked-eye detection”. This technique detects the color change by naked-eye when a ligand reacts with an interesting analyte as metal ion to form a colored complex. Examples of metal ions determination by naked-eye technique are summarized in Table 2.4. This technique is interesting because it is simple and can be applied on-site measurements. Additionally, this simple technique does not require skillful person. Qualitative and quantitative analysis by naked-eye detection can be applied for solution and solid systems. However, the solution system has to use organic solvents which cause expenses. Therefore, the solid system is more interesting.

Table 2.4 Determination of metal ions by naked-eye technique

Metal ions	Ligand	Color	Reference
Cu(II)	quinoline-indene derivertive	purple to orange	[28]
Fe(III)	1-(D-glucopyranosyl-2'-deoxy-2'-imiromethyl)-2H-chromen-2-one	yellow to red	[29]
Ca(II)	bis(spiropyran) podands	reddish purple to bluish purple	[30]
Ag(I)	mesitylene based azo-coupled chromogenic tripodal receptors	yellow to red	[31]
Pb(II)	diaminoanthraquinone-linked polyazamacrocycles	violet to pink	[32]
Zn(II)	hetarylazo derivative	red to faint orange	[33]
Hg(II)	azo coupled macrocyclic receptor on a silica nanotube (SNT)	yellow to violet	[34]
Cd(II)	4-(2-pyridylazo)resorcinol (PAR) on mesoporous silica anchored with TMAC	orange-yellow to light purple	[35]
As(V)	molybdenum adsorbed on the β -hydroxypropyl-di(β -hydroxyethyl)amine) (HDEA) resin	pale yellow to deep greenish blue	[36]
Hg(II), Pb(II), Cd(II), Zn(II), Ni(II) and Cu(II)	4-(2-pyridylazo)-1,3-benzenediol aminated polyacrylonitrile fibers (PAR-PAN _{EF})	red-orange to dark-brown	[37]

Solid supports such as paper strips [38], glass plates [39] and polymers [40] were used for the determination of heavy metals by naked-eye. Silica gel is often used in solid phase extraction for detecting heavy metals [41-42]. It is simple to modify its surface via chemical or physical interactions. Silica gel is an inorganic polymer. The main structure is siloxane (Si-O-Si). The surface of silica is silanol groups (Si-OH) in single, vicinal or germinal forms as shown in Figure 2.1. Silica gel was chosen for this research because of its good selectivity, lack of swelling, quick sorption of metal ions, and high surface area. In addition, it is a white solid. Therefore, the color of target analyte complex adsorbed onto silica gel can be detected by naked-eye without bias.

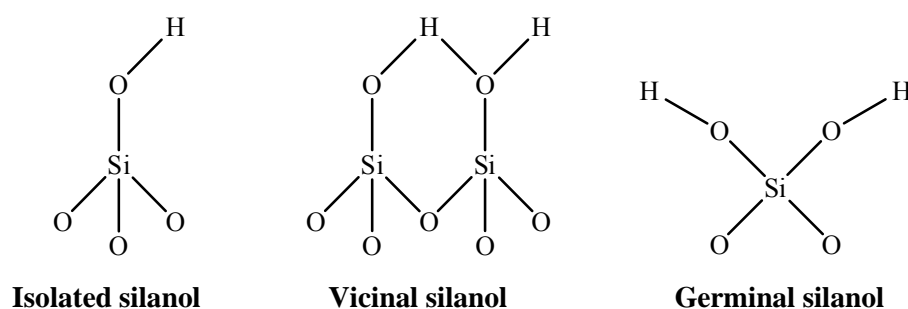


Figure 2.1 Structure of silica gel

Moreover, silica gel is widely used in batch and column systems. Column system is recommended because its sorption capacity is over batch system. The sorbent is contacted continuously by the fresh solution of initial solute concentration in column system. Therefore, the concentration of the solution in contact with a given layer of sorbent in a column remains almost constant. This procedure results in maximum loading of the sorbent at constant solute concentration and is in contrast to continuously declining solute concentration in batch method, thereby decreasing the effectiveness of the sorbent. In addition, column system is possible to use for applying to large sample volumes. These reasons are given the lower limit of detection.

2.4 Xylenol orange

Xylenol orange (XO) exists as H_9XO^{3+} , H_8XO^{2+} , H_7XO^+ , H_6XO , H_5XO^- , H_4XO^{2-} , H_3XO^{3-} , H_2XO^{4-} , HXO^{5-} and XO^{6-} depending on solution pH. The distribution of species of xylenol orange and structures of some xylenol orange species in acidic solution are shown in Figure 2.2 and 2.3 respectively.

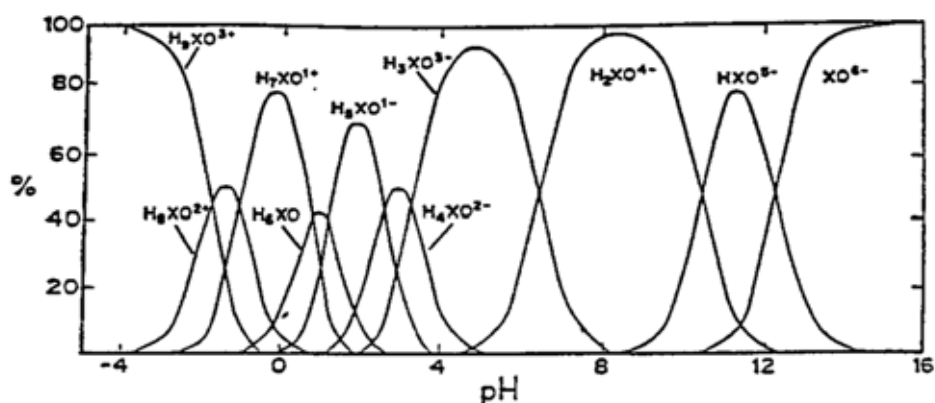


Figure 2.2 Distribution of xylenol orange species at various pH [43]

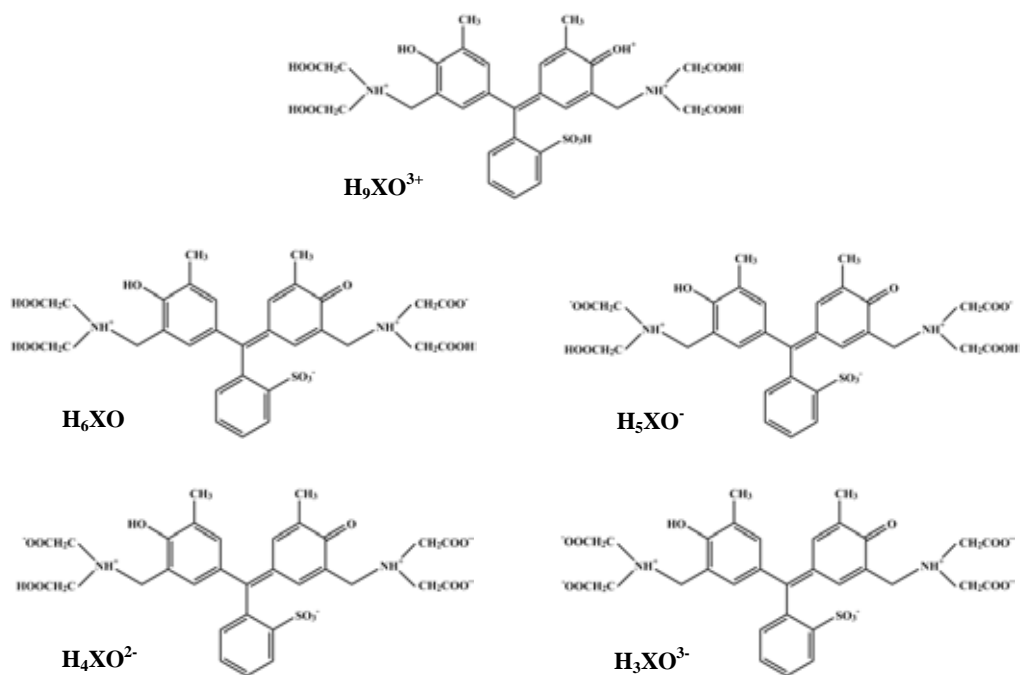


Figure 2.3 Structures of some xylenol orange species in acidic solution

In acidic solution (pH 1-5), H_6XO , H_5XO^- , H_4XO^{2-} and H_3XO^{3-} become the dominant species of xylenol orange attributed to the equilibria and its pKa as shown in Table 2.5.

Table 2.5 Acidic dissociation reaction of xylenol orange species and its pKa
(t = 25°C, $\mu = 0.1$, KNO_3) [44]

Reaction		pKa
H_6XO	$\rightleftharpoons H_5XO^- + H^+$	0.76
H_5XO^-	$\rightleftharpoons H_4XO^{2-} + H^+$	1.15
H_4XO^{2-}	$\rightleftharpoons H_3XO^{3-} + H^+$	2.58
H_3XO^{3-}	$\rightleftharpoons H_2XO^{4-} + H^+$	3.23

Xylenol orange is a metallochromic indicator which forms colored complex with various metal ions. The colors of metal-xylenol orange complexes are shown in Table 2.6 [45].

Table 2.6 Colors of metal-xylenol orange complexes

Metal ions	Color of complex
Cd(II)	Violet
Cr(III)	Red
Fe(III)	Purple
Hg(II)	Violet
Ni(II)	Violet
Pb(II)	Violet
Zn(II)	Violet

Xylenol orange was used as a chelating ligand modified onto various solid materials for determination of heavy metal ions as shown in Table 2.7. From these researches, xylenol orange has been used for extraction of various heavy metal ions but it was not used to detect the heavy metal on solid support by naked-eye. Therefore, xylenol orange was selected to use as a chelating ligand in this research.

Table 2.7 Xylenol orange modified on sorbents for heavy metal ions determinations

Metal ions	Solid material	Reference
Hg(II)	silica gel	[18]
Pb(II)	amberlite XAD-7	[46]
Ni(II)	nanocrystalline ZnO thin film	[47]
Cd(II)	amberlite XAD-2	[48]
Pb(II)	activated carbon	[49]
U(VI)	silica gel	[50]
Cd(II), Co(II), Cu(II), Fe(III)	hydrophobic silica (C ₁₆ , C ₈ , phenyl and C ₁)	[51]
Cd(II), Co(II), Cu(II), Ni(II), Zn(II), Fe(III)	amberlite XAD-7	[52]
Cu(II), Ni(II), Co(II), Pb(II), Fe(III), Cd(II)	amberlite XAD-16	[53]

2.5 Hard-soft acid base

The binding preferences of metal ions with ligand are predicted by the principle of hard-soft acids bases (HSAB). Table 2.8 shows a list of hard-soft acid base natures of main group of metal ions [54]. The binding selectivity of metal ions for ligands depends on charge, size and donor atoms of ligand. In generally, hard acids can bind to hard bases and soft acids to soft bases.

Table 2.8 List of hard-soft acid base

	Hard	Borderline	Soft
Lewis acids	H^+ , Li^+ , Na^+ , K^+ Be^{2+} , Mg^{2+} , Ca^{2+} , Sr^{2+} , Mn^{2+} Al^{3+} , Sc^{3+} , Ga^{3+} , In^{3+} La^{3+} , Gd^{3+} , Lu^{3+} Cr^{3+} , Co^{3+} , Fe^{3+} , As^{3+} , H_3Sn^{3+}	Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Pb^{2+} , Sn^{2+} , Sb^{3+} , Bi^{3+} Rh^{3+} , Ir^{3+} , SO_2 Ru^{2+} , Os^{2+} , GaH_3	Cu^+ , Ag^+ , Au^+ , Tl^+ , Hg^+ Pd^{2+} , Cd^{2+} , Pt^{2+} , Hg^{2+} CH_3Hg^+ , $Co(CN)_5^{2-}$, Pt^{4+} , Te^{4+}
Lewis bases	H_2O , OH^- , F^- CH_3COO^- , PO_4^{3-} , SO_4^{2-} , Cl^- , CO_3^{2-} , ClO_4^- , NO_3^- ROH , RO^- , R_2O NH_3 , RNH_2 , N_2H_4	$C_6H_5NH_2$, C_5H_5N , Br^- , NO_2^- , SO_3^{2-}	R_2S , RSH , RS^- I^- , SCN^- , $S_2O_3^{2-}$ R_3P , R_3As , $(RO)_3P$ CN^- , RNC , CO C_2H_4 , C_6H_6 , H^- , R^-

CHAPTER III

EXPERIMENTAL

3.1 Apparatus

- **Inductively coupled plasma-optical emission spectrometer (ICP-OES)**

Inductive coupled plasma-optical emission spectrometer model iCAP 6000 series (Thermo fisher scientific) was used for determination of metal ions in water samples. The operating conditions are shown in Table 3.1.

Table 3.1 Operating conditions for ICP-OES

Operating conditions	Co	Ni	Fe
Wavelength (nm)	228.6	221.6	259.9
RF power (W)	1150	1150	1150
Pump rate (rpm)	50	50	50
Auxiliary gas flow (L/min)	0.5	0.5	0.5
Nebulizer gas flow (L/min)	0.60	0.60	0.60
Coolant gas flow (L/min)	12	12	12

- **Diffuse reflectance ultraviolet visible spectrophotometer (DR-UV-Vis)**

Reflectance spectra of xylenol orange and complex adsorbed onto silica gel were recorded by a diffuse reflectance ultraviolet visible spectrophotometer (DR-UV-Vis) model UV-2500PC (Shimadzu).

- **Ultraviolet visible spectrophotometer (UV-Vis)**

The mole ratio of xylene orange and metal ion was determined by an ultraviolet visible spectrophotometer (UV-Vis) model HP 8453 (Hewlett packard).

- **Peristaltic pump**

A peristaltic pump was used to control a flow rate of solution in a column system (REGLO Analog MS-4/8 model ISM 827 ISMATEC®).

- **pH meter**

A Metrohm pH meter model 744 was used to measure the solution pH.

3.2 Chemicals

All experiment chemicals were analytical grade and used without any purification. The chemicals are listed in Table 3.2.

Table 3.2 Chemicals in the experiment

Chemicals	Supplier
Acetic acid	Merck
Calcium nitrate	Sigma-aldrich
Glycine	Sigma-aldrich
Hydrochloric acid 37%	Merck
Magnesium chloride	Merck
Nitric acid 65%	Merck
Potassium nitrate	BDH
Silica gel	Merck
Sodium acetate	Carlo Erba
Sodium chloride	Carlo Erba

Table 3.2 Chemicals in the experiment (continued)

Chemicals	Supplier
Xylenol orange	Fluka
Standard solution Co(II) (1000 mg/L)	BDH
Standard solution Ni (II) (1000 mg/L)	Merck
Standard solution Fe (III) (1000 mg/L)	Merck
Cadmium nitrate	Merck
Chromium nitrate	Sigma-aldrich
Cobalt nitrate	BDH
Copper nitrate	Merck
Ferric ammonium sulfate	Carlo Erba
Lead nitrate	May & Baker Ltd
Nickel nitrate	Merck
Zinc nitrate	Merck

3.3 Purification of silica gel

Because metal ions can be adsorbed onto the surface of silica gel from silica gel production process, the purification of silica gel before use is necessary. The purification of silica gel was performed according to a method reported by Fan et al. [55]. Metal ions were removed by soaking silica gel in 6 M hydrochloric acid for two days. Silica gel was filtered and cleaned by Milli-Q water until the neutral solution was obtained. Silica gel was then dried at 100 °C in an oven for 12 hours. It was kept into a desiccator before use.

3.4 Preparation of solutions

All solutions in this experiment were prepared in Milli-Q water.

- **Metal ion standard solutions**

0.1 M of each metal ion standard solutions (Cd(II), Co(II), Cu(II), Ni(II), Pb(II), Zn(II), Fe(III) and Cr(III)) were prepared from an exact weight of metal salt dissolved in Milli-Q water.

Stock standard solutions (Co(II), Ni(II) and Fe(III)) were diluted from the solution of 1000 mg/L to appropriate concentrations in Milli-Q water for ICP-OES method.

- **Xylenol orange solution**

Xylenol orange sodium salt was freshly dissolved by Milli-Q water.

- **Acetate buffer**

0.1 M acetate buffer pH 3-5 was prepared from an amount of sodium acetate and acetic acid in Milli-Q water.

- **Glycine buffer**

0.1 M glycine buffer pH 1-2 was prepared from an amount of glycine and hydrochloric acid in Milli-Q water.

3.5 Preliminary study for metal ions naked-eye detection in batch method

Silica gel (25 mg) was added into 200 μ L of solution containing 0.5 mM xylenol orange and 10^{-2} M Cd(II) under pH ranging from 1 to 5 in a well-plate. After 1 day, the color change of silica gel was detected by naked-eye in comparison with a control without Cd(II). The experiments were performed in the same manner using Co(II), Cu(II), Ni(II), Pb(II), Zn(II), Fe(III) and Cr(III).

3.6 Column method

PVC tube was used as a mini-column (4 mm o.d., 2 cm length). 25 mg of silica gel was packed into the mini-column for all experiments.

3.6.1 Effect of pH

5 mL of solution containing 0.5 mM xylenol orange and 10^{-2} M individual metal salt solution (Cd(II), Co(II), Cu(II), Ni(II), Pb(II), Zn(II), Fe(III) or Cr(III)) at pH 3-5 was passed through a mini-column by a peristaltic pump at a flow rate of 0.5 mL/min. A control solution was performed in the same manner without metal ion. The colors of the complexes and the control solution adsorbed onto silica gel were detected by naked-eye.

3.6.2 Effect of concentration of xylenol orange

Five concentrations of xylenol orange (0.001, 0.005, 0.01, 0.05 and 0.1 mM) were tested with 10^{-5} M of individual metal salt solution e.g. Cd(II), Co(II), Cu(II), Ni(II), Pb(II), Zn(II), Fe(III) and Cr(III) at pH 5. 10 mL of solution was allowed through a mini-column at a flow rate of 0.5 mL/min. A control solution was performed in the same manner without metal ion. The colors of the complexes and the control solution adsorbed onto silica gel were detected by naked-eye.

3.6.3 Effect of flow rate

The flow rate of solution was studied from 0.5 to 5 mL/min using 0.01 mM of xylenol orange and 10^{-5} M of individual metal salt solution (Co(II), Ni(II) or Fe(III)) at pH 5. 10 mL of solution was allowed to a mini-column by a peristaltic pump. A control solution was performed in the same manner without metal ion. The colors of the complexes and the control solution adsorbed onto silica gel were detected by naked-eye.

3.6.4 Effect of sample volume

The volume of sample solution was varied from 10 to 100 mL spiked with 10 μ L of 10^{-2} M for Co(II), Ni(II) and Fe(III) at pH 5 using 0.01 mM of xylenol orange. The solution was passed through a mini-column by a peristaltic pump at a flow rate of 1.0 mL/min. A control solution was performed in the same manner without metal ion. The colors of the complexes and the control solution adsorbed onto silica gel were detected by naked-eye.

3.6.5 Naked-eye detection limit for Co(II), Ni(II) and Fe(III)

In this experiment, the naked-eye detection limit of individual metal salt solution (Co(II), Ni(II) or Fe(III)) was investigated by varying the concentration of metal ion between 10^{-7} to 10^{-4} M complexed with 0.01 mM of xylenol orange in 10 mL of solution at pH 5 compared with a control. This solution was allowed to a mini-column at a flow rate of 1.0 mL/min. A control solution was performed in the same manner without metal ion. The colors of the complexes and the control solution adsorbed onto silica gel were detected by naked-eye. The naked-eye detection limit of metal ions was considered.

3.6.6 Effect of interfering ions

The naked-eye detection of Co(II) in the presence of Ni(II) or Fe(III) as an interfering ion was studied. 10 mL of solution containing 0.01 mM of xylenol orange, 10^{-5} M of Co(II) and 3×10^{-6} , 10^{-5} , 10^{-4} M for Ni (II) (or 10^{-6} , 10^{-5} , 10^{-4} M for Fe(III)) at pH 5 was passed through a mini-column at a flow rate of 1.0 mL/min. A control solution was performed in the same manner without metal ion. The color of the complex in the presence of the interfering ion adsorbed onto silica gel was detected by naked-eye compared with the color of the control solution and the color of the complex in the absence of the interfering ions. The naked-eye detection of Ni(II) in the presence of Co(II) or Fe(III) and the naked-eye detection of Fe(III) in the presence

of Co(II) and Ni(II) were also studied in the similar manner described above using the concentrations of metal ions as follows:

<u>target metal ion</u>	<u>interfering ion</u>
Ni(II) : 10^{-5} M	Co(II) : 3×10^{-6} , 10^{-5} , 10^{-4} M Fe(III) : 10^{-6} , 10^{-5} , 10^{-4} M
Fe(III) : 10^{-5} M	Co(II) : 3×10^{-6} , 10^{-5} , 10^{-4} M Ni(II) : 3×10^{-6} , 10^{-5} , 10^{-4} M

Na^+ , K^+ , Ca^{2+} and Mg^{2+} as interfering ions were spiked into the sample solution at four concentrations (0.6, 10, 100 and 1000 mg/L). 10 mL of solution containing 0.01 mM xylenol orange and 10^{-5} M of individual metal salt solution (Co(II), Ni(II) or Fe(III)) at pH 5 was passed through a mini-column by a peristaltic pump at a flow rate of 1.0 mL/min. A control solution was performed in the same manner without metal ion. The color of the complex in the presence of the interfering ion adsorbed onto silica gel was detected by naked-eye compared with the color of the control solution and the color of the complex in the absence of the interfering ions.

3.7 Application to real samples

The real water samples used in this work were Chulalongkorn University's pond water, Physics building water, drinking water and tap water. All real water samples were filtered through 0.45 μm cellulose membrane except drinking water and kept in polyethylene bottle. They were adjusted the pH with nitric acid solution until pH = 3. The concentrations of Co(II), Ni(II) and Fe(III) in real water samples were determined by ICP-OES. The standard solutions of Co(II), Ni(II) and Fe(III) for ICP-OES calibration curve were prepared at 0, 0.5, 1, 5, 10 and 15 mg/L. The operating conditions of ICP-OES for Co(II), Ni(II) and Fe(III) are shown in Table 3.1. The standard solution of Co(II), Ni(II) and Fe(III) for the naked-eye color calibrations were prepared at 0, 0.2, 0.6, 1.2, 6, 12 and 60 mg/L for Co(II), Ni(III) and 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 6, 12, 20, 40, 80 and 120 mg/L for Fe(III).

The determination of Co(II), Ni(II) and Fe(III) in real water samples by naked-eye method were divided into two procedures:

Procedure I, 10 mL of a real water sample containing 0.01 mM of xylenol orange at pH 5 was passed through a mini-column at a flow rate of 1.0 mL/min. A control solution was performed in the same manner without metal ion. The colors of the complexes and the control solution adsorbed onto silica gel were detected by naked-eye compared with the color calibration of each concentration of metal ion.

Procedure II, 10 mL of a real water sample containing 0.01 mM of xylenol orange spiked with 10^{-5} and 10^{-4} M of an individual metal salt solution was adjusted pH to 5. This sample solution was passed through a mini-column at a flow rate of 1.0 mL/min. A control solution was performed in the same manner without metal ion. The colors of the complexes and the control solution adsorbed onto silica gel were detected by naked-eye compared with the color calibration of each concentration of metal ion.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Preliminary study for metal ions naked-eye detection in batch method

The preliminary naked-eye detection test of metal ions e.g. Cd(II), Co(II), Cu(II), Ni(II), Pb(II), Zn(II), Fe(III) and Cr(III) complexed with xylenol orange adsorbed onto silica gel were investigated at pH 1-5 by batch method. After adding silica gel into the solutions, colors of complex adsorbed onto silica gel were detected and the results are shown in Table 4.1.

From Table 4.1, the color of complexes adsorbed onto silica gel was observed by naked-eye in comparison with control. The color of xylenol orange at pH 1-5 adsorbed onto silica gel at different pH was orange. The color change of only Fe(III) was observed at pH 1. The color change of Co(II), Fe(III) and Cr(III) complexes were observed at pH 2. At pH 3-5, the color of Zn(II) complex, Cd(II) complex and Co(II), Cu(II), Ni(II), Pb(II), Cr(III) complexes were pink, red and purple, respectively while the color of Fe(III) complex was black. As the results, xylenol orange can bind with several metal ions at pH 3-5. Therefore, pH 3-5 was selected to study the metal ions naked-eye detection by column method in next experiment.

Table 4.1 Colors of complex adsorbed onto silica gel at pH 1-5

Metal ions	pH 1	pH 2	pH 3	pH 4	pH 5
Control					
Cd(II)					
Co(II)					
Cu(II)					
Ni(II)					
Pb(II)					
Zn(II)					
Fe(III)					
Cr(III)					

Xylenol orange (XO) = 0.5 mM, metal ion = 10^{-2} M in glycine buffer at pH 1-2 and acetate buffer at pH 3-5.

4.2 Column method





















A mini-column (4 mm o.d., 2 cm length) was made from PVC tube and filled with 25 mg of silica gel. Parameters such as pH of the solution, concentration of xylenol orange, flow rate of solution, sample volume and interference ions were studied in column method. The detection limit of naked-eye method was also investigated.

4.2.1 Effect of pH

The pH of solution is an important parameter which can affect on the complexation because the oxygen donor sites of ligand can be protonated in acidic solution and the precipitation of metal ions can appear in basic solution.

The solution of xylenol orange and metal ion was passed through a mini-column at a flow rate controlled by a peristaltic pump. Effect of pH on the colors of complexes at pH 3-5 are shown in Table 4.2. It was found that the color change of Co(II), Ni(II), Pb(II), Zn(II), Fe(III), Cr(III) complexes at pH 3 in comparison with control was observed while no color change of Cd(II) and Cu(II) complexation was obtained. The colors of all metal ion complexes adsorbed onto silica gel were observed at pH 4 and 5 to change from orange to red for Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II), from orange to purple for Co(II) and Cr(III) and from orange to black for Fe(III). The color change of complex was obviously observed at pH 5 more than pH 3 and 4 in comparison with control. As described in section 2.4 Chapter II, the percentages of H_3XO^{3-} and H_4XO^{2-} at pH 4 are 80% and 20%, respectively and the percentages of H_2XO^{4-} , H_3XO^{3-} and H_4XO^{2-} at pH 3 are 25%, 50% and 25% while H_3XO^{3-} is more than 90% at pH 5.

Table 4.2 Colors of metal ion complexes adsorbed onto silica gel at pH 3-5

Metal ions	pH 3	pH 4	pH 5
Control			
Cd(II)			
Co(II)			
Cu(II)			
Ni(II)			
Pb(II)			
Zn(II)			
Fe(III)			
Cr(III)			

Xylenol orange (XO) = 0.5 mM, metal ions = 10^{-2} M in acetate buffer at pH 3-5, flow rate 0.5 mL/min, sample volume = 10 mL.

At pH 5, xlenol orange gives four coordinating sites of carboxylate groups to bind with metal ion. The formation of 1:1 or 1:2 complexes are probably occurred as shown in Figure 4.1.

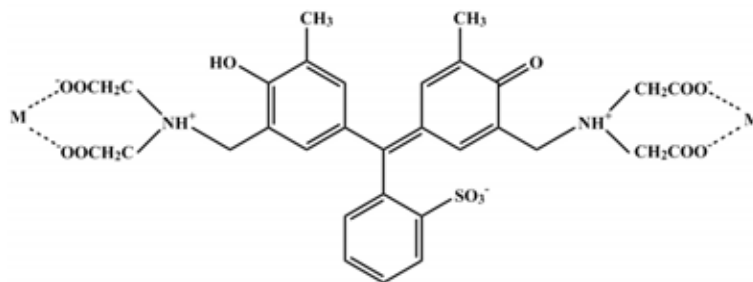
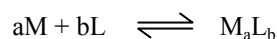


Figure 4.1 Possible formation of 1:1 and 1:2 complexes

Job's plot method was used to determine the stoichiometry of the complex in a solution. In general, the metal-ligand complex is written as the following equilibrium:



The ratio of the mole fraction of metal ion (X_M) and the mole fraction of ligand (X_L) is defined as:

$$\frac{X_M}{X_L} = \frac{a}{b}$$

$$X_M = n_M / n_M + n_L$$

$$X_L = n_L / n_M + n_L$$

$$X_M + X_L = 1$$

when X_M = mole fraction of metal ion

X_L = mole fraction of ligand

n_M = mole of metal ion

n_L = mole of ligand

The formula of metal-ligand complex is obtained from a plot of a maximum absorbance against a mole fraction of metal ion (n_M) or ligand (n_L) as shown in Figure 4.2 [56].

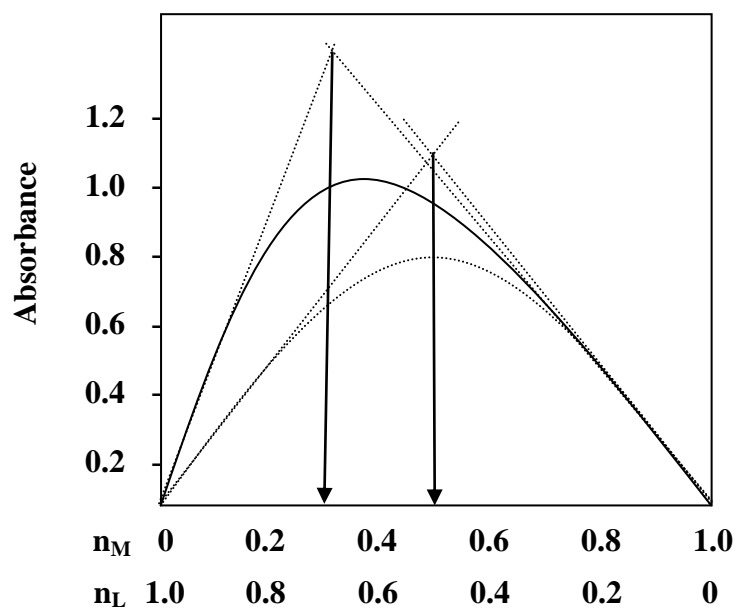


Figure 4.2 Job's plot method for formula of complex ML and ML₂

In this work, the stoichiometry of the complex between only Co(II), Ni(II) and Fe(III) with xylenol orange in solution was studied. 5×10^{-5} M of xylenol orange and 5×10^{-5} M of individual metal salt solution (Co(II), Ni(II) or Fe(III)) at pH 5 were mixed into the total volume of 3 mL. Volume ratios of the mixtures are shown in Table 4.3. The absorbances of the mixtures were measured by UV-Visible spectrophotometer in a range of 200–800 nm and the maximum absorption was recorded at the wavelength of 580 nm for Co(II) and Ni(II) and at 595 nm for Fe(III). The stoichiometry of the complex was obtained from the plot of maximum absorbance against the mole fraction of metal ion.

Table 4.3 Volume ratios of xylenol orange and metal ion solutions

Volume of xylenol orange (mL)	Volume of metal ion (mL)	Mole fraction of xylenol orange and metal ions ($X_{XO} : X_M$)
3.0	0	1.0 : 0
2.7	0.3	0.9 : 0.1
2.4	0.6	0.8 : 0.2
2.1	0.9	0.7 : 0.3
1.8	1.2	0.6 : 0.4
1.5	1.5	0.5 : 0.5
1.2	1.8	0.4 : 0.6
0.9	2.1	0.3 : 0.7
0.6	2.4	0.2 : 0.8
0.3	2.7	0.1 : 0.9
0	3.0	0 : 1.0

The plots of absorbance of the complex against the mole fraction of metal ions are shown in Figure 4.3-4.5 for Co(II), Ni(II) and Fe(III), respectively. The absorbance (A) was calculated by the equation as following:

$$\text{Absorbance (A)} = (A - A_0) \times (1 - X_M)$$

when A = absorbance of metal complex

A_0 = absorbance of ligand

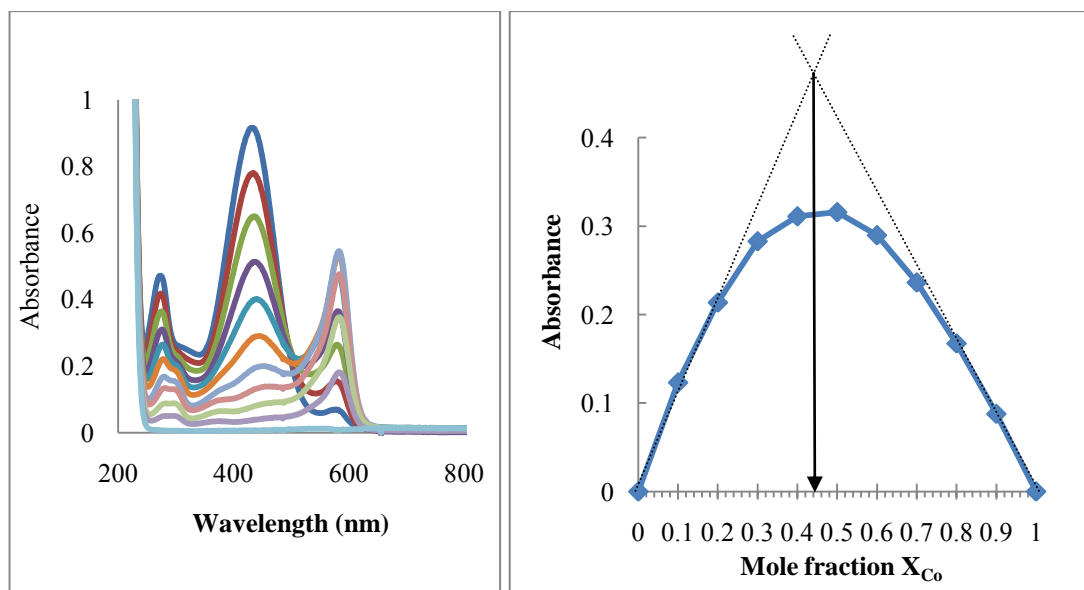


Figure 4.3 UV-Vis spectra and Job's plot of Co(II)-XO complex

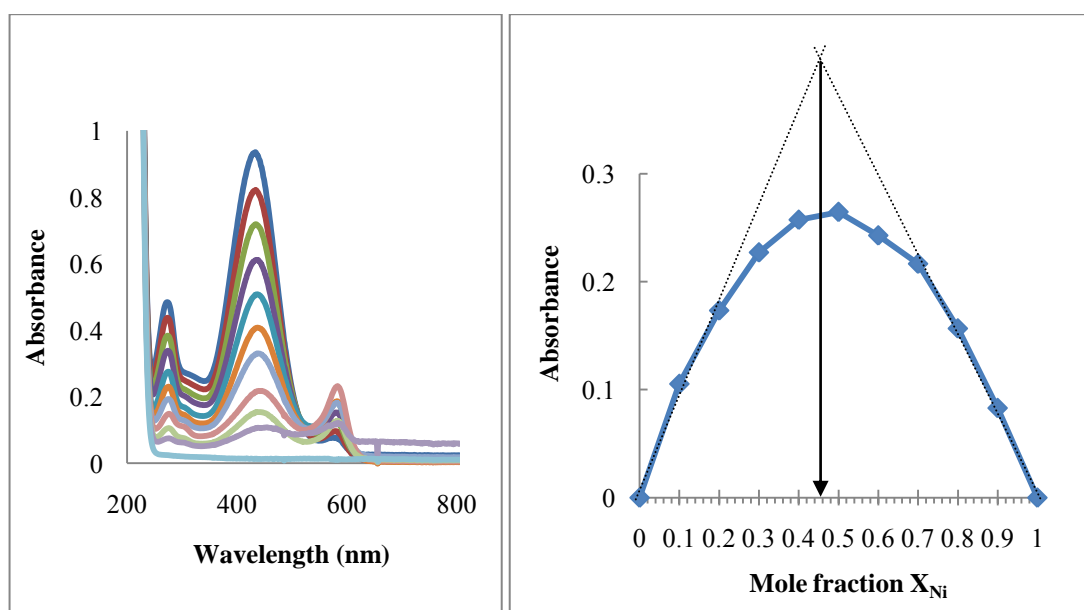


Figure 4.4 UV-Vis spectra and Job's plot of Ni(II)-XO complex

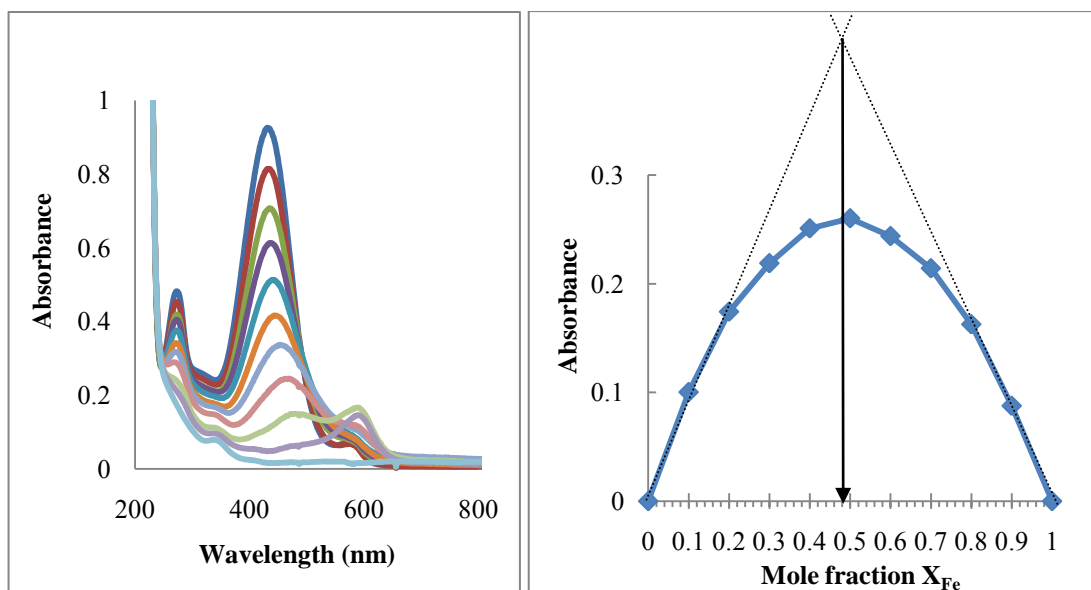


Figure 4.5 UV-Vis spectra and Job's plot of Fe(III)-XO complex

The intersection of two lines was a ratio of mole fraction of metal ion and xylenol orange. It was found that the stoichiometry of metal-xylenol orange complex was 1:1 for Co(II), Ni(II) and Fe(III). Therefore, the proposed structure of Co(II), Ni(II) and Fe(III)-xylenol orange complexes is shown in Figure 4.6.

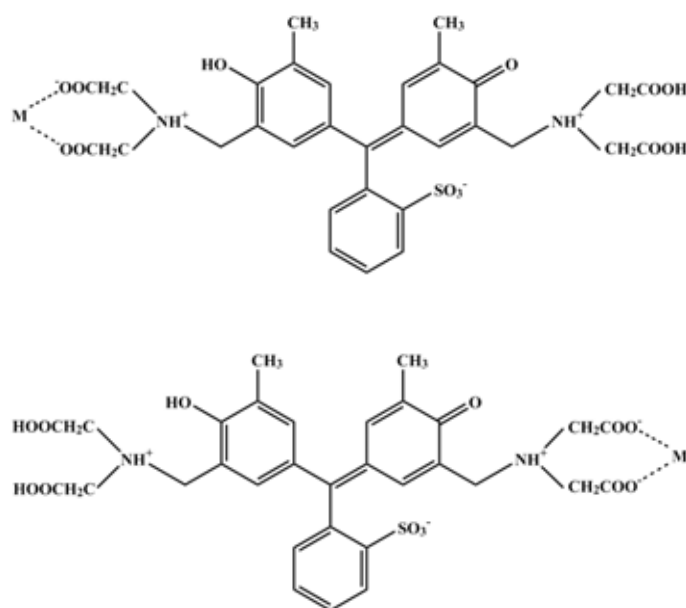


Figure 4.6 Proposed structure of metal complex

The possible interactions between silica gel and metal complexes as shown in Figure 4.7 was the hydrogen bonding between silanol groups and carboxylate groups of xylenol orange.

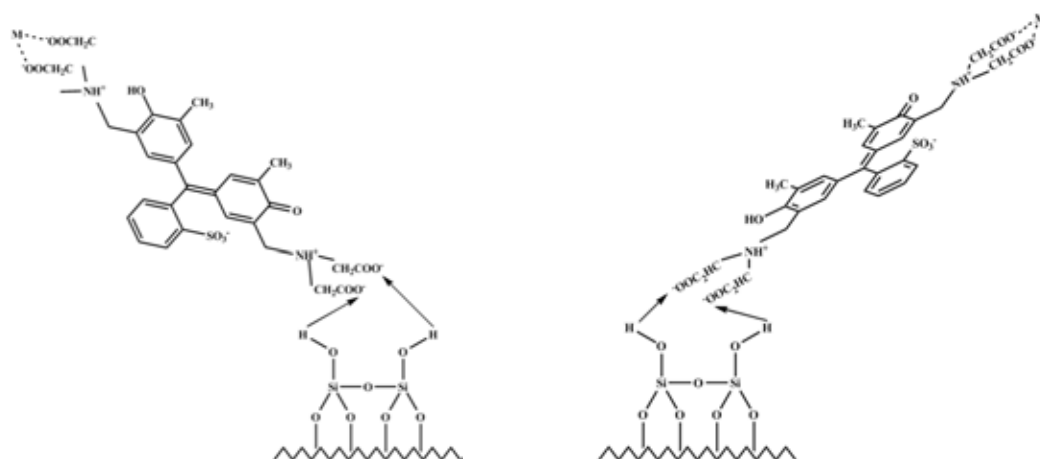


Figure 4.7 Possible interactions between silica gel and metal complexes

Moreover, the metal complexes of Co(II), Ni(II) and Fe(III) adsorbed onto silica gel was measured by diffuse reflectance ultraviolet visible spectrometric technique (DR-UV-Vis). The DR-UV-Vis spectra of the complexes were recorded at wavelength 200-800 nm in comparison with silica gel and silica gel-xylenol orange (Si-XO). The spectra of sample and reference standard were plotted between Kubelka-Munk function; $F(R)$ and wavelength when $F(R)$ was calculated from the following equation [57]:

$$F(R) = \frac{(1-R)^2}{2R} = \frac{k}{s}$$

- when $F(R)$ = Kubelka-Munk function
 R = reflectance of sample
 k = absorption coefficient
 s = scattering coefficient

From Figure 4.8-4.10, the maximum absorption of xylenol orange onto silica gel was found at 468 nm corresponding to di(carboxymethyl)aminomethyl group [58] while the maximum absorption of metal complexes on silica gel was shifted from 468 nm to 580, 580 and 595 nm for Co(II), Ni(II) and Fe(III), respectively indicating that the maximum absorption wavelength was shifted by the coordination between xylenol orange and metal ions. As the results, the color of Si-XO-Co(II) changed from orange-brown to purple, the color of Si-XO-Ni(II) changed from orange-brown to purple and the color of Si-XO-Fe(III) changed from orange-brown to purple-blue.

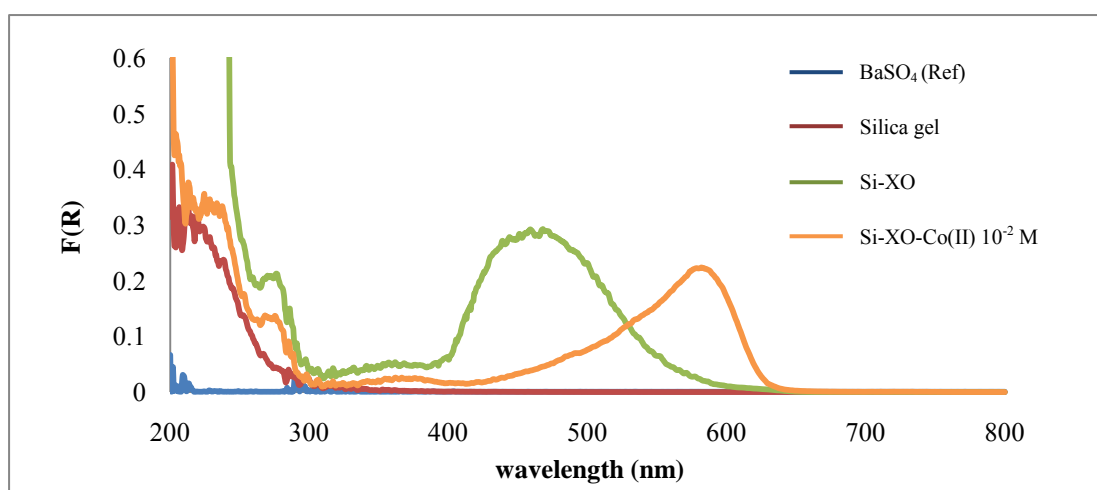


Figure 4.8 DR-UV-Vis spectra of Co(II) complex onto silica gel at pH 5 compared with silica gel and silica gel-xylenol orange (Si-XO)

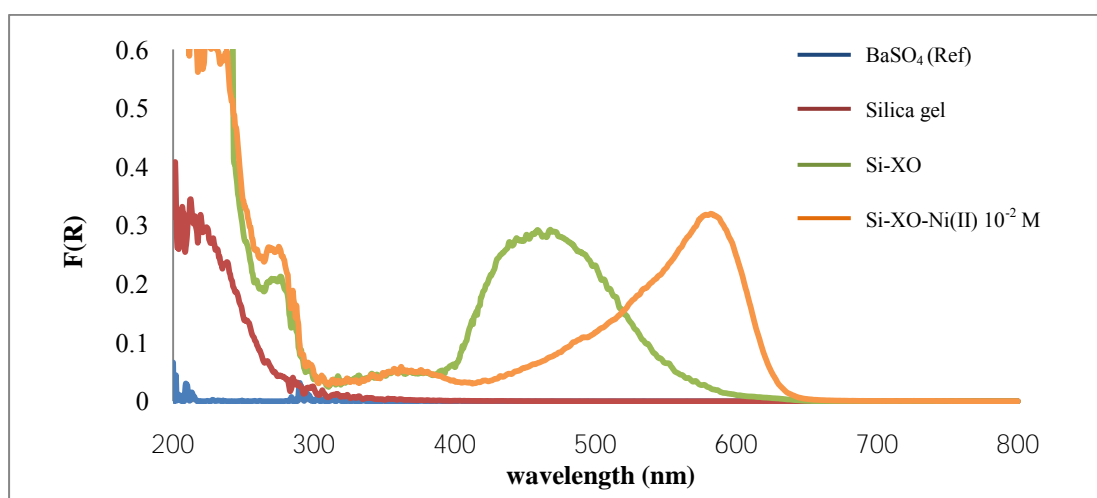


Figure 4.9 DR-UV-Vis spectra of Ni(II) complex onto silica gel at pH 5 compared with silica gel and silica gel-xylenol orange (Si-XO)

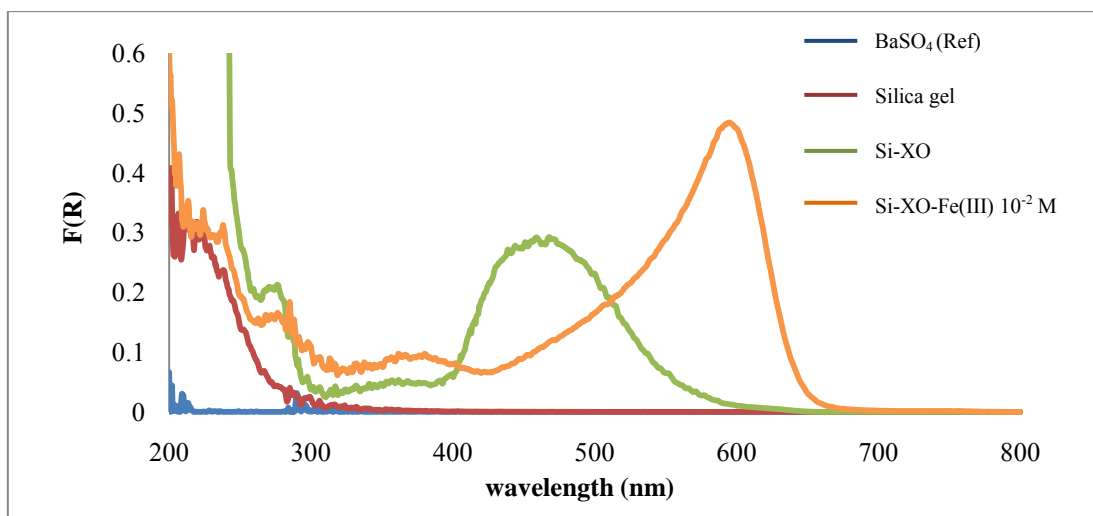















































Figure 4.10 DR-UV-Vis spectra of Fe(III) complex onto silica gel at pH 5 compared with silica gel and silica gel-xylenol orange (Si-XO)

4.2.2 Effect of concentration of xylenol orange

The concentration of xylenol orange affected to the naked-eye detection at low level concentration of metal ions. Five concentrations of xylenol orange (0.001, 0.005, 0.01, 0.05 and 0.1 mM) were tested with Cd(II), Co(II), Cu(II), Ni(II), Pb(II), Zn(II), Fe(III) and Cr(III) at pH 5. From Table 4.4, the results showed that the colors of Co(II), Ni(II) and Fe(III) complexes were obviously changed from brown-orange to red-orange, pink and purple-blue, respectively when 0.01 mM of xylenol orange was used. However, no color changes of Cd(II), Cu(II), Pb(II), Zn(II) and Cr(III) complexes at all concentrations of xylenol orange were appeared. The color of complexes may be masked from the color of xylenol orange at the high concentration of xylenol orange. So, it is indistinct. The intensity of the color of xylenol orange at the low concentration was less than that of high concentration resulting in less colored complex. Therefore, 0.01 mM of xylenol orange and only Ni(II), Co(II) and Fe(III) were selected for further studies due to the colors of Ni(II), Co(II) and Fe(III) complexes were observed obviously at low concentration while no color of other metal complexes was obtained.

Table 4.4 Effect of concentration of xylenol orange for metal ion complexation at pH 5

Metal ions	Concentration of XO (mM)				
	0.001	0.005	0.01	0.05	0.1
Control					
Cd(II)					
Co(II)					
Cu(II)					
Ni(II)					
Pb(II)					
Zn(II)					
Fe(III)					
Cr(III)					



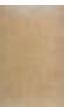













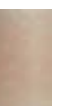







Metal ions = 10^{-5} M, flow rate = 0.5 mL/min, pH = 5, sample volume = 10 mL.

4.2.3 Effect of flow rate

The flow rate of the solution affected to the complex adsorbed onto silica gel. Thus, the adsorption of the complex onto silica gel depended on time. The complex adsorbed onto silica gel at high flow rate was poor adsorption. It should use short-time analysis but the contact time may not be sufficiency to entire adsorption. In contrast, the complex adsorbed onto silica gel at low flow rate was good adsorption resulting in a good contact time but it increased analysis time.

The flow rate of solution was studied between 0.5 to 5 mL/min. The colors of complexes in function of flow rate are shown in Table 4.5. In this experiment, the colors of Co(II) and Ni(II) complexes adsorbed onto silica gel were obviously observed in comparison with only xylenol orange adsorbed onto silica gel at the flow rate of 0.5 and 1 mL/min while the color of Fe(III) complex was clearly observed at the flow rate of 0.5-5 mL/min. This indicated that the colors of all metal complexes distinctly appeared on silica gel at the flow rate of 0.5 and 1 mL/min. Thereby, the flow rate of 1 mL/min was selected for further experiments due to shortening-time analysis.

Table 4.5 Effect of flow rate on the color of the complexes















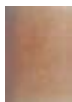





Metal ions	Flow rate (mL/min)					
	0.5	1	2	3	4	5
Control						
Co(II)						
Ni(II)						
Fe(III)						

Xylenol orange (XO) = 0.01 mM, metal ions = 10^{-5} M, pH = 5, sample volume = 10 mL.

4.2.4 Effect of sample volume

A large sample volume was mostly used in the column system to obtain high preconcentration factor. In this experiment, the sample volume was varied from 10 to 100 mL. An amount of metal ions (Co(III), Ni(II) or Fe(III)) was spiked into different volumes of sample solution. The colors of complexes obtained in the mini-columns are shown in Table 4.6.

Table 4.6 Effect of sample volume on the color of the complexes

Metal ions	Sample volume (mL)				
	10	25	50	75	100
Control					
Co(II)					
Ni(II)					
Fe(III)					

Xylenol orange (XO) = 0.01 mM, metal ions = 10^{-5} M, flow rate = 1.0 ml/min, pH = 5.

The results showed that the colors of complexes were detected by naked-eye until the maximum sample volume was 50 mL for Co(II) and 100 mL for Ni(II) and Fe(III). However, The faded color of Co(II), Ni(II) and Fe(III) complexes was observed when the sample volume was increased, indicating that the metal ion was desorbed from silica gel to the solution. This is called break through volume. Therefore, the sample volume between 10 to 50 mL for Co(II) and 10 to 100 mL for Ni(II) and Fe(III) were used in the further experiments.

4.2.5 Naked-eye detection limit for Co(II), Ni(II) and Fe(III)

The optimum conditions such as pH of the solution, concentration of xylenol orange, flow rate of solution, sample volume for metal ion naked-eye detection were obtained. After that the naked-eye detection limit was investigated by varying the concentration of metal ion between 10^{-7} to 10^{-4} M. From Table 4.7, it was found that the lowest concentrations of metal ions detected by naked-eye method were 10^{-5} M (0.6 ppm), 10^{-5} M (0.6 ppm) and 1×10^{-6} M (0.06 ppm) for Co(II), Ni(II) and Fe(III), respectively.

Table 4.7 Naked-eye detection limit of Co(II), Ni(II) and Fe(III)

Metal ions	Concentration of metal ions ($\times 10^{-6}$ M)																
	0	0.1	1	2	3	4	5	6	7	8	9	10	100	1000	2000	5000	10000
Co(II)																	
Ni(II)																	
Fe(III)																	

Xylenol orange (XO) = 0.01 mM, flow rate = 1.0 mL/min, pH = 5, sample volume = 10 mL.

The naked-eye detection limits of Ni(II) and Fe(III) in comparison with the regulated quality of water by Pollution Control Department are shown in Table 4.8. As the results, the naked-eye detection limit was given as same as the level of the concentration of Ni(II) in regulated surface water and was lower than the level of the concentration of Ni(II) in regulated waste water. Moreover, the naked-eye detection limit of Fe(III) was lower than that of regulated drinking water and tap water.

Table 4.8 Naked-eye detection limit compared with the maximum acceptable concentration of Pollution Control Department for Ni(II) and Fe(III)

Metal ions	LOD of this work (mg/L)	Maximum acceptable concentration (mg/L)			
		Surface water	Waste water	Drinking water	Tap water
Co(II)	0.6	-	-	-	-
Ni(II)	0.6	0.1	1.0	-	-
Fe(III)	0.06	-	-	0.3	0.5

Moreover, the DR-UV-Vis spectra of Co(II), Ni(II) and Fe(III) complexes at the concentration range of 10^{-6} - 10^{-4} M were recorded by DR-UV-Vis spectrophotometer at 250-650 nm. The results are shown in Figure 4.11-4.13.

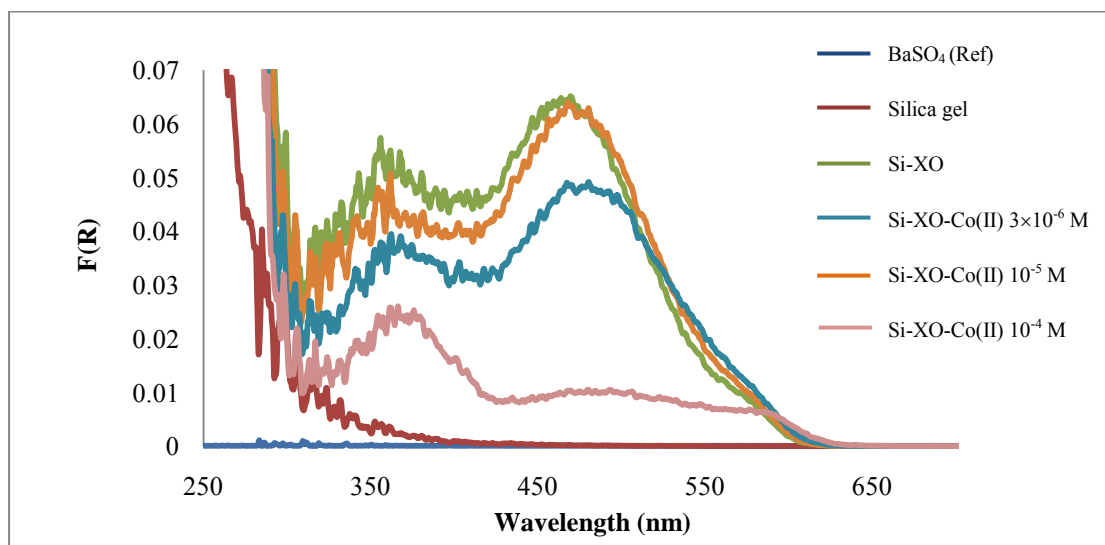


Figure 4.11 DR-UV-Vis spectra of Co(II) complex adsorbed onto silica gel at pH 5 in function of the concentration of Co(II) compared with silica gel and silica gel-xylene orange (Si-XO)

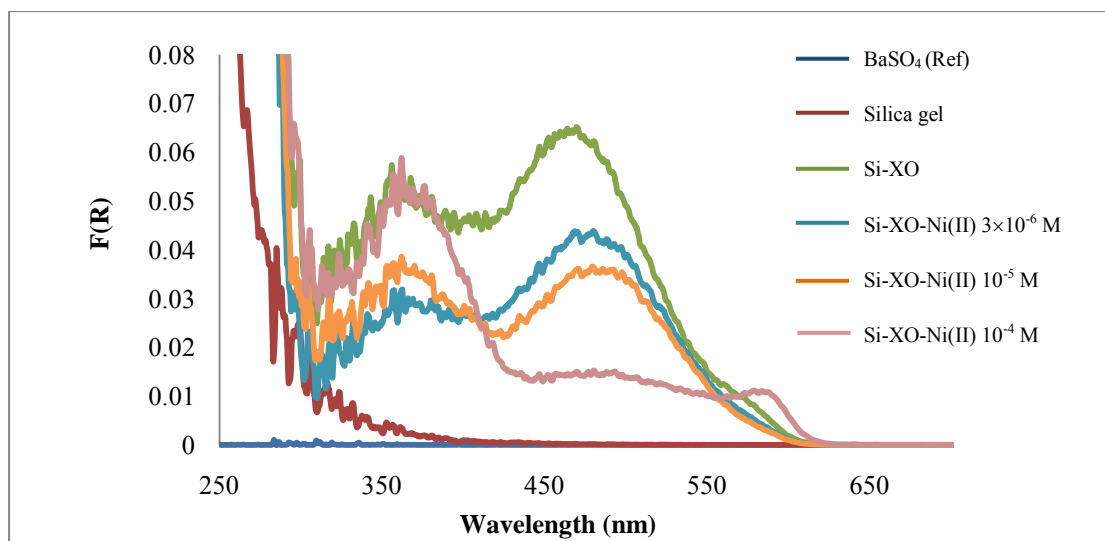


Figure 4.12 DR-UV-Vis spectra of Ni(II) complex adsorbed onto silica gel at pH 5 in function of the concentration of Ni(II) compared with silica gel and silica gel-xylene orange (Si-XO)

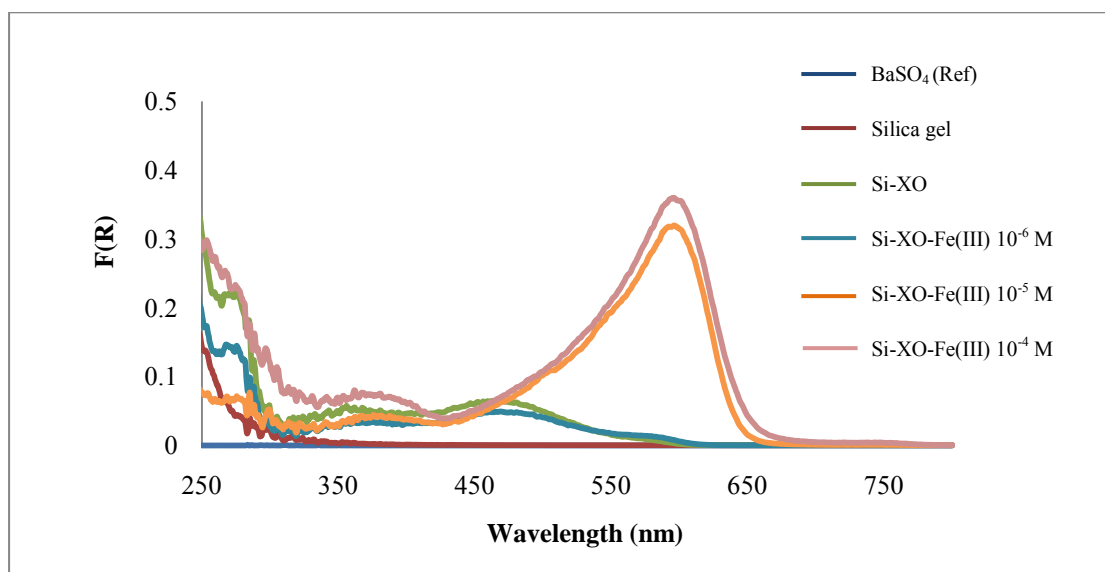


Figure 4.13 DR-UV-Vis spectra of Co(II) complex adsorbed onto silica gel at pH 5 in function of the concentration of Co(II) compared with silica gel and silica gel-xylene orange (Si-XO)









From Figure 4.11-4.13, the F(R) signals of metal complexes onto silica gel at maximum wavelength (580 nm for Co(II) and Ni(II) complexes, 595 nm for Fe(III) complex) were clearly observed at 1×10^{-4} M for Co(II) and Ni(II) and 1×10^{-5} M for Fe(III). These concentrations were higher than the naked-eye detection limit of Co(II), Ni(II) and Fe(III). This indicated that the naked-eye method can detect Co(II), Ni(II) and Fe(III) at the concentration lower than the instrumental method.

4.2.6 Effect of interfering ions

The colors of Co(II), Ni(II) and Fe(III) complexes were red-orange, pink and purple-blue, respectively. The color of a complex may be interfered by the color of another complex affecting to the naked-eye detection. Thus, the tolerance limit of Co(II), Ni(II) and Fe(III) under the optimum conditions were investigated. The results are shown in Table 4.9-4.11.









In case of Co(II), Ni(II) and Fe(III) complexes interfered to the color of Co(II) complex at the concentration of 10^{-5} - 10^{-4} M for Ni(II) and 10^{-6} - 10^{-4} M for Fe(III). In case of Ni(II), Co(II) and Fe(III) complexes interfered to the color of Ni(II) complex at the concentration of 10^{-4} M for Co(II) and 10^{-6} - 10^{-4} M for Fe(III). In case of Fe(III), Co(II) and Ni(III) complexes did not interfere to the color of Fe(III) complex at the concentration of 3×10^{-6} - 10^{-4} M for Co(II) and Ni(II).

Table 4.9 Tolerance limit of Ni(II) and Fe(III) for Co(II) naked-eye detection

Metal ions	XO	Co(II)	Co(II)	Co(II)	Co(II)	Co(II)	Co(II)	Co(II)
			+	+	+	+	+	+
			Ni(II)	Ni(II)	Ni(II)	Fe(III)	Fe(III)	Fe(III)
		3×10^{-6}	10^{-5}	10^{-4}	10^{-6}	10^{-5}	10^{-4}	
Co(II)								









Xylenol orange (XO) = 0.01 mM, flow rate = 1.0 mL/min, pH = 5,
sample volume = 10 mL.

Table 4.10 Tolerance limit of Co(II) and Fe(III) for Ni(II) naked-eye detection

Metal ions	XO	Ni(II)	Ni(II)	Ni(II)	Ni(II)	Ni(II)	Ni(II)	Ni(II)
			+	+	+	+	+	+
			Co(II)	Co(II)	Co(II)	Fe(III)	Fe(III)	Fe(III)
		3×10^{-6}	10^{-5}	10^{-4}	10^{-6}	10^{-5}	10^{-4}	
Ni(II)								

Xylenol orange (XO) = 0.01 mM, flow rate = 1.0 mL/min, pH = 5,
sample volume = 10 mL.

Table 4.11 Tolerance limit of Co(II) and Ni(II) for Fe(III) naked-eye detection





















Metal ions	XO	Fe(III)	Fe(III)	Fe(III)	Fe(III)	Fe(III)	Fe(III)	Fe(III)
			+	+	+	+	+	+
			Co(II)	Co(II)	Co(II)	Ni(II)	Ni(II)	Ni(II)
		3×10^{-6}	10^{-5}	10^{-4}	3×10^{-6}	10^{-5}	10^{-4}	
Fe(III)								

Xylenol orange (XO) = 0.01 mM, flow rate = 1.0 mL/min, pH = 5,
sample volume = 10 mL.

Na^+ , K^+ , Ca^{2+} and Mg^{2+} were studied for interfering ions because they were found in various water sources. In addition, xylenol orange can bind with alkaline earth. The formation complex and formation constant between xylenol orange with alkaline earth were reported [44]. Moreover, these cations may show the competitive coordination with the target metal ion (Co(II) , Ni(II) and Fe(III)) affecting to the naked-eye detection.





















Metal ion solution containing individual interfering ion at 0.6, 10, 100, 1000 mg/L was studied. The results are shown in Table 4.12-4.14. The results showed that Na^+ , K^+ , Ca^{2+} and Mg^{2+} at all concentrations did not interfere to the detection of Co(II) , Ni(II) and Fe(III) by naked-eye method.

Table 4.12 Effect of interfering ions for Co(II) naked-eye detection

Interfering ions	Concentration (mg/L)				
	0	0.6	10	100	1000
K^+					
Na^+					
Ca^{2+}					
Mg^{2+}					





















Xylenol orange (XO) = 0.01 mM, metal ions = 10^{-5} M, flow rate = 1.0 mL/min, pH = 5, sample volume = 10 mL.

Table 4.13 Effect of interfering ions for Ni(II) naked-eye detection

Interfering ions	Concentration (mg/L)				
	0	0.6	10	100	1000
K⁺					
Na⁺					
Ca²⁺					
Mg²⁺					

Xylenol orange (XO) = 0.01 mM, metal ions = 10^{-5} M, flow rate = 1.0 mL/min, pH = 5, sample volume = 10 mL.

Table 4.14 Effect of interfering ions for Fe(III) naked-eye detection

Interfering ions	Concentration (mg/L)				
	0	0.6	10	100	1000
K⁺					
Na⁺					
Ca²⁺					
Mg²⁺					

Xylenol orange (XO) = 0.01 mM, metal ions = 10^{-5} M, flow rate = 1.0 mL/min, pH = 5, sample volume = 10 mL.

4.3 Application to real samples

The proposed naked-eye method was applied to determine Co(II), Ni(II) and Fe(III) in real water samples e.g. Chulalongkorn University's pond water, Physics building water, drinking water and tap water. The concentrations of Co(II), Ni(II) and Fe(III) in four water samples by ICP-OES are shown in Table 4.15. The results showed that only Fe(III) was found in all real water samples.

Table 4.15 Concentrations of Co(II), Ni(II) and Fe(III) in real water samples by ICP-OES

Water samples	Concentration of Co(II) (mg/L)	Concentration of Ni(II) (mg/L)	Concentration of Fe(III) (mg/L)
Chula pond (A ₁)	n.d.	n.d.	0.02
Chula pond (A ₂)	n.d.	n.d.	0.11
Physics building (B ₁)	n.d.	n.d.	0.02
Physics building (B ₂)	n.d.	n.d.	0.36
Drinking (C)	n.d.	n.d.	0.02
Tap (D)	n.d.	n.d.	0.02

n.d. = not detectable

Moreover, the determination of Fe(III) by naked-eye method was performed for only sample A₂ and B₂ because the concentrations of Fe(III) in sample A₁, B₁, C and D were lower than the Fe(III) naked-eye detection limit (0.06 mg/L). The colors of Fe(III) complex of sample A₂ and B₂ were compared with the Fe(III) naked-eye color calibration at the working concentration range of 0.1-120 mg/L as illustrated in Figure 4.14. The results showed that 0.1 mg/L of Fe(III) was found for sample A₂ while 0.3-0.4 mg/L of Fe(III) was found for sample B₂. The concentration of Fe(III) by naked-eye method did not differ from ICP-OES method.

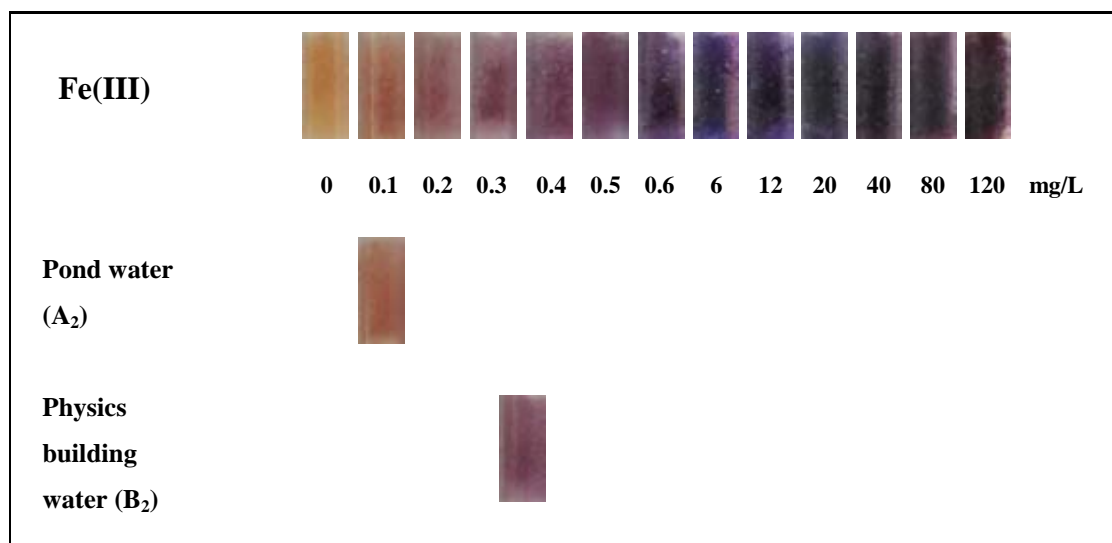


Figure 4.14 Colors of Fe(III) complex in real water samples

The matrix effect on the proposed naked-eye method were studied with real water samples. The water samples (A₁, B₁, C and D) were spiked with Co(II), Ni(II) and Fe(III) in two levels of concentration at 10^{-5} M (or 0.6 mg/L) and 10^{-4} M (or 6 mg/L). The concentrations of Co(II), Ni(II) and Fe(III) in spiked water samples were compared with the Co(III), Ni(II) and Fe(III) naked-eye color calibration at the working concentration range of 0.1-60 mg/L for Co(II) and Ni(II) 0.1-120 mg/L for Fe(III) as illustrated in Figure 4.15 – 4.17. The results showed that 0.6 and 6 mg/L of Co(III), Ni(II) and Fe(III) were observed in comparison with naked-eye color calibration. Therefore, this proposed naked-eye method can be used to determine Co(III), Ni(II) and Fe(III) in real water samples without matrix effect. Moreover, The concentrations of Co(II), Ni(II) and Fe(III) in spiked water samples were determined by ICP-OES as shown in Table 4.16. It was found that the concentrations of Co(II), Ni(II) and Fe(III) by naked-eye method did not differ from ICP-OES method, indicating that the proposed naked-eye method can be applied for determination of Co(II), Ni(II) and Fe(III) in various water sources without special equipment as ICP-OES.

Table 4.16 Concentrations of Co(II) Ni(II) and Fe(III) in spiked real water samples by ICP-OES

Sample	Co(II)		Ni(II)		Fe(III)	
	added (mg/L)	found (mg/L)	added (mg/L)	found (mg/L)	added (mg/L)	found (mg/L)
A₁	0.6	0.6	0.6	0.6	0.6	0.6
	6	5.5	6	5.7	6	5.8
B₁	0.6	0.6	0.6	0.6	0.6	0.6
	6	5.4	6	5.5	6	5.8
C	0.6	0.7	0.6	0.6	0.6	0.6
	6	6.4	6	6.0	6	6.2
D	0.6	0.6	0.6	0.5	0.6	0.6
	6	6.0	6	5.3	6	5.7

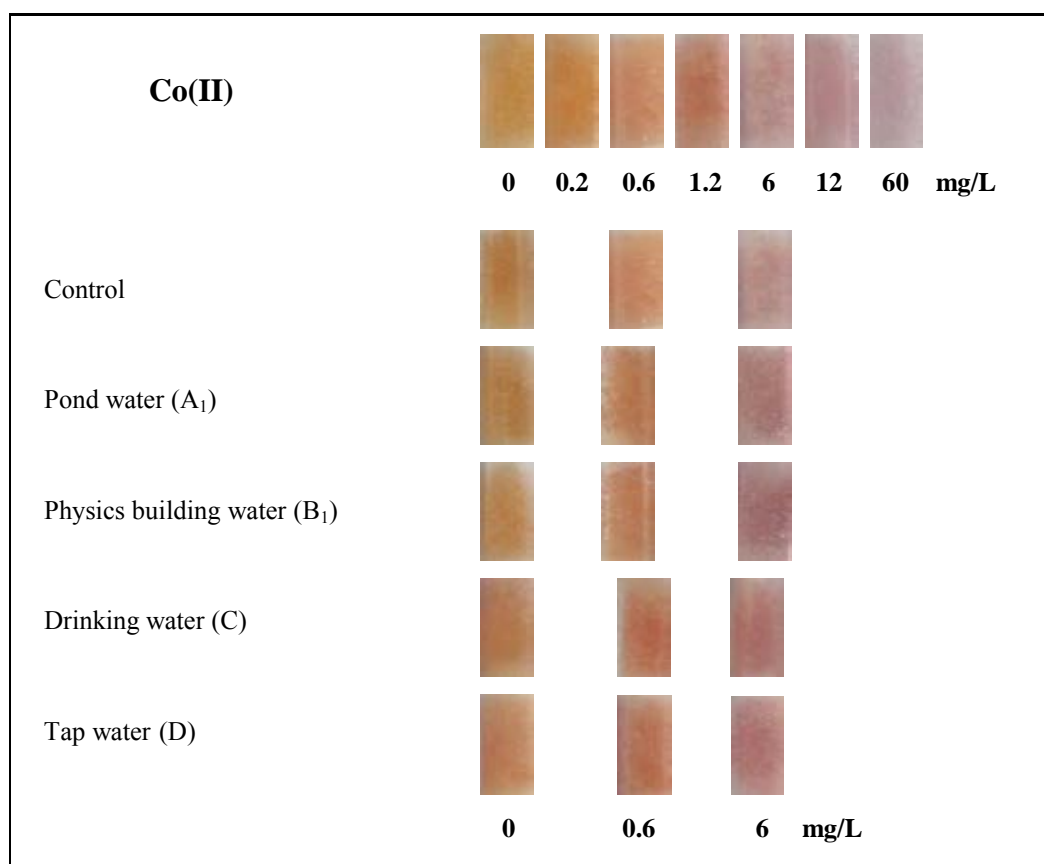


Figure 4.15 Colors of Co(II) complex in spiked real water samples

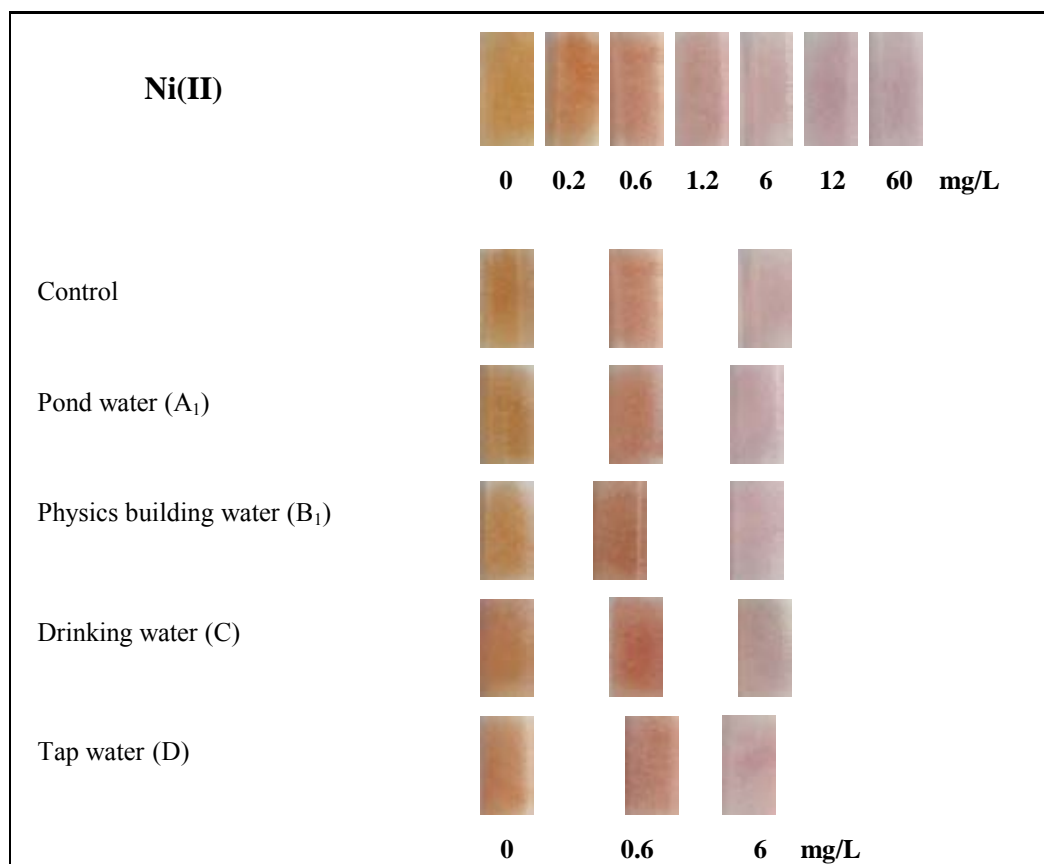


Figure 4.16 Colors of Ni(II) complex in spiked real water samples

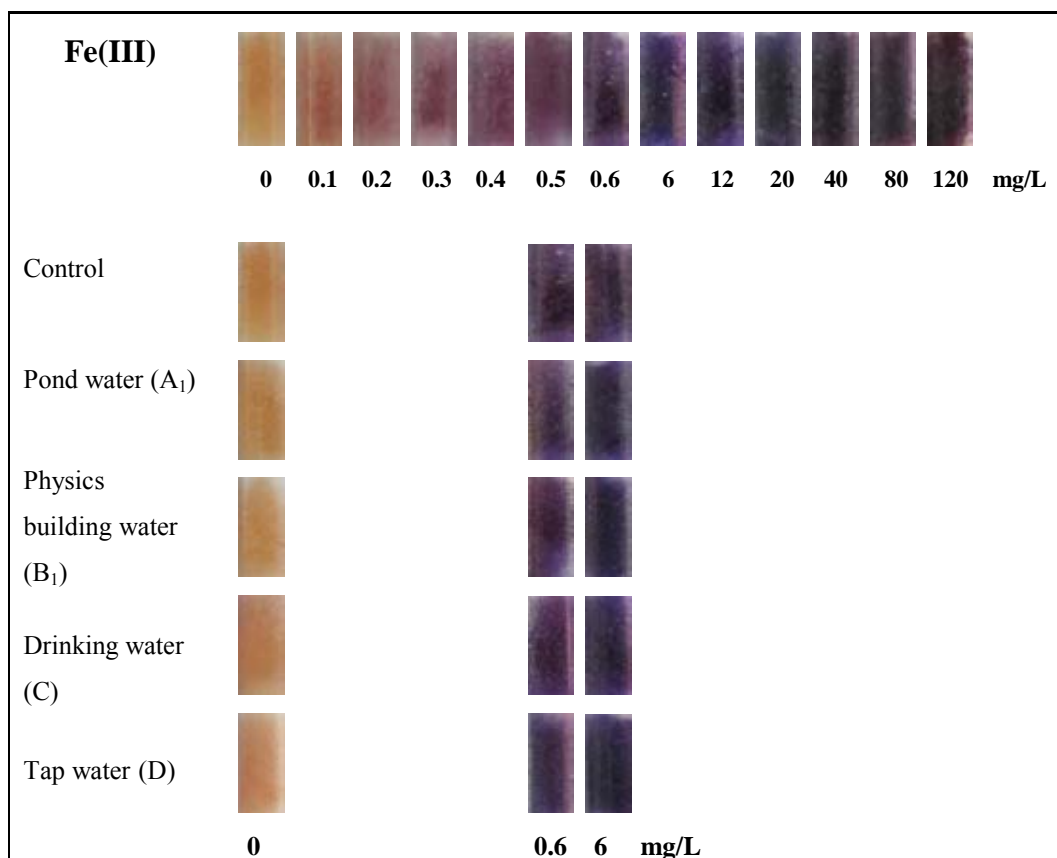


Figure 4.17 Colors of Fe(III) complex in spiked real water samples

The naked-eye detection limit was compared with the naked-eye detection limit reported in previous literatures as shown in Table 4.17. It was found that the naked-eye detection limits of Co(II), Ni(II) and Fe(III) in this work were lower than those of the same metal ions. The prominent point of the proposed naked-eye method is the use of column system which gave high preconcentration factor. Therefore, this work can give the lower limit of detection and can detect heavy metal ions at trace level in water resources.

Table 4.17 Comparison of naked-eye detection limits for heavy metal ion determinations

Metal ions	Ligands	Colors	LOD (mg/L)	Reference
Co(II), Ni(II) and Fe(III)	xylanol orange adsorbed onto silica gel	brown-orange to red, pink and purple-blue	Co(II) = 0.6 Ni(II) = 0.6 Fe(III) = 0.06	This work
Ni(II)	coumarin derivatives coated with filter paper	colorless to pink	Ni(II) = 2.9	[10]
Ni(II)	2-aminocyclopent-1-ene-1-carbodithioic acid coated with dip-sticks	yellow to red	Ni(II) = 1	[59]
Ni(II)	poly(caprolactone) electrospun fibers	colorless to pink	Ni(II) = 1	[60]
Fe(III)	sensor G3	slight yellow to red	Fe(III) = 0.3	[61]
Fe(II), Fe(III) and Cu(II)	1-(D-Glucopyranosyl-20-deoxy-20-iminomethyl)-2-hydroxynaphthalene	yellow to light, dark purple and green	Fe(III) = 0.3	[62]
Fe(III) and Ru(III)	per-6-amino- β -cyclodextrin	yellow to colorless	Fe(III) = 3 Ru(III) = 3	[63]
Fe(III) and Mg(II)	7-hydroxy-4-methyl-8-((pyridine-2-yl-imino)methyl)-2H-chromen-2-one	colorless to brown	Fe(III) = 0.1 Mg(II) = 0.002	[64]

CHAPTER V

CONCLUSION

Heavy metal ion detection was studied by the solid naked-eye method after forming complexes with xylenol orange adsorbed onto silica gel. The preliminary results showed that the colors of Zn(II) complex, Cd(II) complex and Co(II), Cu(II), Ni(II), Pb(II), Cr(III) complexes were pink, red and purple, respectively while the color of Fe(III) complex was black at pH 3-5 in batch method.

In column method, the solution containing xylenol orange and an individual metal ion at pH 3-5 was passed through a mini-column packed with 25 mg of silica gel controlled by a peristaltic pump. It was found that the colors of all metal ion complexes adsorbed onto silica gel were clearly observed at pH 5 changing from orange to red for Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II), to purple for Co(II) and Cr(III) and to black for Fe(III). The metal complex adsorbed onto silica gel was also measured by DR-UV-Vis spectrophotometry. The maximum f(R) signal of xylenol orange adsorbed onto silica gel was found at 468 nm while the maximum f(R) of metal complexes adsorbed onto silica gel were found at 580, 580 and 595 nm for Co(II), Ni(II) and Fe(III), respectively. It was exposed the coordination of metal ion and xylenol orange. The formation ratio of the complex of Co(II), Ni(II) and Fe(III) and xylenol orange in aqueous solution was found to be 1:1 by Job's plot method. The concentration of xylenol orange suitable for solid naked-eye metal ion detection was 0.01 mM. The colors of only Ni(II), Co(II) and Fe(III) complexes were obviously changed from brown-orange to red-orange, pink and purple-blue, respectively while no color change of other metal complexes was observed. The flow rate of solution was appropriately 1.0 mL/min. The maximum sample volumes were 50 mL for Co(II) and 100 mL for Ni(II) and Fe(III). The naked-eye color calibrations were prepared at the working concentration range of 0.1-60 mg/L for Co(II) and Ni(II) 0.1-120 mg/L for Fe(III). The lowest concentration of metal ions observed by naked-eye method was 10^{-5} M (or 0.6 ppm), 10^{-5} M (or 0.6 ppm) and 1×10^{-6} M (or 0.06 ppm) for Co(II), Ni(II) and Fe(III), respectively. The naked-eye detection limit was given as same as

the level of concentrations of Ni(II) and Fe(III) in the regulated water by Pollution Control Department as shown in Table 4.8. Ni(II) and Fe(III) complexes interfered to the color of Co(II) complex with the tolerance limits of 10^{-5} M for Ni(II) and 10^{-6} M for Fe(III). Moreover, Co(II) and Fe(III) complexes also interfered the color of Ni(II) complex with the tolerance limits of 10^{-4} M for Co(II) and 10^{-6} M for Fe(III). High concentrations of Na^+ , K^+ , Ca^{2+} and Mg^{2+} did not interfere the naked-eye detection of Co(II), Ni(II) and Fe(III).

The proposed naked-eye method was applied to determine Co(II), Ni(II) and Fe(III) in real water samples e.g. Chulalongkorn University's pond water, Physics building water, drinking water and tap water. The naked-eye detection of Co(II), Ni(II) and Fe(III) in spiked and non spiked real water samples did not differ from ICP-OES method. Therefore, the naked-eye detection method can be applied for determination of Co(II), Ni(II) and Fe(III) in various water sources without special equipment as ICP-OES.

Suggestion for future work

From the tolerance limit of Co(II), Ni(II) and Fe(III), the mixed colors of metal complexes interfered by naked-eye detection. Thus, a suitable masking agent to eliminate this effect should be studied.

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