CHAPTER IV

RESULTS AND DISCUSSION

4.1   Extraction of cyanidin and its characterization

Cyanidin was extracted from red cabbage under methanolic hydrochloric acid solution. Other nonpolar molecules were removed from extracted solution by using chloroform. Then, the extracted was hydrolyzed any sugar molecules consisted in anthocyanin structure under acidic condition. The final solution was allowed to stand in a dark refrigerator until dark-brown powder of the extract (Figure 4.1) was obtained. The dark-brown powder was purified through sephadex LH-20 using methanol as eluent. The clearly red solution fraction was collected and dried using rotary evaporator. The cyanidin powder should be kept in dark refrigerator to avoid either oxidation or degradation by light.

Figure 4.1 Cyanidin extracted from red cabbage

The characteristics of the extract were proved by UV-vis spectrophotometry, [1]H-nuclear magnetic resonance spectrometry (^{1}H-NMR) and mass spectrometry (MS).

The absorption spectrum of extracted cyanidin dissolved in 0.3% v/v HCl/MeOH (Figure 4.2) showed the absorption band at 280 nm and 330 nm corresponding to flavoniod and phenolic structure, respectively. Moreover, the absorption band at 535 nm corresponded to the anthocyanin chromophore. These

results were agreed with Shinoda's test. The bathochromic shift from 535 nm to 557 nm was obtained after adding $AlCl_3$ into the solution.
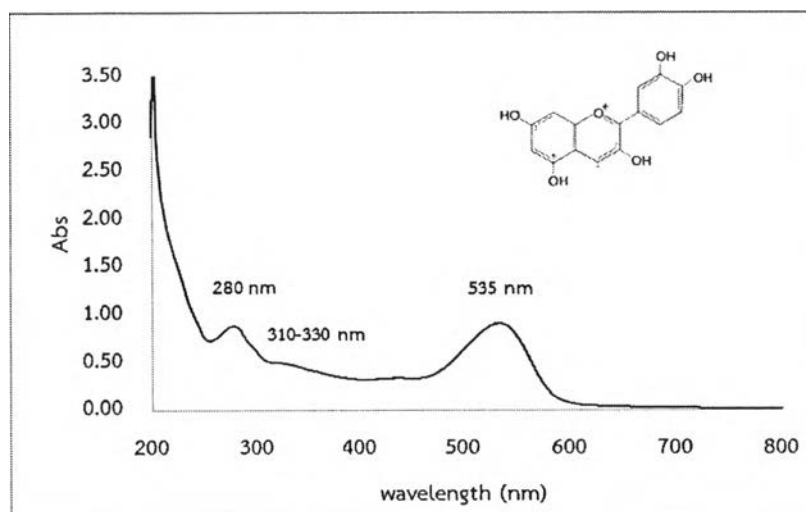

Figure 4.2 UV-Visible spectrum of extracted cyanidin

Mass spectrum of the extracted cyanidin (Figure 4.3) showed a major molecular ion peak at m/z = 286.3 which was nearly the molecular ion mass of cyanidin.
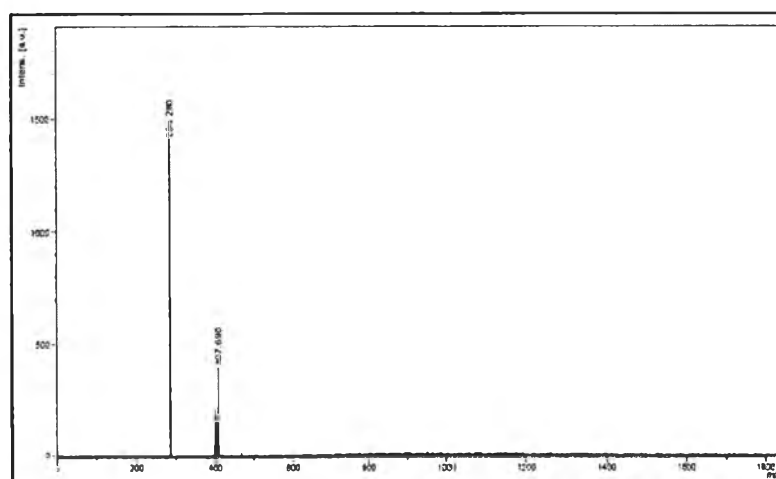

Figure 4.3 Mass spectrum of cyanidin extracted from red cabbage

^{1}H-NMR spectrum (Figure 4.4) also exhibited signals corresponding to cyanidin molecule. A 1H singlet at δ 8.57 ppm was tropical of H-4 of cyanidin structure. Four 1H doublets at δ 6.61 (J=2.0), 6.87 (J=1.0), δ 8.03 (J=2.4) and δ 6.92 (J=8.4) ppm corresponded, respectively, to H-6, H-8, H-2' and H-5'of cyanidin. A 1H doublet of doublet at δ 8.12 (J=8.4, 2.0) ppm was corresponded to H-6'.

Figure 4.4 ^{1}H-NMR spectrum of cyanidin extracted from red cabbage

The results showed that cyanidin was the main kind of anthocyanin in red cabbage which agreed with the research of Wiczkowski and co-workers [62]. They used high resolution HPLC to isolate anthocyanin in red cabbage. They also identified about 20 types of cyanidin glucoside derivatives.

pH of solution affecting on cyanidin structure and causing visible color change is shown in Figure 2.3. However, *ortho*-dihydroxyl group at B ring is still potential coordinated with metal ions at pH lower than 8.

The maximum absorption shifting to the longer wavelength (λ_{max}) in visible region is observed along with increase pH value as shown in Table 4.1. Because anthocyanin can be easily deprotonated at low pH resulting structural changes in the anthocyanin chromophore. The reversible reaction can also be taken even in acid or basic solution. Furthermore, decoloration of anthocyanin is occurred in the aqueous solution due to the hydration at the 2 or 4 hydroxyl position of anthocyanin structure.

Table 4.1 Maximum wavelength (λmax) of cyanidin at various pH

pH	λ_{max} (nm)
3	521
4	540
5	550
6	560
7	570
8	603

Cyanidin powder should be kept in dark refrigerator at all times to avoid light and high temperature degradation of cyanidin structure. Cyanidin solution was prepared by dissolving 1.6 mg of cyanidin powder in 5 mL of methanol then leaved to equilibrium in a dark refrigerator for 1 hour. To study the stability of cyanidin solution, it was measured the maximum absorption wavelength and absorbance unit by UV-Vis spectrophotometer in different periods of time as shown in Table 4.2.

Table 4.2 Stability of cyanidin solution

Period	λ_{max}	Abs
30 days	535	0.478
		0.477
		0.472
120 days	535	0.451
		0.450
		0.452
150 days	535	0.464
		0.467
		0.467
Average		0.464
SD		0.011
%RSD		2.4

The λ_{max} and absorbance unit of cyanidin solution was measured over a long time periods. The results still present the maximum absorption wavelength (λ_{max}) at 535 nm which is the characteristic of cyanidin structure and the absorbance quite stable (%RSD=2.4). Thus, this reagent could be efficiently used as colorimetric reagent for metal ions at least for 150 days keeping in the dark refrigerator.

4.2   Qualitative determination of metal ions using cyanidin solution

4.2.1   pH of solution

pH of solution affecting on cyanidin structure and causing visible color change is shown in Figure 2.3. However, ortho-dihydroxyl group at B ring is still potential coordinated with metal ions at pH lower than 8. From Figure 4.5 it was found that cyanidin molecule clearly formed complex with only four metal ions because the different colors solution comparing with blank solution was observed. Color of Cu(II) complex was different from that of blank solution at pH range of 5-7 whereas Pb(II) complex also showed those difference at pH range of 5-6. Fe(III) coordinated with cyanidin at acidic pH 3-4. For Al(III) complex, it performed color different from blank at widely pH range of 3-6.
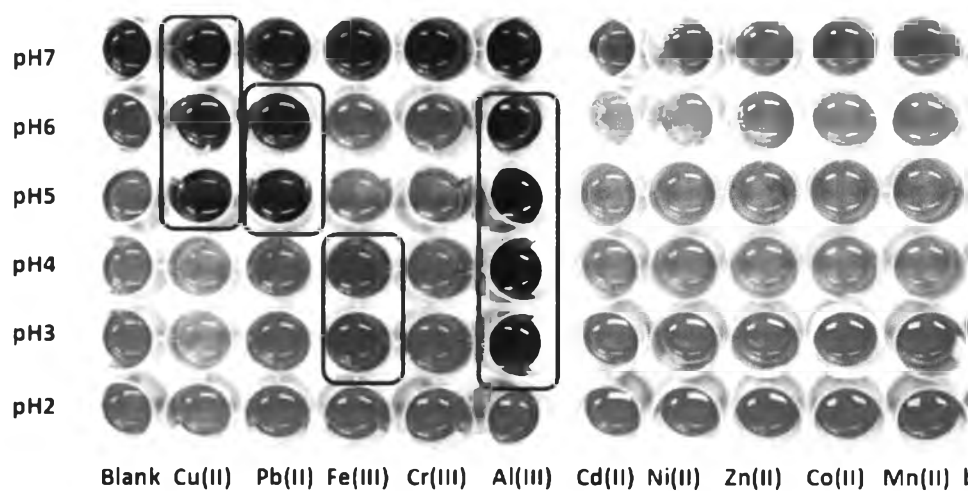

Figure 4.5 Complexation of cyanidin and metal ions depended on pH of solution

From the results, cyanidin extracted from red cabbage showed the selectivity for Cu(II), Pb(II), Fe(III) and Al(III). Therefore, the effective determination using cyanidin as colorimetric reagent for Cu(II), Pb(II), Fe(III) and Al(III) was investigated in the this research.

The formations of cyanidin complex are reported that some metal cations coordinate with *ortho*-dihydroxyl group at B-ring of cyanidin structure. This reaction cause the bathochromic shift appeared. The possibility of M^{n+} ions binding to two OH groups of the A ring is quite unlikely because of the steric effect and both groups are also too far from another one. The complex formation of cyanidin and metal ion is shown in Figure 4.6.
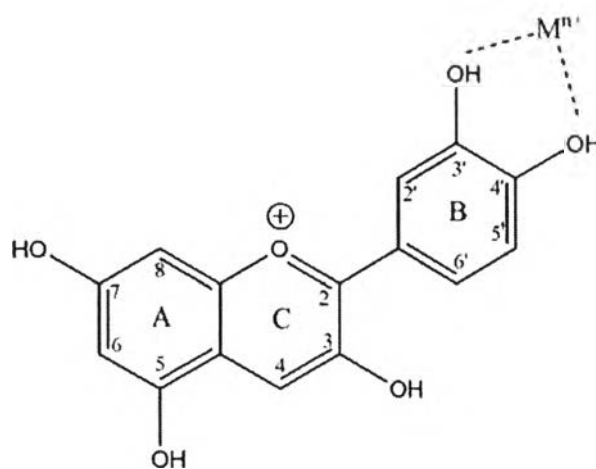

Figure 4.6 Cyanidin-metal complex formation

The complex ratio of cyanidin and Cu(II), Pb(II), Fe(III) and Al(III) was also reported and it was found that the different ratios depended on metal ion target. For Cy 3-glc-Cu(II) complex, the stoichiometry performed 1:1 for high excess of Cu(II) ion as studied by Smyk *et al.* (2008) [14]. The 1:1 complex ratio for Al(III) was reported under the investigation via a simple model using synthetic flavylium salts [15]. The complex ratio of Pb(II)-cyanidin was found to be 1:2 at pH 4.2 [26].

However, 1:2 complex ratio was found in case of complexation of Al(III) with Cy 3,5-glc at pH 4.5 similar to the case of Fe(III)-Cy 3-glc complex reported in the same research [63].

From those results, the maximum wavelength of the complexation of cyanidin and Cu(II), Pb(II), Fe(III) and Al(III) was measured using UV-Visible spectrophotometry. It was found that all of complexes showed the maximum absorption at the longer wavelengths correlated with absorbance unit except Cy-Cu(II) complex which showed lower absorbance after complexation as shown in Figure 4.7. This observation closely agreed with research of Smyk *et al.* (2008) [14].
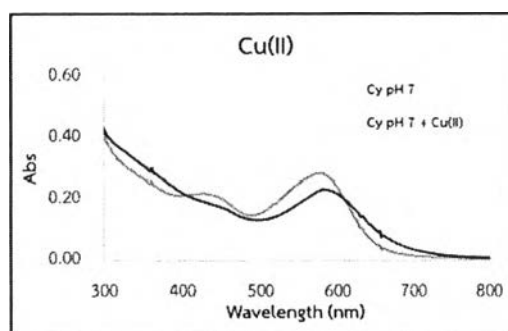

Figure 4.7 Cy-Cu(II) complex

They reported that the complexation between Cu(II) and Cy-3-glc in binary solvent (methanol-water) caused the reduction of Cu(II) to be Cu(I) and oxidation of Cy-3-glc instead. The reaction was rapidly occurred resulting in the bathochromic shift at short period of time and then continued decreasing to the lowest absorbance. The Cy-Cu(II) complex showed longer wavelength shift from 570 nm to 590 nm as the same result as the complexation of Cy-Pb(II) shifting from 560 nm to 580 nm. For Cy-Fe(III) complex, the bathochromic shift appeared from 540 nm to 560 nm. The formation of Cy-Al(III) showed the wavelength shift from 550 nm to 570 nm.

Fe(II) detection was not investigated in this study because Pyysalo and Kuusi (1973) reported that no colored complexes of anthocyanins and Fe(II) were found. They studied the color change of cyanidin from 5 berries with Fe(III), Fe(II) and Sn(II). It was found that Fe(II) caused no significant discoloration nor precipitation in all berries. [64]

4.2.2 Masking agents

In section 4.2.1, some metal ions complexed with cyanidin at the same pH, to discriminate Cu(II), Pb(II), Fe(III) and Al(III) for possible detecting simultaneously, the masking agents were employed.

Interference from unexpected cations can be sometime eliminated by adding a suitable masking agent which is an auxiliary ligand that preferentially forms highly stable complexes with the potential interference. In other words, a masking agent is a complexing agent that reacts selectivity with a component in a solution and also prevents that component from interfering in an analysis. Clearly, a masking agent must not affect the behavior of the analyte significantly [65]. There are several anions or molecular reagents used as masking agent for metal masking [66-68]. Table 4.3 shows example of masking agent for metal ion in water analysis [68]. Moreover, Cu(II) also interacted with DMG as reported by Frasson et al. (1959) [69].

Table 4.3 Some of masking agents

Element	Masking agent
Cu	Citrate, CN^-, EDTA, diethyldithiocarbamate, thiourea, tartrate, glycine, hydrazine
Pb	Acetate, citrate, EDTA, tartrate, triethanolamine,
Fe	Acetylacetone, citrate, CN^-, 1,10-phenanthroline, SCN^-, tartrate, thiourea, EDTA
Al	Acetate, acetylacetone, citrate, EDTA, F^-, tartrate, formate, salicylate

However, the reagent used as masking agents in this study should interact with unexpected metal ions to be colorless complexes which do not interfere naked-eye detection.

From color change results in section 4.2.1, Cu(II) determination at pH 7 would not be interfered from other cations. Though, Al(III) could be determined at pH range of 3-6, Al(III) would be investigated at pH 5-6 to avoid the interference of Al(III) on Fe(III) detection at pH 3-4. In this case, pH 4 was better for Fe(III) determination because of its higher buffer capacity. However, reduction of Al(III) interference at pH 4 should be considered. For Pb(II) determination, pH 5-6 would be investigated.

The naked-eye detection using cyanidin solution was investigated at pH 6 because cyanidin could be coordinated with Cu(II), Pb(II) and Al(III) at this pH solution. Figure 4.8 shows example of KF as Al(III) masking agent. The blue color of Al(III) complex did not appear after adding KF solution because Al(III) interacted with F^- better than cyanidin molecule. Other reagents were investigated and the masking results of metal ions are summarized in Table 4.4.
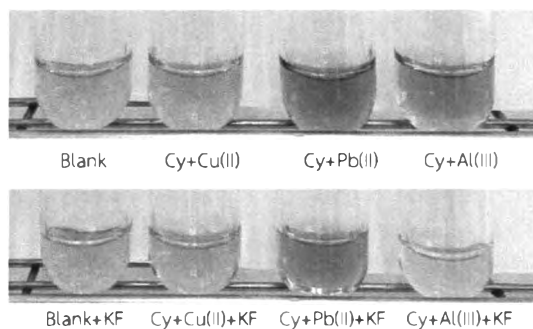
Figure 4.8 Masking agent of Al(III) naked-eye detection at pH 6

Table 4.4 Masking agent tested for Cu(II), Pb(II), Fe(III) and Al(III) detection at pH 6

Masking agent (0.1 M)	Metal ion masked			
	Cu(II)	Pb(II)	Fe(III)	Al(III)
Thiourea	√	-	-	-
EDTA	√	√	√	√
$Na_4P_4O_7$	√	√	√	√
KF	-	-	-	√
Citrate	√	√	√	√
NaH_2PO_4	-	√	-	√
Oxalate	√	√	-	√
Tartrate	√	√	-	√
Sulfamic acid	-	√	-	-
DMG	√	-	-	-

√ = metal ions masked by tested reagent resulting colorless complex

The results in Table 4.4 shows that KF completely masked only Al(III) to be AlF_6^{3-} complexes (log K_f = 19.84) [66, 68] whereas DMG showed potentially mask Cu(II) to be $Cu(DMG)_2$ (log K_f = 33.44) [69, 70]. Sulfamic acid can mask only Pb(II), however, orange solution occurred after adding sulfamic acid into Fe(III) solution. Therefore, sulfamic acid was not suitable to be masking agent for Pb(II). The other masking agents can mask several metal ions including target metal ion except DMG and thiourea. Thiourea also masked Cu(II) ion but the slightly yellow color appeared after leaving for a period of time interfered naked-eye observation. In addition, the complex formation constant of thiourea-Cu(II) (log K_f = 15.4) is also lower than of DMG-Cu(II) [70]. Thus, in this study KF, DMG and the mixture of KF and DMG were used as masking agent for Fe(II), Al(III) and Pb(II) detection, respectively. Thus, in further study, KF and DMG were employed as masking agent for reduction of Al(III) and Cu(II) interference.

From masking results, it can be conclude that Fe(III) and Al(III) could be determined at pH 4 and 5 along with KF and DMG as masking agent, respectively. In case of Pb(II) detection, both KF and DMG were employed under the solution pH 6.

4.2.3 Concentration of cyanidin and reaction time

Though, the bathochromic shift of solution appeared after complexation, the reddish color of cyanidin might interrupt naked-eye detection of complex color so its concentration should be considered. Three concentrations of cyanidin were prepared by dissolving 0.8, 1.6 and 3.2 mg of cyanidin powder in methanol (5 mL). Each cyanidin concentration was mixed with 1 mM Cu(II), Pb(II), Fe(III) and Al(III) standard solution under the conditions as follows:

- 1 mM standard Cu(II) solution, 100 µL/ buffer pH 7, 0.01 M, 1 mL

- 1 mM standard Pb(II) solution, 100 µL/ buffer pH 6, 0.01 M, 1 mL/ 0.1 M KF, 100 µL/ 1.0% w/v DMG,100 µL

- 1 mM standard Fe(II) solution, 100 µL/ buffer pH 4, 0.01 M, 1 mL/ 0.1 M KF, 100 µL

- 1 mM standard Al(III), 100 µL/ buffer pH 5, 0.01 M, 1 mL/ 1.0% w/v DMG, 100 µL

Cyanidin prepared by dissolving cyanidin 1.6 mg in 5 mL of methanol was selected because this concentration less affect the color of complex which is easy to detect by naked-eye and the color change of the solution as a function of cyanidin-metal complex reaction time was stable after 6 minutes of progress as shown in Figure 4.9.
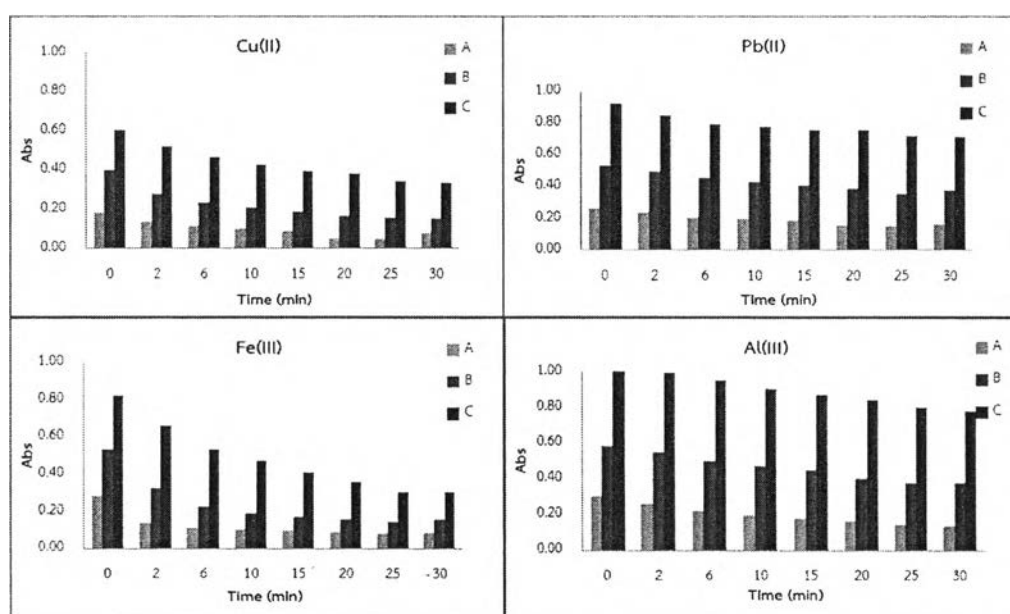

Figure 4.9 Effect of cyanidin concentration along with reaction time on complexation

(Cyanidin powder = 0.8 mg (A), 1.6 mg (B) and 3.2 mg (C))

4.2.4 Interfering ion effect

It is the fact that one ligand can potentially complex with many cations and some anions might also be the anionic ligands associated with the metal ions. Cations and anions (Na^+, K^+, Ca^{2+}, Cl^-, F^-, SO_4^{2-}, NO_3^-) studied in this research based on the assumption that they are normally ions found in natural water. The mixture solution of each metal ion solution (1 mM) and each interfering cation ($CaCl_2$, KCl, $NaNO_3$) and anion ($NaCl$, KF, Na_2SO_4 and $Ca(NO_3)_2 \cdot 4H_2O$) in the concentration of 0.01 M were prepared and developed color as described in section 3.5.4. Each target metal ion in the same concentration of standard metal ions was also prepared as interfering ion.

The absorbance measured by UV-Visible spectrophotometry was compared with that of no interfering ion. Figure 4.10 and 4.11 show the results of interfering ions effect from cations, anions and target metal ions. In case of Pb(II) detection, it was found that the absorbance unit of solution containing SO_4^{2-} was clearly different from others, whereas the solution consisted of F^- shows the difference in Abs value in case of Al(III) determination. It can be proposed that SO_4^{2-} interrupts Pb^{2+} detection due to $PbSO_4$ formation whereas F^- interfere Al^{3+} determination because they can interact to be AlF_6^{3-} complex. For target metal ions, the results showed that no significant difference was observed.
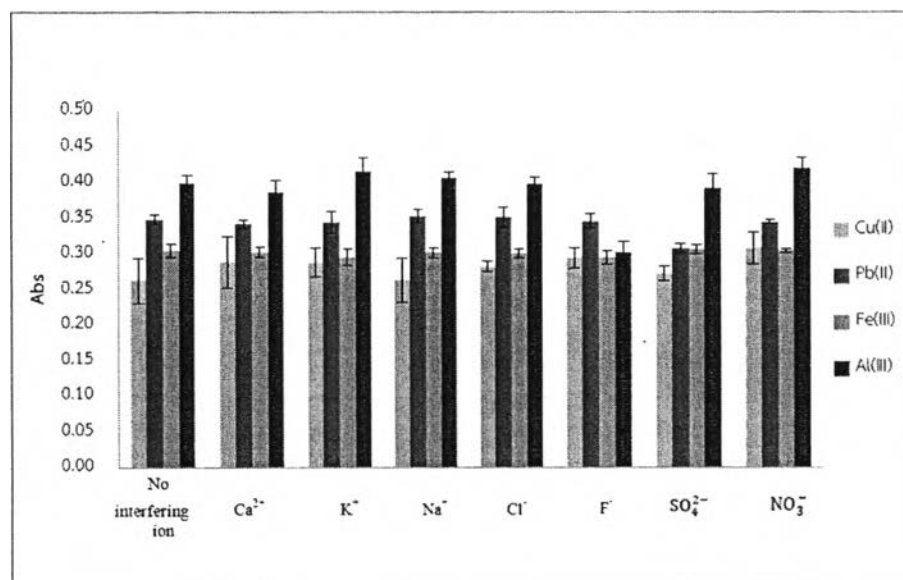
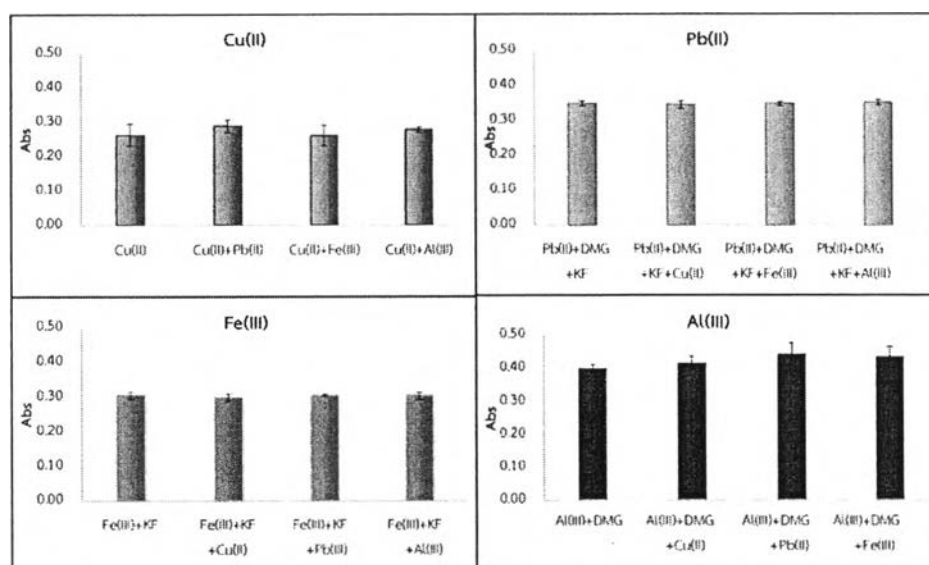**Figure 4.10** Interfering effects from cations and anions for Cy-metal complex

Figure 4.11 Interfering effect from target metal ion for Cy-metal complex

4.2.5 Summarization of the optimum conditions for qualitative determination using cyanidin solution

From the results, it can be summarized the optimum conditions for qualitative determination of Cu(II), Pb(II), Fe(III) and Al(III) using cyanidin solution as shown in Table 4.5. 1.0 mL of buffer solution (0.01 M) was added into 100 µL of sample solution followed by 100 µL of masking agent solution and 100 µL of cyanidin solution (1.6 mg in MeOH 5 mL). Figure 4.12 shows simultaneous qualitative detection of these four metal ions using the optimum conditions summarized in Table 4.5. These conditions would be applied for quantitative determination in the next step.

Table 4.5 Qualitative detection condition for Cu(II), Pb(II), Fe(III) and Al(III) using cyanidin solution

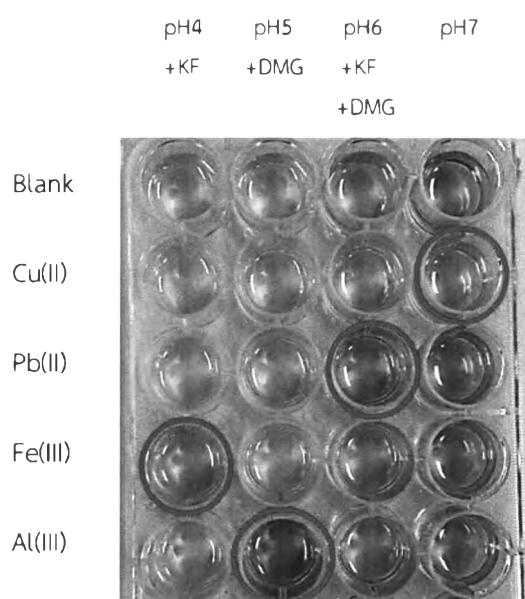| Metal ion	Metal ion/ Sample (µL)	Buffer (0.01 M, 1 mL)	Masking agent (100 µL)	Cyanidin solution (µL)
Cu(II)	100	pH 7	-	100
Pb(II)	100	pH 6	0.1 M KF + 1.0% w/v DMG	100
Fe(III)	100	pH 4	0.1 M KF	100
Al(III)	100	pH 5	1.0% w/v DMG	100

Figure 4.12 Simultaneous qualitative detection of Cu(II), Pb(II), Fe(III) and Al (III)

The determination of mix standard metal ion solutions (unknown 1 and 2) with the proposed method was investigated. The colors of unknown 1 were different from those of blank solutions (Figure 4.13) indicating that this unknown consisted of Cu(II), Pb(II), Fe(III) and Al(III). Whereas the colors of unknown 2 changed only at pH 5 and pH 6 indicating that this unknown consisted of only Al(III) and Pb(II).
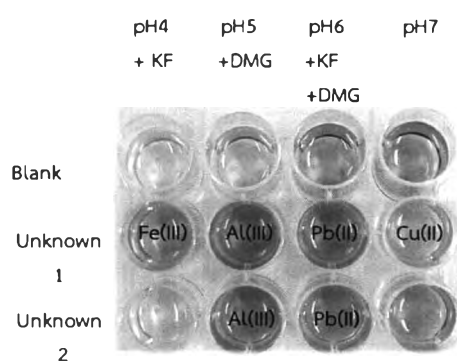

Figure 4.13 Example of simultaneous qualitative determination of mix metal ion using cyanidin solution

4.3 Quantitative determination of metal ions using cyanidin solution

After finding out the optimum conditions for qualitative determination, the quantitative determination was also investigated under the optimum conditions of qualitative determination as described in Table 4.5.

4.3.1 Naked-eye detection

The quantitative analysis by naked-eye detection performed as semi-quantitative detection could be divided into 3 color shades depended on metal ion concentration ranges as shown in Figure 4.14. For Cu(II) detection, at the concentration lower than 50 μM the color of solution was not different from blank solution whereas at the concentration range between 50-80 μM the color of solution was violet-blue and at the concentration higher than 80 to 1000 μM the color of solution was contributed to blue. For Pb(II) detection, the purple color appeared until the concentration up to 80-200 μM the violet-blue was observed. Dark-blue color appeared when the concentration of Pb(II) was higher than 200 to 800 μM. In case of Fe(III) determination, no different color between blank solution and Fe(III) solution at the concentration lower than 200 μM was observed. The Fe(III) solution was developed to violet when the concentration of Fe(III) was up to 200-400 μM and appeared blue color at the concentration 400-600 μM. The Al(III) solution in the concentration lower than 50 μM showed the color not different from blank solution and contributed to violet-blue at the concentration range of 50-100 μM. After increasing Al(III) concentration higher than 100 to 400 μM the blue color was observed.

Table 4.6 shows the summary of color shade depended on metal ion concentration ranges. It was found that the lowest concentrations detected by naked-eye for Cu(II), Pb(II), Fe(III), and Al(III) were 50, 80, 200 and 50 μM, respectively.
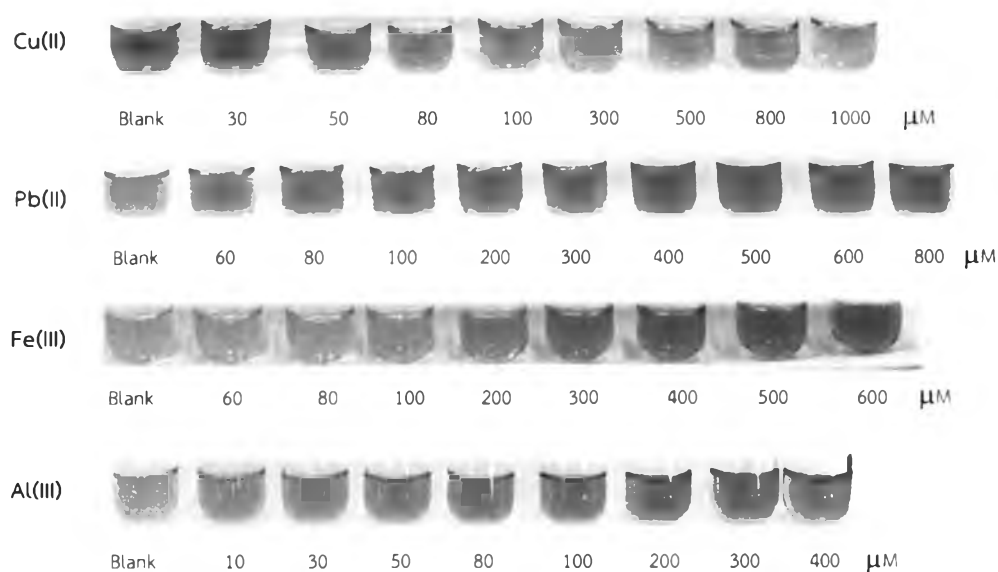
Figure 4.14 Color chart of Cy-metal complex for semi-quantitative analysis

Table 4.6 Semi-quantitative detection and limit of detection of Cu(II), Pb(II), Fe(III) and Al (III) by naked-eye

Metal ion	pH	Quantitative determination			LOD
Cu(II)	7	Violet < 50 µM	Violet-blue 50-80 µM	Blue > 80-1000 µM	50 µM
Pb(II)	6	Purple < 80 µM	Violet-blue 80-200 µM	Blue > 200-800 µM	80 µM
Fe(III)	4	Pink < 200 µM	Violet 200-400 µM	Blue > 400-600 µM	200 µM
Al(III)	5	Purple < 50 µM	Violet-blue 50-100 µM	Blue > 100-400 µM	50 µM

4.3.2 UV-Visible determination

In this research, we tried to use cyanidin as colorimetric reagent for determination of metal ions by UV-Visible spectrophotometry to preliminary compare the detection limit with naked-eye detection.

It was found that cyanidin was probably applied for determination of Pb(II), Fe(III) and Al(III) in the linear range of 0-500 μM, 0-400 μM and 0-100 μM, respectively (Table 4.7). The detection limits of Pb(II), Fe(III) and Al(III) were 48, 81 and 3.7 μM, respectively (Table 4.7), under calculated using following equation [71]:

$$LOD = \frac{y_B + 3S_{y/x}}{slope}$$

where

LOD   = Limit of detection (μM)

y_B = Blank signal

$S_{y/x}$ = Standard deviation obtained from regression equation (Standard deviation of blank)

Table 4.7 Linear concentration and detection limit of Pb(II), Fe(III) and Al(III) determined by UV- Visible spectrophotometry

Metal ion	Regression equation	R^2	Linear concentration	LOD
Pb(II)	Y=0.2096X+0.1962	0.9935	0-500 μM	48 μM
Fe(III)	Y=0.2845X+0.1382	0.9716	0-400 μM	81 μM
Al(III)	Y=1.4277X+0.1037	0.9995	0-100 μM	3.7 μM

It was found that the detection limits by instrumental analysis was lower than naked-eye detection. However, LOD of Cu(II) was not proper determined by this technique because the absorbance of complexes was not proportional to the complex concentration.

From this study, it was suggested that cyanidin could be alternative reagent for determination of Pb(II), Fe(III) and Al(III) by UV-Visible spectrophotometry.

4.4   Method validation for metal ion determination using cyanidin solution

Water samples were filtered through cellulose filter (0.45 µm) and preserved in an acidic medium (HNO_3) before use. Two concentrations of each metal ion were prepared by spiking stock standard solution into unknown water samples (pond and tap water) under the optimum conditions for quantitative analysis.

Table 4.8 shows comparative results between naked-eye detection and ICP-OES method. From this table, it was found that two concentrations of spiked metal ion determined by naked-eye are closed to ICP-OES method and almost results from ICP-OES were in the concentration range of naked-eye detection. Percentage of the recoveries from ICP-OES method were in the acceptable range except Al(III) in both water samples, Cu(II) and Fe(III) in tap water. However, the determination by naked-eye indicated the approximate concentrations of metal ion in both water samples.

Table 4.8 Accuracy of naked-eye detection compared with ICP-OES method using cyanidin solution

Water sample	Metal ion	Concentration (mM)				%Recovery	
		Blank	Spike	ICP-OES	Naked-eye	ICP-OES	Naked-eye
Pond water	Cu(II)	0	0.05	0.045	< 0.05	90.0	< 100
			0.1	0.09	0.05-0.08	90.0	< 100
	Pb(II)	0	0.1	0.092	0.08-0.30	92.0	80-300
			0.3	0.286	0.08-0.30	95.3	26.7-100
	Fe(III)	0	0.2	0.167	<0.20	83.5	< 100
			0.5	0.432	0.20-0.40	86.4	40-80
	Al(III)	0	0.08	0.118	0.05-0.10	147.5	62.5-125
			0.3	0.309	>0.1	103.0	> 33.3
Tap water	Cu(II)	0	0.05	0.043	< 0.05	86.0	< 100
			0.1	0.062	0.05-0.08	62.0	50-80
	Pb(II)	0	0.1	0.091	0.08-0.30	91.0	80-300
			0.3	0.28	0.08-0.30	93.3	26.7-100
	Fe(III)	0	0.2	0.13	<0.20	65.0	< 100
			0.5	0.442	0.20-0.40	88.4	40-80
	Al(III)	0	0.08	0.134	0.05-0.10	167.5	62.5-125
			0.3	0.378	> 0.10	126.0	>33.3

Therefore, it can be concluded that the naked-eye detection using cyanidin solution as colorimetric reagent was potentially applied for determination of Cu(II), Pb(II), Fe(III) and Al(III) determination in real water samples.

4.5 Application in real water sample using cyanidin solution

This developed method was applied for metal ion determination in 30 water samples prepared by spiking 30 different metal ion concentrations, single metal ion or mix metal ions, in pond water. Then, all of water samples were developed color under the optimum conditions of Cu(II), Pb(II), Fe(III) and Al(III) detections as summarized in Table 4.5.

The results from naked-eye detection were compared with spiked value and ICP-OES method in term of both qualitative and quantitative analysis (Table 4.9). The naked–eye detection showed qualitative analysis correctly for 20 samples from 30 samples, representing 67.7% (see appendix A). These 20 correct samples were included with 3 samples which was spiked metal ion at the concentration lower than LOD of naked-eye detection. For quantitative analysis of these 20 water samples, the concentrations of each spiked metal ion were in the acceptable ranges. However, almost concentrations of spiked metal ion in other incorrect samples determined by naked-eye were closed to spiked values. Whereas the most qualitative and quantitative results of ICP-OES detection were closed to spiking method.

Table 4.9 The results of naked-eye detection compared with spiked value and

ICP-OES method

| Unknown | Spike | | | Naked-eye | | ICP-OES | |
	Metal ion	Conc. (µM)	Remark	Metal ion	Conc. (µM)	Metal ion	Conc. (µM)
pond	-	-		-	-	-	-
1	Al(III)	200		Al(III)	50-100	Al(III)	218
				Pb(II)	80-300	-	-
	Cu(II)	60		Cu(II)	50-80	Cu(II)	56
2	Fe(III)	100	spike Fe(III) <LOD	-	-	Fe(III)	98
	Al(III)	200		Al(III)	>100	Al(III)	227
3	Cu(II)	60		Cu(II)	50-80	Cu(II)	57
4	Fe(III)	300		Fe(III)	200-400	Fe(III)	330
				Al(III)	50-100	-	-
				Pb(II)	>300	-	-
5	Fe(III)	300		Fe(III)	200-400	Fe(III)	300
				Al(III)	50-100	-	-
				Pb(II)	>300	-	-
	Cu(II)	90		Cu(II)	>80	Cu(II)	83
6	Pb(II)	90		Pb(II)	80-300	Pb(II)	85
7	Pb(II)	100		Pb(II)	80-300	Pb(II)	95
8	Fe(III)	50	spike Fe(III) <LOD	-	-	Fe(III)	52
9	Fe(III)	300		Fe(III)	200-400	Fe(III)	326
	Al(III)	40		Al(III)	50-100	Al(III)	30
				Pb(II)	>300	-	-
	Cu(II)	100		Cu(II)	>80	Cu(II)	100
10	Pb(II)	90		Pb(II)	>80	Pb(II)	87
	Cu(II)	100		Cu(II)	>80	Cu(II)	90
11	Pb(II)	100		Pb(II)	80-300	Pb(II)	96
12	Al(III)	60		Al(III)	50-100	Al(III)	62
	Pb(II)	90		Pb(II)	80-300	Pb(II)	86
	Cu(II)	60		Cu(II)	50-80	Cu(II)	55
13	Cu(II)	90		Cu(II)	>80	Cu(II)	84

Table 4.9 (continued)

Unknown	Spike			Naked-eye		ICP-OES	
	Metal ion	Conc. (µM)	Remark	Metal ion	Conc. (µM)	Metal ion	Conc. (µM)
14	Al(III)	200		Al(III)	>100	Al(III)	204
15	Al(III)	200		Al(III)	>100	Al(III)	234
	Pb(II)	100		Pb(II)	80-300	Pb(II)	95
16	Al(III)	60		Al(III)	50-100	Al(III)	65
	Cu(II)	100		Cu(II)	>80	Cu(II)	90
17	Al(III)	90		Al(III)	>100	Al(III)	86
	Cu(II)	100		Cu(II)	>80	Cu(II)	95
18	Fe(III)	300		Fe(III)	200-400	Fe(III)	323
				Al(III)	50-100		
				Pb(II)	>300		
	Cu(II)	70		Cu(II)	50-80	Cu(II)	65
19	Fe(III)	400		Fe(III)	200-400	Fe(III)	429
	Al(III)	60		Al(III)	>100	Al(III)	57
				Pb(II)	>300		0
20	Al(III)	100		Al(III)	>100	Al(III)	110
	Pb(II)	100				Pb(II)	85
21	Fe(III)	100		Fe(III)	200-400	Fe(III)	95
	Al(III)	100		Al(III)	>100	Al(III)	114
				Pb(II)	80-300		
	Cu(II)	100		Cu(II)	>80	Cu(II)	94
22	Fe(III)	70		Fe(III)	200-400	Fe(III)	66
	Al(III)	70		Al(III)	>100	Al(III)	76
				Pb(II)	80-300		
	Cu(II)	70		Cu(II)	50-80	Cu(II)	66
23	Al(III)	60		Al(III)	50-100	Al(III)	69
24	Cu(II)	100		Cu(II)	>80	Cu(II)	93
25	-	-					
26	Pb(II)	90		Pb(II)	80-300	Pb(II)	88
	Cu(II)	60		Cu(II)	50-80	Cu(II)	57
27	Pb(II)	50	spike Pb(II) <LOD			Pb(II)	50

Table 4.9 (continued)

| Unknown | Spike | | Remark | Naked-eye | | ICP-OES | |
	Metal Ion	Conc. (µM)		Metal Ion	Conc. (µM)	Metal Ion	Conc. (µM)
28	Pb(II)	200		Pb(II)	80-300	Pb(II)	186
29	Cu(II)	40		Cu(II)	<50	Cu(II)	37
30	Al(III)	90		Al(III)	50-100	Al(III)	84
	Pb(II)	90				Pb(II)	95

In addition, in case of comparison each metal ion determined by naked-eye and spiked value or ICP-OES method, it was found that the minimal errors about 7% for Fe(III) detection. Percentage of the error about 10-30% for Al(III) determination in term of both qualitative and quantitative analysis whereas Pb(II) detection showed the error about 30% both qualitative and quantitative determination. However, there are no percent error for Cu(II) detection

4.6   Modification of solid sorbent

To modify cyanidin as colorimetric reagent on solid sorbent, several parameters have to be realized such as solid sorbent type, coating conditions including its stability.

4.6.1   Solid sorbent type

Solid sorbent used in this study was selected based on its colorless or white color appearance which do not interfere naked-eye detection after modifying. In addition, the structure of solid sorbent should consist of functional group which can interact with cyanidin molecule. Cyanidin corresponds to flavonoid and phenolic compound and consists of *ortho-dihydroxyl* group (Figure 2.4). Several OH-groups of

cyanidin might interact with another OH-group of AXAD-7, silica gel and cellulose via hydrogen bonding while π-π interaction might also be occurred between aromatic ring of cyanidin molecule and AXAD-2 which is polystyrene copolymer resin.

After coating all selected solid sorbents, the surface color turned to pink-red as shown in Figure 4.15. AXAD-7 and silica gel showed homogeneous cyanidin coating in contrast with both AXAD-2 and cellulose paper. However, cyanidin coated on silica gel was easily dissolved in aqueous solution especially in acidic solution due to the protonation at silanol group. Cyanidin coated on AXAD-7 was stable in aqueous medium along with widely pH range. For further study, AXAD-7 was selected as solid sorbent because of its stability especially the homogenous solid surface would contribute to easily detect by naked-eye.
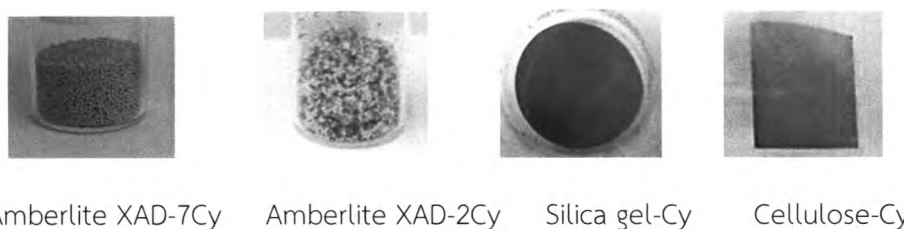
Amberlite XAD-7Cy   Amberlite XAD-2Cy   Silica gel-Cy   Cellulose-Cy

Figure 4.15 Appearance comparison of selected solid sorbent coated with cyanidin

ATR-IR was used to identify the functional group of AXAD-7 coated with cyanidin. It was found that ATR-IR spectrum of AXAD-7Cy was quite similar to that of unmodified AXAD-7 as shown in Figure 4.16. Nevertheless, the observation of pink-red color on modified solid sorbent indicated cyanidin molecules consisted in AXAD-7. The interaction of cyanidin and AXAD-7 was probably via H-bond. In addition, the proposed structure of AXAD-7Cy interacting with metal ions (M^{n+}) at ortho-dihydroxyl group is shown in Figure 4.17.
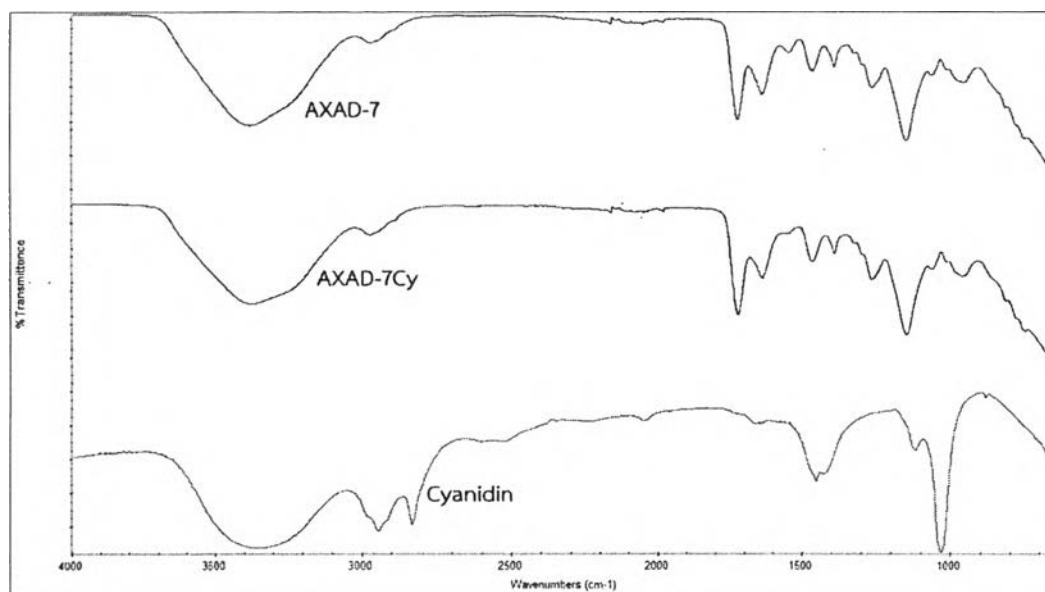
Figure 4.16 IR spectra of AXAD-7, AXAD-7Cy and cyanidin
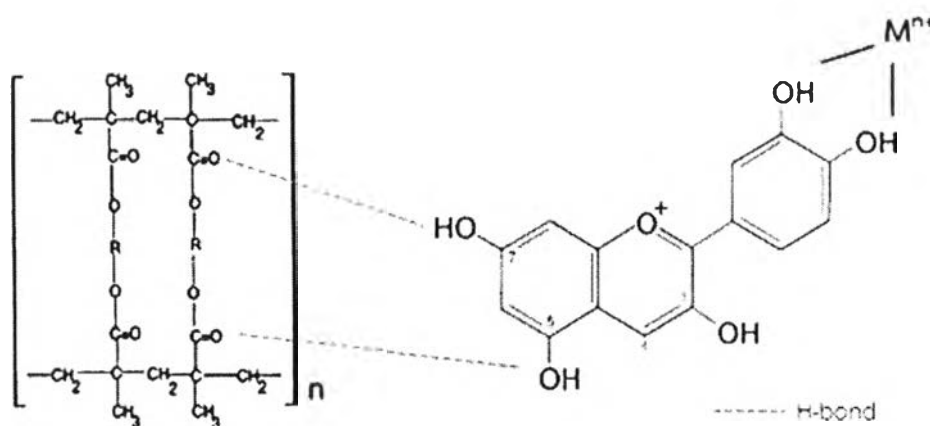

Figure 4.17 Proposed structure of AXAD-7Cy

4.6.2 Coating conditions

After finding out an appropriate solid sorbent, coating condition was the main influences which should be considered. As previous research reported that the flavylium cation is the predominated species in acidic solution (pH < 3) and more stable than other pH [72]. Effect of methanolic hydrochloric acid solution of cyanidin

coating on solid sorbent was investigated. In acidic methanolic hydrochloric acid solution, cyanidin can be retained in form of flavylium cation which highly stable from light and oxygen in ambient air as described before. However, storing AXAD-7Cy in the dark and air tight container would be better for long time using.

AXAD-7Cy was coated by two coating conditions which prepared from cyanidin dissolved in 0.3% and 0.6% v/v HCl/MeOH. It was found that, AXAD-7Cy from both conditions showed homogeneous pink-red color surface. The stability of AXAD-7Cy was also monitored using DR-UV-Vis spectrophotometry for determination of its reflectance (%R) which correlated with absorbance unit as shown in equation below.

$$A = -\log R$$

where: A is absorbance

R is reflectance

The reflectance was measured for a period of time until the significant difference in % reflectance was observed. The data was expressed as mean $\pm$ SD (n=3). The results in Figure 4.18 shows that the color of AXAD-7Cy coating under condition 1 was obviously decreased after storage for a short period of time whereas AXAD-7Cy coating under condition 2 showed better stability in the color than another one. The better stability was probably due to the stronger acid condition contributed higher flavylium cation predominated specie in coating solution. In addition, Figure 4.19 shows that AXAD-7Cy could be stored without any effects for at least 60 days.
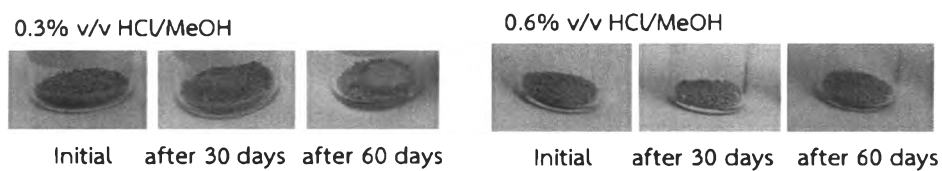
0.3% v/v HCl/MeOH   0.6% v/v HCl/MeOH

Initial   after 30 days after 60 days   Initial   after 30 days after 60 days

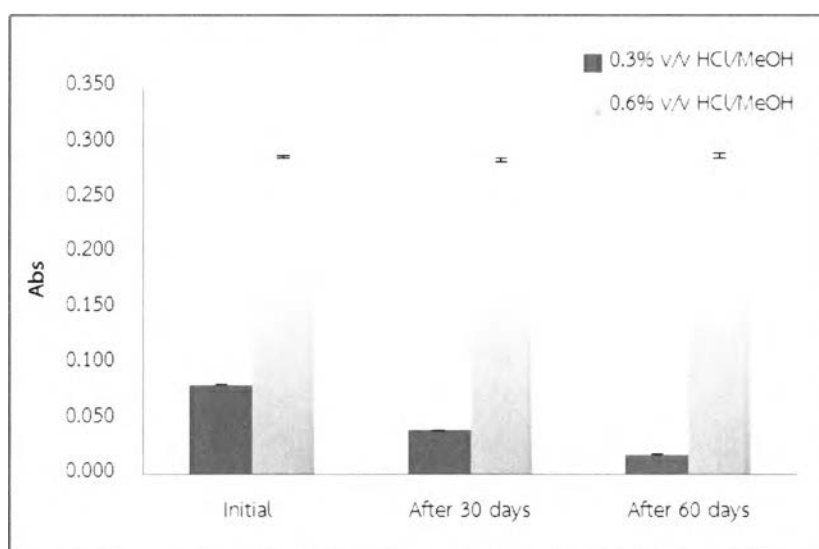**Figure 4.18** Comparison of AXAD-7 coating

Figure 4.19 Stability of AXAD-7Cy

For further study, AXAD-7Cy coating by dissolving cyanidin in 0.6% v/v HCl/MeOH was chosen as solid sorbent for determination of Cu(II), Pb(II), Fe(III) and Al(III) by naked-eye detection.

4.6.3 Cyanidin concentration and coating time

AXAD-7 (1 g) was mixed with 3 different concentrations of cyanidin solutions (5 mL) and then left in the dark for 30 minutes, 12 and 24 hours. The reflectance (%R) of all modified solid sorbents was measured. The results in Figure 4.20 shows the maximum absorption wavelength of all AXAD-7Cy at around 535 nm,

and the absorbance was direct proportional to cyanidin concentration and coating time. However, in this research AXAD-7Cy coated for 24 hours was chosen because the highest absorbance of cyanidin of each condition was obtained from this coating time which was the maximum coating time studied in this section.
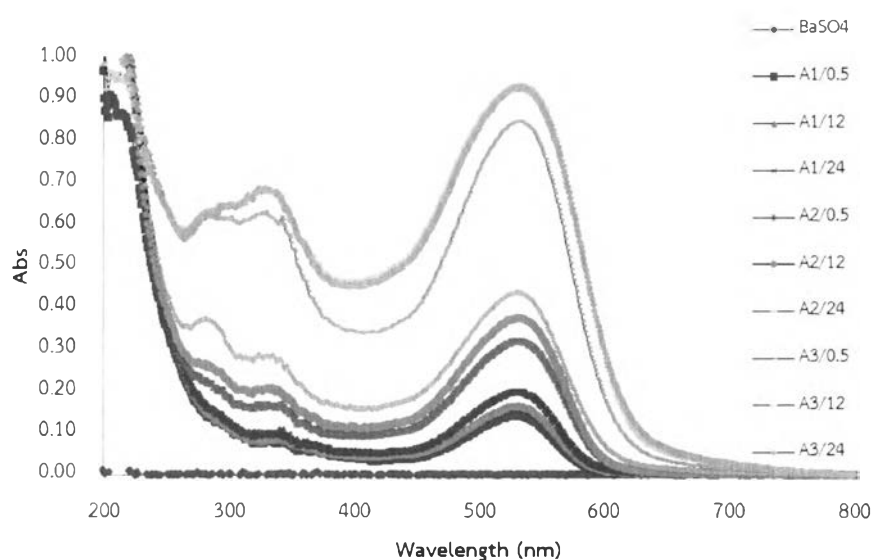
Figure 4.20 Spectra of modified solid sorbents at various cyanidin concentrations and coating times determined by DR-UV-Vis spectrophotometry

(A1=AXAD-7Cy1 (Cy 0.8 mg), A2=AXAD-7Cy2 (Cy 1.6 mg), A3=AXAD-7Cy3 (Cy 3.2 mg))

AXAD-7 modified with cyanidin (AXAD-7Cy) for 24 hours of each concentration were investigated under the optimum producing condition by comparing the naked-eye detectable at various concentrations of Cu(II). It was found that the color of Cu(II)-Cy complex on AXAD-7Cy1 was not clearly different from blank unlike those results from AXAD-7Cy2 and AXAD-7Cy3. However, the color of Cu(II)-Cy complex at low concentration was interfered with the intense color of cyanidin on AXAD-7Cy3 sorbent.

Cu(II) was detected by AXAD-7Cy2 better than AXAD-7Cy1 and can also be separated concentration range clearly than AXAD-7Cy3 due to the reddish color of cyanidin could interfere naked-eye observation as shown in Figure 4.21. Therefore, AXAD-7Cy2 was the optimum modified solid sorbent used for further study.
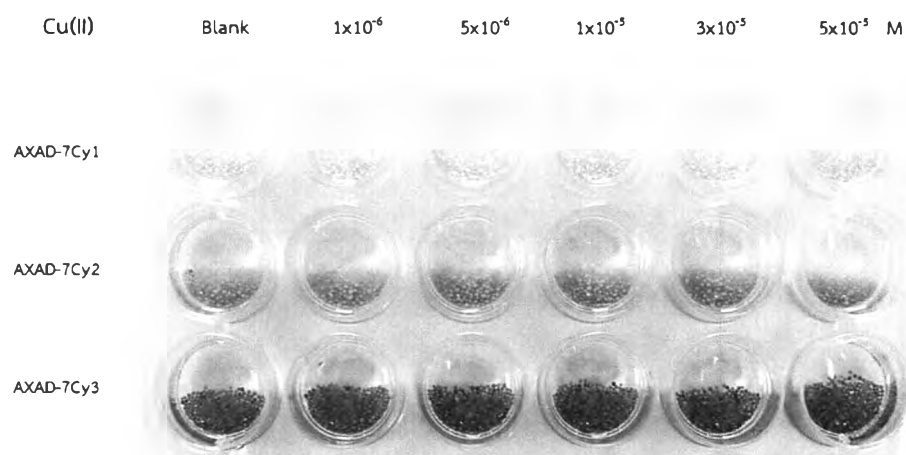

| Cu(II) | Blank | 1×10^{-6} | 5×10^{-6} | 1×10^{-5} | 3×10^{-5} | 5×10^{-5} M |

AXAD-7Cy1

AXAD-7Cy2

AXAD-7Cy3

Figure 4.21 Comparison of AXAD-7Cy at different cyanidin concentrations for Cu(II) naked-eye detection

4.7   Qualitative determination of metal ions using modified solid sorbent

Various parameters investigated in qualitative determination using cyanidin solution were also studied to achieve the optimum conditions for solid naked-eye detection at the lowest level. In this study we considered parameters such as pH of the solution, masking agents including interfering ion effect.

4.7.1 pH of the solution

Because pH of solution effects on cyanidin structure and causes visible color change. Therefore, the pH of the solution was first studied.

The colors of AXAD-7Cy in Cu(II), Pb(II), Fe(III) and Al(III) solutions were different from those in blank solution as shown in Figure 4.22. These results indicated that *ortho*-dihydroxyl group still potential coordinate with metal ions at pH lower than 8. The pH ranges of the complexation were quite similar to those studied in form of cyanidin solution. Cyanidin clearly formed complex with Cu(II) at pH range of 5-7 while Pb(II) complexed with cyanidin at only pH 6. Fe(III) and Al(III) coordinated with cyanidin in the pH range of 4-5 and 5-6, respectively.

Blank Cu(II)   Pb(II)   Fe(III)   Al(III)   Cr(III)   Cd(II)   Zn(II)   Ni(II)   Co(II)   Mn(II)   Blank
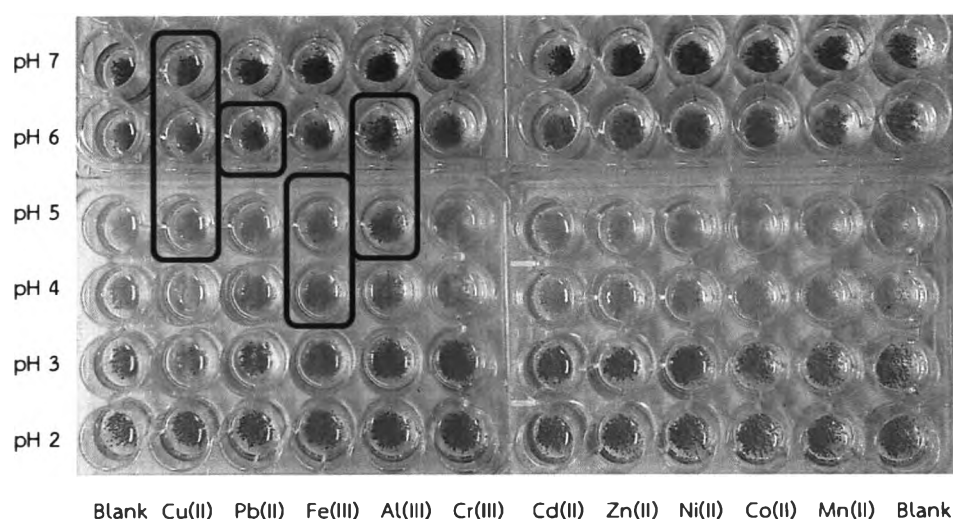
Figure 4.22 Complexation of cyanidin on AXAD-7Cy and metal ions depended on pH solution

4.7.2   Masking agent

The masking agent used in this section followed those studied in section 3.5.2. From Figure 4.22 Fe(III) and Cu(II) could be determined at pH 4 and 7, respectively, without any effect from other metal ions. Therefore, masking agents were investigated in case of Al(III) and Pb(II) determination at pH 5 and 6, respectively. Cu(II) and Fe(III) should be masked for Al(III) detection at pH 5 while

Cu(II) and Al(III) have to be masked for Pb(II) determination at pH 6. The results also showed that F can completely mask Al(III) as the same results described before. Figure 4.23 shows the comparison of using 1.0% w/v DMG and 0.1 M thiourea as masking agent. It was found that thiourea showed potentially mask Cu(II) better than DMG and no interfering effect from slightly yellow color solution. It was also found that Fe(III) was less interfered at this condition. Thus, thiourea and the mixture of thiourea and KF were used for Al(III) and Pb(II) determination.
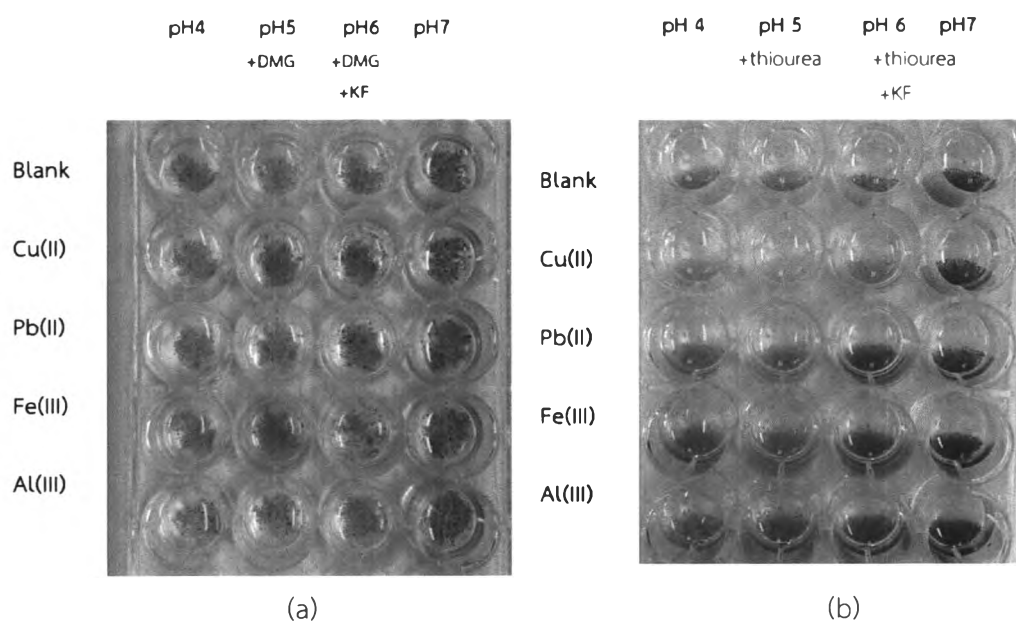
Figure 4.23 Comparison of 1.0% DMG (a) and 0.1 M thiourea (b) as masking agent for simultaneous naked-eye detection using AXAD-7Cy

4.7.3 Buffer concentration and the volume ratio of all reagents

The AXAD-7Cy was conditioned in a buffer solution. However, the molecular ion from buffer or even interference from sample water might interrupt complexation of target metal ions and cyanidin.

From the preliminary test, 500 μL of 0.01 M buffer solution, 100 μL of 0.1 M masking agent and 500 μL of standard metal ion solution mixing with 20 mg of AXAD-7Cy showed that limits of detection by naked-eye were not as low as determined by using cyanidin solution. Thus, to achieve the lowest concentration as studied in previous section, the variation of concentration and volume used of buffer solution and masking agent was investigated while AXAD-7Cy was fixed at about 20 mg. Buffer solution at pH 4, 5, 6 and 7 were used for Fe(III), Al(III), Pb(II) and Cu(II), respectively. Thiourea and the mixture of thiourea and KF were used as masking agent for Al(III) and Pb(II) analysis, respectively.

The concentrations of buffer solution were 0.01 M and 0.10 M and the volume of buffer solution was varied from 100 μL to 1000 μL. Sample volume was varied between 250 μL - 1000 μL and the masking agent solution was also varied between 50 μL -100 μL. Cu(II), Fe(III) and Al(III) in the concentration of 0.1 mM and Pb(II) in the concentration of 0.5 mM were selected as representative of sample solution because these concentrations are closed to the limit of detection examined by using cyanidin solution. Table 4.10 summarizes the studied conditions and the naked-eye observation of four target metal ions.

Table 4.10 Optimization of the volume ratio of all reagents

Condition	Metal ion	Sample (µL)	Buffer solution (M)	pH	Buffer solution (µL)	Masking agent (µL)	Naked-eye detectable metal ion
1	Cu(II)	500	0.1	7	500	-	√
	Pb(II)	500	0.1	6	500	100 (KF) 100 (Thiourea)	-
	Fe(III)	500	0.1	4	500	-	-
	Al(III)	500	0.1	5	500	100 (Thiourea)	√
2	Cu(II)	500	0.1	7	250	-	√
	Pb(II)	500	0.1	6	250	50 (KF) 50 (Thiourea)	√ (slightly change)
	Fe(III)	500	0.1	4	250	-	√ (slightly change)
	Al(III)	500	0.1	5	250	50 (Thiourea)	√
3	Cu(II)	250	0.1	7	250	-	√
	Pb(II)	250	0.1	6	250	25 (KF) 25 (Thiourea)	√ (slightly change)
	Fe(III)	250	0.1	4	250	-	√ (slightly change)
	Al(III)	250	0.1	5	250	25 (Thiourea)	√
4	Cu(II)	1000	0.1	7	100	-	√
	Pb(II)	1000	0.1	6	100	100 (KF) 100 (Thiourea)	√
	Fe(III)	1000	0.1	4	100	-	√
	Al(III)	1000	0.1	5	100	100 (Thiourea)	√

Table 4.10 (continued)

Condition	Metal ion	Sample volume (µL)	Buffer solution (M)	pH	Buffer solution (µL)	Masking agent (µL)	Naked-eye detectable metal ion
5	Cu(II)	500	0.01	7	500	-	√
	Pb(II)	500	0.01	6	500	100 (KF) 100 (Thiourea)	√ (slightly change)
	Fe(III)	500	0.01	4	500	-	√
	Al(III)	500	0.01	5	500	100 (Thiourea)	√
6	Cu(II)	250	0.01	7	250	-	√
	Pb(II)	250	0.01	6	250	25 (KF) 25 (Thiourea)	√ (slightly change)
	Fe(III)	250	0.01	4	250	-	√ (slightly change)
	Al(III)	250	0.01	5	250	25 (Thiourea)	√
7	Cu(II)	250	0.01	7	1000	-	√
	Pb(II)	250	0.01	6	1000	25 (KF) 25 (Thiourea)	√
	Fe(III)	250	0.01	4	1000	-	√
	Al(III)	250	0.01	5	1000	25 (Thiourea)	√

√ = metal ions detected by naked-eye

The results from this table show that all of metal ions could be observed under condition 4 and 7. However, the results from condition 4 was better because it showed the obviously detection by naked-eye. Moreover, using more sample volume as in condition 4 contributed more complexation resulting in better observation. For example, Figure 4.24 shows the comparison of Pb(II) detection under

condition 4 and 7. It was found that Pb(II) potentially formed complex and showed the color change under condition 4 better than another one.
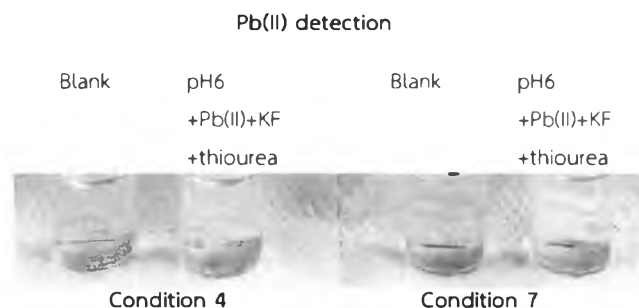
Pb(II) detection

Figure 4.24 Comparison of Pb(II) detection under condition 4 and 7

4.7.4 Reaction time for determination of metal ions using AXAD-7Cy

The reaction time of this research was operated under condition 4 as studied in section 4.7.3. Each metal ion concentration was varied from 5 to 1000 μM whereas the contact time was varied from 5 to 15 minutes. Figure 4.25 shows that the colors of each metal ion complex at the reaction time between 5-15 minutes were not different. This indicated that 5 minutes was enough to reach equilibrium.
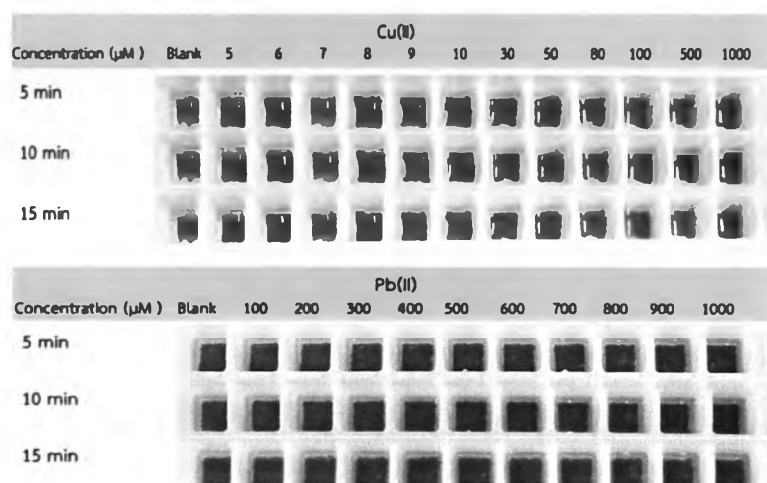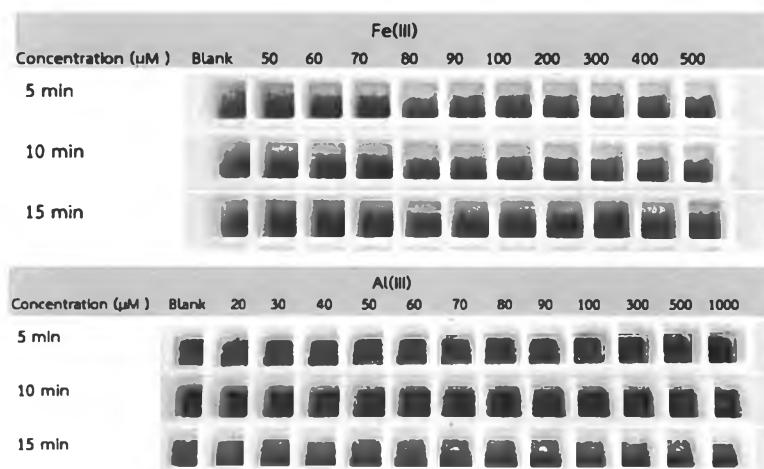
Figure 4.25 Reaction time of Cu(II), Pb(II), Fe(III) and Al(III) determination using AXAD-
7Cy

4.7.5 Interfering ion effect

The interfering ion effect from cations and anions were investigated
under the assumption as described in section 4.2.4. The mixture solution of each
metal ion concentration of 1 mM (except Pb(II) was prepared in the concentration of
0.5 mM) and cations ($CaCl_2$, KCl, $NaNO_3$) or anions (NaCl, KF, Na_2SO_4 and
$Ca(NO_3)_2 \cdot 4H_2O$) were prepared. Cations and anions in the concentration 10 times of
target metal ions whereas, each target metal ions in the same concentration of
standard metal ions were also prepared as interfering ion. Then, the mixture solution
was determined under the optimum qualitative determination of each metal ion.

For Cu(II) detection, naked-eye detection was not effected from any
cations and anions except after adding $CaCl_2$ and $Ca(NO_3)_2 \cdot 4H_2O$, the color of AXAD-
7Cy turned from blue to purple. It can be concluded that Ca^{2+} could affect Cu(II)
detection. It was also found that Ca^{2+} interfered Cu(II) analysis in case of its
concentration higher than 10 times of Cu(II). F can interrupt Al(III) detection to be
AlF_6^{3-} complex as described in cyanidin solution section. However, for Pb(II) and Fe(III)

determination, there were not any interfering effects from both cations and anions including target metal ions as shown in Figure 4.26.
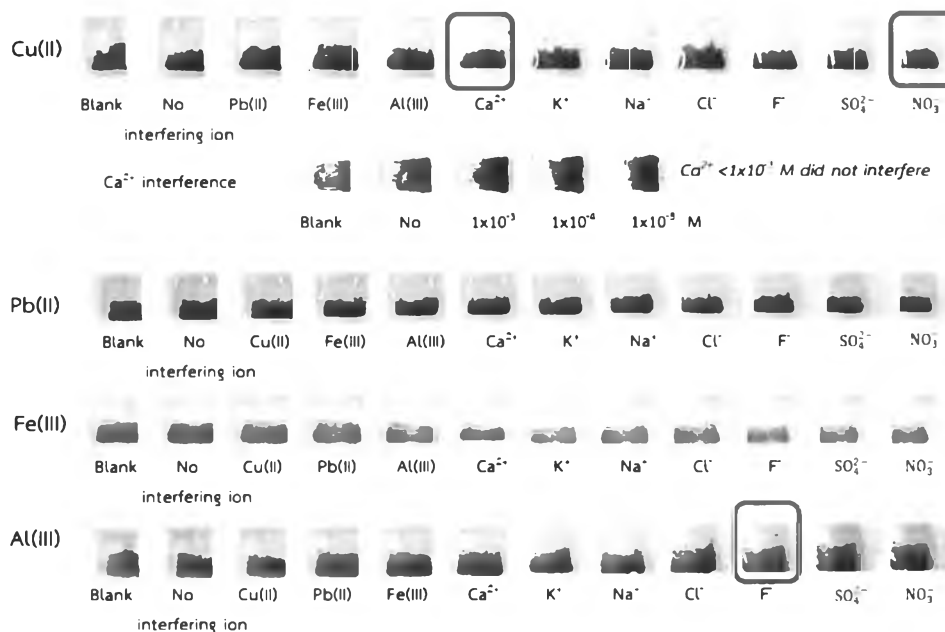

Figure 4.26 Interfering ion effect for determination of Cu(II), Pb(II), Fe(III) and Al(III) using AXAD-7Cy

4.7.6 Summarization of the optimum conditions for qualitative determination using modified solid sorbent

After investigation of several parameters influencing naked-eye detection using AXAD-7Cy, effectively simultaneous determination of these 4 metal ions were summarized. The AXAD-7Cy (condition 2) was first rinsed out with 500 µL of 0.10 M desired buffer solution and then was conditioned again in 100 µL of the same buffer. 1000 µL of water sample was completely mixed with 100 µL of 0.10 M masking agent and then gently poured into conditioned solid sorbent and mixed

together. The optimum conditions of all reagents for simultaneous determination of Cu(II), Pb(II), Fe(III) and Al(III) were summarized in Table 4.11.

Table 4.11 Optimum conditions for simultaneous determination of Cu(II), Pb(II), Fe(III) and Al(III) using AXAD-7Cy

Metal ion	AXAD-7Cy (mg)	0.10 M buffer (μL)	Masking agent (0.10 M, 100 μL)	Standard metal/Sample (μL)
Cu(II)	20	100	-	1000
Pb(II)	20	100	Thiourea + KF	1000
Fe(III)	20	100	-	1000
Al(III)	20	100	Thiourea	1000

The color changes of AXAD-7Cy under the optimum conditions in Table 4.11 are shown in Figure 4.27.
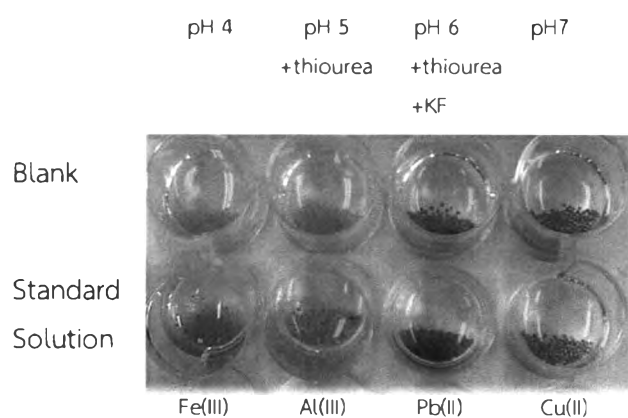
Figure 4.27 Simultaneous determination of Cu(II), Pb(II), Fe(III) and Al(III) using AXAD-7Cy

This developed method was applied for determination of mixed standard metal ions solutions e.g. Pb(II)+Cu(II), Fe(III)+Cu(II), Al(III)+Cu(II) and Al(III)+Pb(II)+Cu(II). It was found that this method can effectively identify all of metal ions in sample solutions as shown in Figure 4.28. For example, the test sample 1 consisted of Pb(II) and Cu(II) the color change was different from blank solution at only pH 6 and 7, respectively.

Sample water	pH4	pH5 +thiourea	pH6 +thiourea + KF	pH7	Metal ion found
Blank					Blank
Test sample 1					Pb(II) +Cu(II)
Test sample 2					Fe(III)+Cu(II)
Test sample 3					Al(III)+Cu(II)
Test sample 4					Al(III)+Pb(II) +Cu(II)

Figure 4.28 Simultaneous determination of mixed standard metal ions

4.8 Quantitative analysis of metal ions using AXAD-7Cy

After finding out the optimum conditions for qualitative determination, the quantitative analysis was also investigated under the optimum conditions for qualitative determination as described in Table 4.11.

The quantitative of naked-eye determination performed as semi-quantitative detection can be divided into 3 color shades depended on metal ion concentration ranges. The quantitative results are shown in Figure 4.29 and Table 4.12.
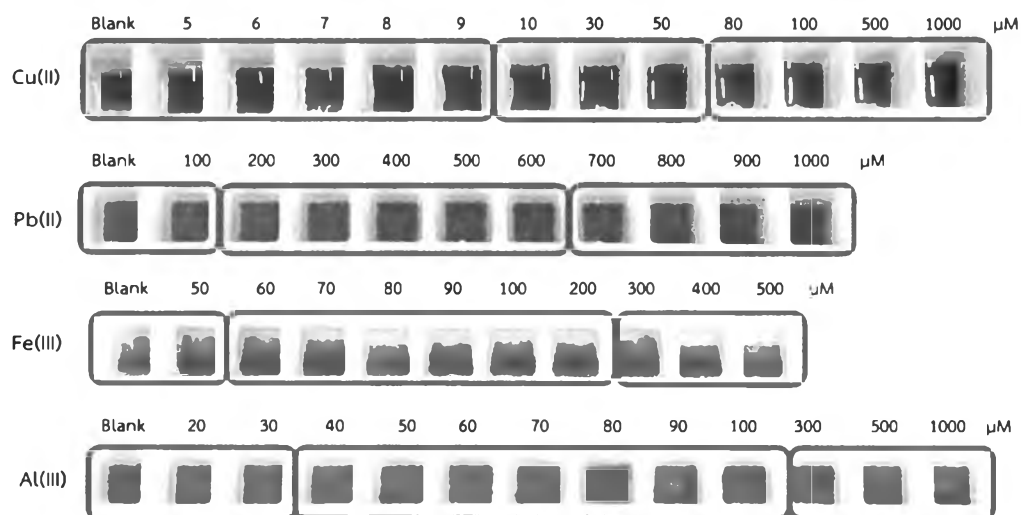
Figure 4.29 Quantitative determination of Cu(II), Pb(II), Fe(III) and Al(III) using AXAD-7Cy

Table 4.12 Semi-quantitative detection and limit of detection of Cu(II), Pb(II), Fe(III) and Al (III) by using AXAD-7Cy

Metal ion	pH	Quantitative determination			LOD
Cu(II)	7	Violet < 10 µM	Violet-blue 10-50 µM	Blue > 50 µM	10 µM
Pb(II)	6	Purple < 200 µM	Violet-blue 200-600 µM	Blue > 600 µM	200 µM
Fe(III)	4	Pink < 60 µM	Pink-Purple 60-200 µM	Purple > 200 µM	60 µM
Al(III)	5	Purple < 40 µM	purple-blue 40-100 µM	Blue > 100 µM	40 µM

For Cu(II) detection, the color change of AXAD-7Cy was observed at the concentration higher than 10 µM. Then, violet-blue color of AXAD-7Cy was turn to blue at the concentration higher than 50 µM. In case of Pb(II) determination, the color of AXAD-7Cy was not different from blank when the concentration of Pb(II) was lower than 200 µM. The violet-blue color of AXAD-7Cy was observed in the Pb(II) concentration range of 200-600 µM whereas the blue color was occurred when the Pb(II) concentration was higher than 600 µM. For Fe(III) detection, the pink-purple color of AXAD-7Cy was observed indicating the concentration of 60-200 µM. The purple color was observed at the concentration higher than 200 µM. For Al(III) detection, the AXAD-7Cy would be turn to purple-blue and blue at the concentration of 40-100 µM and higher than 100 µM, respectively. Limit of detections

(LOD) by naked-eye for Cu(II), Pb(II), Fe(III) and Al(III) using AXAD-7Cy were 10, 200, 60 and 40 µM, respectively. According to the difference in the amount of cyanidin on 20 mg of AXAD-7Cy and in 100 µL of cyanidin solution, and the types of masking agent used, thus, these systems might affect the interaction of cyanidin-metal complex resulting in different LOD of naked-eye detection of both detection methods.

In case the color of AXAD-7Cy in water sample was not different from those in blank solution, it was concluded in 2 points that water sample might be not contaminated with metal ions or might be contaminated at the concentration lower than LOD.

4.9   Method validation for metal ion determination using AXAD-7Cy

This method was validated in the same way of cyanidin solution (section 3.7). Two sample concentrations of each metal ion were detected by naked-eye under the optimum conditions as summarized in Table 4.11 and then compared the results with the actual concentration determined by ICP-OES method.

Table 4.13 shows comparative results between using AXAD-7Cy and ICP-OES method. From this table, it was found that two concentrations of spiked metal ion determined by using AXAD-7Cy were closed to ICP-OES method and almost results from ICP-OES are in the concentration range of naked-eye detection using AXAD-7Cy. Percentage of the recoveries from ICP-OES method were in the acceptable range except Al(III) in both water samples. However, the determination by naked-eye indicated the approximate concentrations of metal ion in both water samples.

The results in Table 4.13 show that the concentrations determined by naked-eye detection are closed to those determined by ICP-OES method. Thus, this developed method could be applied for real water analysis with high accuracy.

Table 4.13 Comparative results of metal ion concentration determined by naked-eye
detection and ICP-OES method using AXAD-7Cy

Water sample	Metal ion	Blank	Concentration (µM)			%Recovery	
			Spiked	ICP-OES	Naked-eye	ICP-OES	Naked-eye
Pond water	Cu(II)	0.005	40	43	10-50	106.7	25-125
			100	94	> 50	94.5	> 50
	Pb(II)	0.001	100	97	< 200	97.0	< 200
			400	389	200-300	97.3	50-75
	Fe(III)	0.005	80	68	60-200	85.5	75-250
			200	192	> 200	95.9	>100
	Al(III)	0.000	80	113	40-100	141.0	50-125
			200	259	> 100	129.4	50
Tap water	Cu(II)	0.005	40	43	10-50	107.9	25-125
			100	98	> 50	98.1	> 50
	Pb(II)	0.001	100	99	< 200	98.5	< 200
			400	397	200-300	99.4	50-75
	Fe(III)	0.008	80	66	60-200	82.5	75-250
			200	193	> 200	96.3	>100
	Al(III)	0.000	80	116	40-100	145.1	50-125
			200	265	> 100	132.6	50

4.10   Application in real water sample using AXAD-7Cy

This developed method was applied for metal ion determination in 30 water samples prepared by spiking 30 different metal ion concentrations, single metal ion or mix metal ions, in pond water. Then, all of water samples were developed color under the optimum conditions of Cu(II), Pb(II), Fe(III) and Al(III) detections as summarized in Table 4.11.

The results from naked-eye detection were compared with spiked value and ICP-OES method in term of both qualitative and quantitative analysis (Table 4.14). The naked–eye detection showed qualitative analysis correctly for 21 samples from 30 samples, representing 70% (see appendix B). For quantitative analysis of these 21 water samples, the concentrations of each spiked metal ion were almost in the acceptable ranges. However, the concentrations of spiked metal ion in other incorrect samples determined by naked-eye were closed to spiked values. Whereas the most qualitative and quantitative results of ICP-OES detection were close to spiking method.

In addition, in case of comparison each metal ion determined by naked-eye and spiked value or ICP-OES method, it was found that the error of both comparisons was quite similarly. Fe(III) detection showed no error for qualitative determination comparing with both spiking and ICP-OES method and the minimal errors about 10% for quantitative was obtained. For Al(III) detection, it showed an error about 10-30% in term of both qualitative and quantitative analysis. Pb(II) and Cu(II) detection showed similarly error about 7% and 20% for qualitative and quantitative determination, respectively when compared with both spiking and ICP-OES methods.

Table 4.14 The results of naked-eye detection using AXAD-7Cy compared with

spiked value and ICP-OES using AXAD-7Cy

Unknown	Spike		Remark	Naked-eye		ICP-OES	
	Metal ion	Conc. (μM)		Metal ion	Conc. (μM)	Metal ion	Conc. (μM)
pond	-	-		-	-		
1				Pb(II)	200-300		
	Cu(II)	50		Cu(II)	10-50	Cu(II)	46
2	Pb(II)	300		Pb(II)	200-300	Pb(II)	258
3	Fe(III)	100		Fe(III)	60-200	Fe(III)	101
				Al(III)	40-100		
4	Al(III)	100				Al(III)	101
5	Pb(II)	100	spike Pb(II) < LOD			Pb(II)	93
	Cu(II)	50		Cu(II)	10-50	Cu(II)	51
6	Al(III)	60		Al(III)	40-100	Al(III)	61
	Cu(II)	100		Cu(II)	> 50	Cu(II)	107
7	Fe(III)	200		Fe(III)	> 200	Fe(III)	194
	Cu(II)	30		Cu(II)	10-50	Cu(II)	32
8	Al(III)	100		Al(III)	40-100	Al(III)	142
	Pb(II)	500		Pb(II)	40-600	Pb(II)	428
9	Fe(III)	50		Fe(III)	60-200	Fe(III)	54
	Al(III)	100				Al(III)	101
10	Al(III)	40		Al(III)	40-100	Al(III)	43
	Pb(II)	400		Pb(II)	200-300	Pb(II)	349
	Cu(II)	10				Cu(II)	13
11	Fe(III)	100		Fe(III)	60-200	Fe(III)	112
	Al(III)	100		Al(III)	40-100	Al(III)	103
	Cu(II)	10		Cu(II)	10-50	Cu(II)	12
12	Al(III)	20	spike Al(III) < LOD	Al(III)	40-100	Al(III)	21
	Cu(II)	20		Cu(II)	10-50	Cu(II)	21
13	Cu(II)	50		Cu(II)	> 50	Cu(II)	53
14	Al(III)	300		Al(III)	> 100	Al(III)	351
	Pb(II)	400		Pb(II)	200-300	Pb(II)	408
15	Fe(III)	300		Fe(III)	> 200	Fe(III)	324

Table 4.14 (continued)

Unknown	Spike			Naked-eye		ICP-OES	
	Metal Ion	Conc. (µM)	Remark	Metal ion	Conc. (µM)	Metal Ion	Conc. (µM)
16	Al(III)	300		Al(III)	40-100	Al(III)	254
	Pb(II)	400		Pb(II)	> 600	Pb(II)	430
17	Fe(III)	70		Fe(III)	60-200	Fe(III)	74
	Al(III)	100				Al(III)	105
				Cu(II)	10-50		
18	Al(III)	300		Al(III)	40-100	Al(III)	351
19	Pb(II)	300		Pb(II)	200-300	Pb(II)	263
20	Al(III)	30	spike Al(III) < LOD			Al(III)	33
	Pb(II)	300				Pb(II)	264
	Cu(II)	30		Cu(II)	10-50	Cu(II)	31
21				Al(III)	40-100		
	Pb(II)	50	spike Pb(II) < LOD			Pb(II)	53
	Cu(II)	50		Cu(II)	10-50	Cu(II)	53
22	Pb(II)	100				Pb(II)	97
23	Al(III)	300		Al(III)	> 100	Al(III)	312
	Pb(II)	400		Pb(II)	400-600	Pb(II)	349
24	Cu(II)	50		Cu(II)	> 50	Cu(II)	53
25	Al(III)	50		Al(III)	40-100	Al(III)	55
	Cu(II)	50		Cu(II)	> 50	Cu(II)	52
26	Fe(III)	200		Fe(III)	> 200	Fe(III)	222
	Pb(II)	200		Pb(II)	200-300	Pb(II)	190
27	Fe(III)	100		Fe(III)	60-200	Fe(III)	115
	Cu(II)	30		Cu(II)	> 50	Cu(II)	32
28	-	-					
29	Al(III)	100		Al(III)	40-100	Al(III)	107
	Cu(II)	100		Cu(II)	> 50	Cu(II)	104
30	-	-					

CHAPTER V

CONCLUSIONS

This research focused on using a natural product as colorimetric reagent modified on suitable solid sorbent for determination of some metal ions. Cyanidin from red cabbage was the best choice because its structure consists of *ortho*-dihydroxyl group at B-ring which can coordinate with various metal ions. Cyanidin was extracted under methanolic hydrochloric acid solution and its characteristics were proved by UV-vis spectrophotometry, [1]H-nuclear magnetic resonance spectrometry (^{1}H-NMR) and mass spectrometry (MS). The UV-visible spectrum at 280 nm and 330 nm showed flavonoid and phenolic structure, respectively. Moreover, spectrum at 535 nm was illustrated the anthocyanin chromophore.

At first step, metal ion detection was studied in the form of cyanidin solution. It was found that only complexation of Cu(II), Pb(II), Fe(III) and Al(III) and cyanidin could be appeared at various pH ranges, for example, pH 5-8, pH 5-6, pH 3-4 and pH 3-6 for Cu-Cy, Pb-Cy, Fe-Cy and Al-Cy complex, respectively. As the results some metal ions could be determined at the same conditions. To discriminate these target metal ions individually, masking agents have also been investigated. It was found that KF showed completely mask Al(III) to be Al-F complexes whereas DMG shows potentially mask Cu(II) to be $Cu(DMG)_2$. Thus, the simultaneous determination of Cu(II), Pb(II), Fe(III) and Al(III) were obtained under the following optimum conditions. Cu(II) detection, the solution should be controlled at pH 7 without any masking agent. Pb(II) determination, the solution pH 6 operated together with KF and DMG as the masking agent was appropriate. For Fe(III) determination, it was detected in buffer solution pH 4 employed KF as masking agent. Finally, Al(III) was analyzed in buffer solution pH 5 along with DMG as masking agent. No interfering effects from cations,

anions and even target metal ions were observed. The quantitative analyses were performed as semi-quantitative determination depended on metal ion concentration ranges. It was found that the lowest naked-eye detections for Cu(II), Pb(II), Fe(III), and Al(III) were 50, 80, 200 and 50 µM, respectively.

Next step, cyanidin was modified on AXAD-7 resin to be AXAD-7Cy by soaking AXAD-7 in cyanidin solution for 24 hours and drying using rotary evaporator. Cyanidin was prepared in methanolic hydrochloric acid solution, in this study, 0.6% v/v HCl/MeOH was appropriate. It was found that Cu(II), Pb(II), Fe(III) and Al(III) contributed the color change on AXAD-7Cy due to the complexation. The simultaneous determination of these four metal ions was operated under pH 7, pH 6, pH 4 and pH 5 for Cu(II), Pb(II), Fe(III), and Al(III), respectively, as the same results testing in cyanidin solution form. Thiourea and the mixture of thiourea and KF were used as masking agents for Al(III) and Pb(II) determination, respectively. F^- interfered Al(III) detection whereas Ca^{2+} in the concentration more than 1 mM also affected Cu(II) analysis. The quantitative reults were silmilar to those reported in using cyanidin solution which performed as semi-quantitative detection. The detection limit observed by naked-eye was 10, 200, 60 and 40 µM for Cu(II), Pb(II), Fe(III), and Al(III) analysis, respectively. The stability of AXAD-7Cy kept in dark and dry container was at least 60 days.

In addition, cyanidin in form of both solution and modified on AXAD-7 could be potentially applied in real water sample (tap and pond water) without interfering effects and showed high accuracy. It also determined the target metal ions at the low level (micromolar) with simplicity, rapidity, low cost, no sample preparation requirement, and environmental friendly detection due to using low volume of all reagents. Interestingly, the colorimetric reagent used is also very cheap because cyanidin is a natural product and easily extracted from any plants.

When compared this research with others previous study that also using natural pigment as colorimetric reagent, it was found that this study showed the detection limit at the micromolar level but still higher than those reported in others previous research. However, previous studies could be only applied for a few selectively metal ions target determination and have to operate under instrumental operation. In this present study, it can be applied for simultaneous determination up to 4 metal ions along with simply naked-eye detection. Moreover, it also showed alternative application for using this reagent in form of both solution and modified solid sorbent.

Suggestions for further work

This naked-eye detection method should validate using more different operators for more consistency.

Although this research proposed that cyanidin in form of both solution and solid sorbent can be applied for determination of these four metal ions, the use of the modified solid sorbent seems to be easier and faster for onsite detection than cyanidin solution form. Moreover, it was prepared in high mass quantity and kept for a long time period.