



## Simple Detection Kit for Copper (II) Ion in Water using Solid Sorbent Modified with Cyanidin Extracted from Red Cabbage

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### Abstract

A simple copper (II) ion ( $\text{Cu}^{2+}$ ) test kit was developed in this work using a cheap and environmental-friendly solid sorbent modified with cyanidin extracted from red cabbage. The best solid sorbent was found to be a cation-exchange resin (Pall Tech: PTNR 8715, Nuclear Grade ion Exchange Resin). The optimum ratio of cyanidin : resin was 1.6 mg : 2 g giving a clear naked-eye detection of interactive  $\text{Cu}^{2+}$ . The detection of  $\text{Cu}^{2+}$  could be able to change the color of modified resin at their different concentrations along with double rinsing the modified resin with a few solution of pH 7 buffer and mixing with 1 mL of water sample. To evaluation, the results from naked-eye detection were compared with those of Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP-OES), and there was no significant difference noticed. The lowest detection limit of  $\text{Cu}^{2+}$  was 0.70 ppm which was lower than the drinking water contaminant standards. The modified resin might probably not able to change the color in case of high concentration of Calcium ion ( $\text{Ca}^{2+}$ ). However, this novel test kit could be applied in real water sample for preliminary screening and detecting the  $\text{Cu}^{2+}$  contamination with simplicity and also environmental-safety.

**Keywords:** red cabbage, copper (II) ion, cyanidin, test kit

### Introduction

Copper bioavailability is the amount of copper forms available for intake by living organisms. Such copper forms could be delivered to other organisms including human through food chains and cause health risks when they absorb too much copper

Copper is released into water sources by natural processes and also human activities such as mining operations, industrial discharge, and copper-containing waste disposal. Exposure to copper causes health problems because it can interfere with cell permeability at the cell membrane and may disrupt enzyme activities and cell division. The toxicity of copper depends on the chemical composition of different copper forms and exposure routes, for example, inhalation, skin contact, and digestive system. (Wang, Fan, Li, & Feng, 2008)

The detection of copper in water is currently carried out by using well-known techniques such as UV-Visible Spectrophotometry, Atomic Absorption Spectrophotometry (AAS), and Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP-OES). However, these standard techniques need skills, knowledge, and experience in laboratory. Moreover, they are not portable, expensive, chemical-consuming, and environmental-unfriendly.

The effect of metal ions on pigment stability in plants has been widely studied. The blue pigment comes from the complex formation between anthocyanins and some metals such as Al, Fe, Cu, Sn, Mg, Mo (Kerry et al., 2001, Khaodee, Wongkiti & Madang, 2017). Many researchers have also explored the color changes in cabbage tissues. It was found that a stable blue pigment is due to the anthocyanin-molybdenum complexation. Additionally, the effect of pH on the complex formation between *o*-di-hydroxyl anthocyanins and Fe(III) or

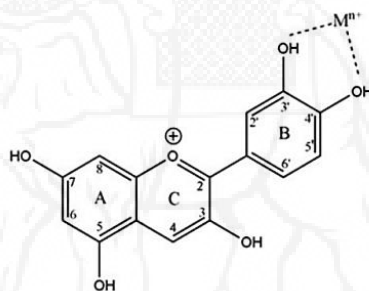
Mg(II) ions has been investigated. The optimum pH of 5 is suitable for a blue pigment formation in most plants with a stoichiometric ratio of anthocyanin: Fe(III) of more than or equal to 1 : 6 (Castaneda, Pacheco, Paez, Rodriguez, & Galan, 2009).

Cyanidin can form complexes with metal ions because of its electron-donating nucleophilic property. Khaodee, Aeungmaitrepirom, and Tuntulani (2014) reported the pH-dependent simultaneous naked-eye detection of Cu(II), Pb(II), Al(III) and Fe(III) using cyanidin extracted from red cabbage (*Brassica Oleraceae Var. Rubra*, Figure 1) as chelating agent.



**Figure 1** Red cabbage

Qualitative and quantitative naked-eye analysis of such metal ions were carried out based on the interaction between metal ions and *o*-dihydroxyl group (ring B) of cyanidin as presented in Figure 2, giving a complex absorbed the light at higher wavelength.



**Figure 2** Metal-Cyanidin complex formation (Khaodee et al., 2014)

Besides that, there are many reports applying reagent on solid sorbent to be many proposes such as coloring sensor, solid phase extraction. Solid sorbents such as AXAD-7 (Hosseini et al., 2010), silica gel (Shabani, Dadfarnia, & Dehghani, 2009), activated carbon (Lyubchik et al., 2008, Ahn, Woo, & Park, 2008), agricultural waste (Shukla & Pai, 2005) were normally because there are high porous and stability.

Therefore, the aim of this work was to develop a cheap test kit protocol based on the red cabbage extracts modified on a local material for a low chemical consuming, safe, and environmental-friendly detection of copper in water.

## Methods and materials

### 1. Instruments

4-decimal balance (METTLER TOLEDO, AG204, Switzerland), 5-decimal balance (OHAUS, AX224, USA), Rotary Evaporator (BÜCHI, B-480/R-124, Switzerland), pH Meter (Metrohm, 827 pH lab, Switzerland), UV-Visible spectrophotometer (UV-1601, Shimadzu, Japan)



## 2. Chemicals

Copper (II) Nitrate Trihydrate ( $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ , AR grade, 98.0%, MW = 241.60 g/mol, UNIVAR, Australia), Copper standard solution 1,000 ppm (Merck, Germany), Nitric Acid ( $\text{HNO}_3$ , AR grade, 65.0%, MW = 63.01 g/mol,  $d = 1.40 \text{ g/cm}^3$ , LAB-SCAN, Ireland), Acetic Acid Glacial ( $\text{CH}_3\text{COOH}$ , AR grade, 99.7%, MW = 60.05 g/mol,  $d = 1.05 \text{ g/cm}^3$ , RCI Labscan, Thailand), Hydrochloric Acid ( $\text{HCl}$ , AR grade, 37.0%, MW = 36.46 g/mol,  $d = 1.19 \text{ g/cm}^3$ , RCI Labscan, Thailand), Sodium Dihydrogen Phosphate Monohydrate ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ , AR grade, 99.0%, MW = 137.99 g/mol, BDH, England), Sodium Acetate Trihydrate ( $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ , AR grade, 99.5%, MW = 136.08 g/mol, RCI Labscan, Thailand), Sodium Hydroxide ( $\text{NaOH}$ , AR grade, 97.0%, MW = 40.00 g/mol, UNIVAR, Australia), Disodium Hydrogen Phosphate ( $\text{Na}_2\text{HPO}_4$ , AR grade, 99.0%, MW = 141.96 g/mol, MERCK, Germany), Methanol ( $\text{CH}_3\text{OH}$ , AR grade, 99.9%, MW = 32.04 g/mol,  $d = 0.79 \text{ g/cm}^3$ , RCI Labscan, Thailand) Resin Pall Tech (PTNR 8715, Nuclear Grade ion Exchange Resin)

## 3. Reagent preparation

This extraction method was slightly modified from Khaodee et al. (2014). Red cabbage 200 g was cleaned and sliced to be small pieces and then was soaked in 400 mL of the mixture of 1% w/w HCl in methanol for 72 hours. After that, the solution was slowly filtered and purified using  $\text{CHCl}_3$  to remove non polar molecules. The purified solution was then refluxed under acid condition until deep violet solution appeared, kept in refrigerator immediately. The dark brown powder would be precipitated. It was then filtered and kept in desiccators until use. Characterization was done by UV-Visible spectrophotometry using 1% w/w HCl in methanol as solvent.

## 4. Preparation of Cation exchange resin coated with cyanidin (CRCC)

Dark brown powder 1.6, 3.2 and 6.4 mg were dissolved in 5 mL of the mixed solvent (1% w/w HCl in MeOH). These 3 solutions were gently poured into 2 g of cation exchange resin, mixed well and leaved in the dark overnight. All 3 conditions of the coated resin (CRCC) were then rotary evaporated to remove residual solvent and should be kept in dark and airtight container.

## 5. pH effect of CRCC

Three conditions of CRCC were checked the color appearance under difference pH solutions (3-7). CRCC 20 mg were double rinsed for 30 seconds with 1 mL of tested buffer solution (0.1 M). Then, 1 mL of those tested buffer solution were mixed with CRCC again. Observed the color of CRCC in difference pH solutions.

## 6. Determination of $\text{Cu}^{2+}$ by naked eye detection

CRCC about 20 mg were weighted and transferred into 10 cleaned wells before conditioned by double rinsing with 1 mL of pH7 buffer solution. To determination of  $\text{Cu}^{2+}$  concentrations, 1 mL of pH7 buffer solution was mixed with CRCC for 30 seconds, then, 1 mL of standard  $\text{Cu}^{2+}$  solutions in the concentrations of 0.30, 0.40, 0.50, 0.70, 1.20, 2.00, 5.00, 12.0 and 20.00 ppm were added into wells number 2-10. All color changed were compared with CRCC in the first well which using deionized water as blank solution.

## 7. Interference effect of $\text{Cu}^{2+}$ determination

CRCC were prepared in 5 sets (one for control set and another 4 for test sets). All sets were conditioned and mixed with  $\text{Cu}^{2+}$  concentrations follow by mention above. For 4 test sets, standard  $\text{Cu}^{2+}$  of all

concentrations were added with each 0.1 M of 4 interfered ions ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ) in each test set. All color changed were compared with CRCC in control set.

### 8. $\text{Cu}^{2+}$ test kit preparation and method validation

Buffer solution (0.1 M, pH7) was contained in brown bottle. CRCC in amount of 20 mg were packed in tightly small glass bottle. In test kit box, there was 1 mL of syringe for sample solution.  $\text{Cu}^{2+}$  in water sample in 3 concentrations (0.37 ppm, 12.4 ppm and 20.0 ppm) were determined by this test kit compared the results with ICP-OES method

## Results and Discussion

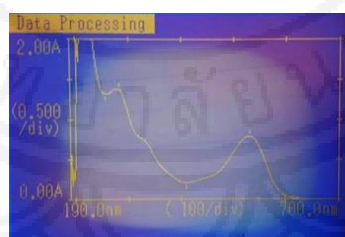
### 1. Reagent extraction and purification

After red cabbage was soaked in solvent (1% w/w HCl in MeOH) and refluxed under acid condition, the dark brown powder was obtained as shown in Figure 3 which could be filtered by filter paper no 1. This powder should be kept in dark desiccator until use.



**Figure 3** Dark brown powder of cyanidin

UV-Visible spectrum of dark brown powder in 1% w/w HCl in MeOH solution was shown in Figure 4. It was reported that cyanidin is the major type of anthocaynin found in red cabbage. Cyanidin is nucleophile property which can interact with  $\text{Cu}^{2+}$  at ortho-di hydroxyl group of phenolic structure. This complex would change cyanidin color into the longer wavelength.



**Figure 4** UV-Visible spectrum of dark brown powder in 1% w/w HCl in MeOH solution

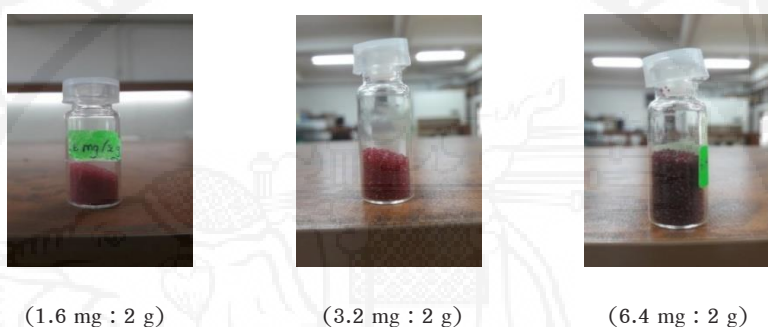
### 2. Modification of resin

Resin used in this research was Pall Tech (PTNR 8715, Nuclear Grade ion Exchange Resin) which is cation exchange resin (Figure 5) for high water purification. It is colorless to light yellow.



**Figure 5** Pall Tech (PTNR 8715, Nuclear Grade ion Exchange Resin)

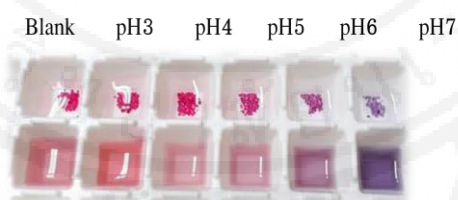
Cation exchange resin coated with cyanidin (CRCC) was done by comparing 3 ratios of dark brown powder and resin. It was found that cyanidin could be modified on cation resin perfectly via hydrogen bonding. It could not diffuse to water phase for long time soaking. The colors of modified resins were pink to dark purple as shown in Figure 6.



**Figure 6** Appearance of modified resin with cyanidin

### 3. pH effect of CRCC

























After buffer solution pH 3–7 were added into CRCC, the colors depended on acid or basic condition (Figure 7). Red color appeared under acid solution, whereas, purple color showed in neutral solution. This result indicated that dark brown powder was cyanidin that could be modified on cation exchange resin.



**Figure 7** CRCC in difference pH solution

Khaodee et al. (2014) reported that cyanidin is one type of anthocyanin extracted from red cabbage which could be formed complex with  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$  contaminated in water sample. The detection limits of  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$  detection were 50, 80, 50 and 200  $\mu\text{M}$ , respectively under various pH solution. In this report,  $\text{Cu}^{2+}$  was determined in 0.1 M pH 7 buffer solution by naked eye detection. So in this work, 3 conditions of CRCC would be tested followed this report. It was found that CRCC made from 1.6 mg of dark brown powder and 2 g of resin was clearly observed the difference color shade depending on  $\text{Cu}^{2+}$  concentrations as shown in Figure 8.

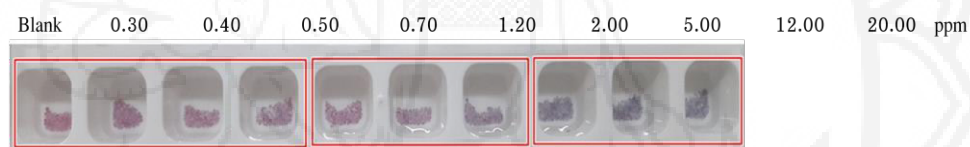


Cyanidin : Resin (by weight)	Cu <sup>2+</sup> concentration (ppm)							
	Blank	50.0	25.0	12.5	5.0	3.0	2.0	1.0
1.6 mg : 2 g								
3.2 mg : 2 g								
6.4 mg : 2 g								

**Figure 8** Color shades of all CRCC in various Cu<sup>2+</sup> concentrations

#### 4. Determination of Cu<sup>2+</sup> by naked eye detection

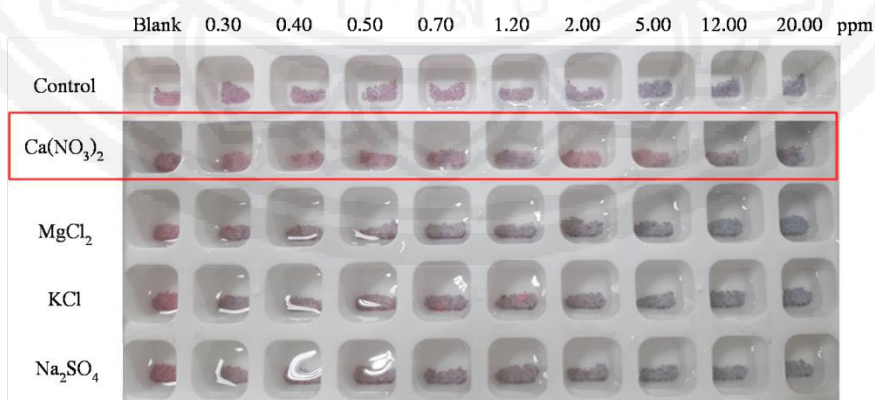
The results showed that CRCC in pH 7 solution of various concentrations of Cu<sup>2+</sup> could be separated into 3 color shades depended on Cu<sup>2+</sup> concentration (Figure 9). For Cu<sup>2+</sup> concentration lower than 0.70 ppm, the CRCC color was not difference from blank, whereas, light purple color were observed in the concentrations range of 0.70 – 2.00 ppm. Moreover, blue color would be appeared in Cu<sup>2+</sup> concentration more than 2.00 ppm. This research showed the high efficiency that the detection limit of Cu<sup>2+</sup> determination by naked eye detection (0.70 ppm) was lower than the standard level of drinking water which control Cu<sup>2+</sup> concentration not over 1 ppm.



**Figure 9** CRCC in various Cu<sup>2+</sup> concentrations

#### 5. Interference effect of Cu<sup>2+</sup> determination

The interfering ions studied in this research were Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup> and Na<sup>+</sup> which were normally found in natural water. The result showed that all cations could not interfere Cu<sup>2+</sup> determination except Ca<sup>2+</sup> which could remain CRCC color although increasing of Cu<sup>2+</sup> content (Figure 10).



**Figure 10** Interference effect of Cu<sup>2+</sup> determination



$\text{Ca}^{2+}$  was divalent ion and it might has been strongly exchangeable efficiency more than  $\text{Cu}^{2+}$ . The reaction of  $\text{Ca}^{2+}$  and exchangeable resin is shown below. Thus, to get rid other divalent ions, water sample might be pass through cationic exchange resin before determination of  $\text{Cu}^{2+}$ .



## 6. Determination of $\text{Cu}^{2+}$ by using test kit and method validation

This test kit was prepared by using all small reagent volume and could be used for screening  $\text{Cu}^{2+}$  contaminated in water sample as shown in Figure 11.



Figure 11  $\text{Cu}^{2+}$  test kit

For method validation, 3 concentrations of  $\text{Cu}^{2+}$  were accurately determined by ICP-OES method. It was found that, 3 concentrations of  $\text{Cu}^{2+}$  were 0.37 ppm, 12.4 ppm and 20.0 ppm. CRCC in amount of 20 mg contained in 3 glass bottles were double rinsed using 0.1 M of buffer pH7 and soaked in this solution again for 30 seconds. After that, 1 mL of water sample was added into each those CRCC and mixed well. Then the colors of all CRCC were observed concentration range shade followed Figure 10. The results indicated that  $\text{Cu}^{2+}$  concentrations determined by the test kit were related with the results obtained from ICP-OES method as shown in Table 1.

Table 1  $\text{Cu}^{2+}$  concentration determined by ICP-OES method and the test kit

Item	$\text{Cu}^{2+}$ concentration (ppm)	
	ICP-OES method	$\text{Cu}^{2+}$ test kit
1	0.37	< 0.70
2	12.4	0.70 – 5.0
3	20.0	> 5.0



## Conclusion

Cation exchange resin modified with cyanidin extracted from red cabbage called CRCC could be used for determination of  $\text{Cu}^{2+}$  in water sample.  $\text{Cu}^{2+}$  determination by naked eye detection was operated by double rinsing CRCC with 0.1 M of buffer pH 7 and soaked in this solution again for 30 seconds before adding 1 mL of water sample. Light purple to blue color would be observed at  $\text{Cu}^{2+}$  different concentrations. The lowest of detection limit of  $\text{Cu}^{2+}$  was 0.7 ppm which was lower than the standard level of drinking water contaminant (1 ppm). The  $\text{Cu}^{2+}$  test kit fabricated by packing in a portable box with user manual and standard color chart was then used in real applications for preliminary screening and detecting the metal contamination. Moreover, this heavy metal test kit could be applied to determine the low levels of metal ions in water samples with an easy and environmental friendly.

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