



The Application of Using Natural Reagent Extracted from Purple Sweet Potato for Naked-Eye Detection of Copper in Water Samples

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Abstract

Reagent extracted from purple sweet potato was used to enable detection of Cu^{2+} in water by the naked-eye. The optimum conditions for this were determined by adding 10 μL of reagent to a mixture of 500 μL of buffer pH 7 (0.01 M) and a 200 μL water sample. The lowest concentration of Cu^{2+} able to be detected by the naked-eye was 3×10^{-4} M without any interfering effects. This method was validated by comparing Cu^{2+} concentration detected with the AAS technique. The results show that Cu^{2+} concentrations obtained from both techniques were similar.

Keywords: Purple sweet potato, Copper (II), Anthocyanin, Cyanidin, Naked-eye detection

Introduction

Water contamination or pollution results in lowered water quality, with reduction in dissolved oxygen and high levels of chemicals, such as Lead (Pb), Copper (Cu), mercury (Hg), Arsenic (As), Cadmium (Cd). Contaminated wastewater released from industries involved in the production of copper pipes or copper electric wiring contains Cu, while other manufacturing enterprises are often responsible for releasing chemical-contaminate wastewater, from leather and cloth dyeing operations, and agricultural use of herbicides and pesticides also contributes to environmental pollution, including contamination of ground water supplies. Humans, animals and fish can become ill, and die, from the effects of these chemicals when consumed in drinking water or contaminated food.

Cu^{2+} in appropriate amounts is necessary for muscle and bone growth and development, and is easily absorbed in the stomach and upper gastrointestinal tract, but it is harmful if taken in excessive quantities, resulting in nausea, vomiting, abdomen and muscle inflammation, diarrhea, and abnormal heart function. In addition, it could depress the immune system and may result in mental disorders. Accumulation of Cu^{2+} for a long time, with the liver being unable to regularly remove the Cu^{2+} may result in Wilson's Disease. Metal ions are able to be determined by UV-Visible Spectrophotometer, Atomic Absorption Spectrophotometer (AAS) and Inductively Coupled Plasma (ICP), which are the usual, standard, methods used, with high accuracy and precision. However, these methods are costly, use volumes of highly hazardous chemicals, and require expert operators. Test kit is one of the way to screen target metal ions. There are many chemical using determine some metal ion such as diphenylcarbazide, Triazine derivative and Formaldoxime were used for detection of Cr^{6+} , Fe^{3+} and Mn^{2+} in water, respectively (<http://www.alltestkit.com>). In addition, 1,5-diphenylcarbohydrazide was the reagent using for analysis of Cu^{2+} constituent in serum and urine sample (Mikac-Dević, 1969). These are all synthesized chemicals that could affect the environment in the future.

Some natural reagents were reported that they were a co-pigment which can interact with some metal ions and then be changed original properties such as optical property and high stability. (Castaneda-Ovando, Pacheco-Hernandez, PaezHernandez, Rodriguez, and Galan-Vidal, 2009; Rein, 2005). Furthermore, Moncada et al. (2003) found out the complexation of Al-anthocyanin showed highly stable blue-violet color because of o-di-hydroxyl group of anthocyanin interacting with Al^{3+} ion. This effect also reduced the oxidation reaction of anthocyanin molecule. Ukwueze, Nwadinigwe, Okoye, and Okoye (2009) extracted 3,3',4',5,7-pentahydroxyflavylium from *Hibiscus rosa-sinensis* L. (*Malvaceae*) to be a ligand for Pb(II), Cd(II) and Cr(III) complexation. Using different solvent compositions (methanol and ethanol), and different pH solutions, resulted in several colors of complex compounds. The absorbance was in direct variation with the concentrations of the complex compounds. Yoshida, Kitaharato, and Kondo (2006) studied blue color plant cells and concluded that pH 5 was important for o-di-hydroxyl anthocyanins and Fe(III) or Mg(II) interaction. Whereas, Khaodee, Aeungmaitrepirom, and Tuntulani (2014) used cyanidin extracted from red cabbage as a reagent for simultaneous determination of Cu (II) Pb (II) Al (III) and Fe (III). Color appearance depended on the metal complexation at difference pH levels in the tested solutions. This technique was applied for both qualitative and quantitative naked-eye detection. The detection limits of Cu (II) Pb (II) Al (III) and Fe (III) were 50 μ M, 80 μ M, 50 μ M and 200 μ M, respectively. The interaction of cyanidin and metal ion through o-di-hydroxyl group (B ring) is shown in Figure 1. This action causes longer wavelength absorption.

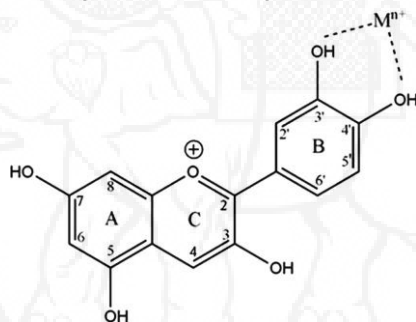


Figure 1 The interaction between cyanidin and metal ion (Khaodee et al., 2014)

Purple Sweet Potato (Figure 2) has a high level of anthocyanin which is a major antioxidant. The **anthocyanin** could help to slow down cell degeneration, and reduce the risk of heart disease and stroke (Kidmose, Edelenbos, Nørbæk, & Christensen, 2002; Steed & Truong, 2008).



Figure 2 Purple sweet potato

In this research focused on using anthocyanin from purple sweet potato as a color reagent for some metal ions. This study used local plants which may provide a low-cost, easy to use and environmentally friendly.



Methods and Materials

Instruments

UV-Visible Spectrophotometer (SHIMADZU, UV-1601, JAPAN), Atomic Absorption Spectrophotometer (AAS, SHIMADZU, AA 6200, JAPAN)

Chemicals

Chromium (III) nitrate ($\text{Cr}(\text{NO}_3)_3$, 96%, AR, UNILAB, Australia), Sodium Sulphate Anhydrous (Na_2SO_4 , 99%, AR, UNIVAR, Australia), Cobalt (II) nitrate ($\text{Co}(\text{NO}_3)_2$, 99%, AR, QRĒCTM, New Zealand), Copper (II) nitrate ($\text{Cu}(\text{NO}_3)_2$, 99.5%, AR, QRĒCTM, New Zealand), Sodium acetate (CH_3COONa , 99%, AR, QRĒCTM, New Zealand), Lead (II) nitrate ($\text{Pb}(\text{NO}_3)_2$, 99.5%, AR, CARLO ERBA, Italy), Nickel(II) nitrate ($\text{Ni}(\text{NO}_3)_2$, 98%, AR, Fluka, Switzerland), Zinc (II) nitrate ($\text{Zn}(\text{NO}_3)_2$, 99%, AR, MERCK, Germany), Manganese (II) nitrate ($\text{Mn}(\text{NO}_3)_2$, 99%, AR, MERCK, Germany), Potassium Chloride (KCl, 99.8%, AR, Fisher Scientific, India), Magnesium Chloride Hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 99.82%, AR, Fisher Chemicals, India), Calcium Nitrate ($\text{Ca}(\text{NO}_3)_2$, 98%, AR, RANKEM, India), Ethanol ($\text{C}_2\text{H}_5\text{OH}$, Commercial, 95%, $d = 0.789 \text{ g/cm}^3$, RCL Labscan Limited, Thailand), Chloroform (CHCl_3 , AR, 99.8%, $d = 1.489 \text{ g/cm}^3$, RCL Labscan Limited, Thailand), Acetic Acid, (CH_3COOH , AR, 99.8%, $d = 1.05 \text{ g/cm}^3$, RCL Labscan Limited, Thailand)

Method

Extracting the Reagent from Purple Sweet Potato

Fresh purple sweet potatoes were cleaned with DI water, then sliced into small pieces. About 100 g of the pieces were placed in each of 3 beakers, and 100 mL of DI water, acetone and ethanol were added into each beaker, which were then placed in a refrigerator for 72 hours, and the solutions were then filtered through Whatman filter paper no.1.

Purification of reagent extracted

A 100 mL volume of the reagent extracted from the sweet purple potatoes was mixed with 50 mL of chloroform in a separation funnel. The chloroform layer was then removed, and more chloroform was added twice more. The purified reagent was characterized by the UV-Visible Spectrophotometry.

Qualitative determination of metal ions

Buffer solution (0.10 M) pH range of 3-6 ($\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$) and 7-8 ($\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$) was prepared. 500 μL of all pH buffers solutions were transferred into each of the well plate followed by 1 drop of 0.01 M of metal ion solution (Co^{2+} , Cu^{2+} , Pb^{2+} , Ni^{2+} , Mn^{2+} , Cr^{3+} , Zn^{2+}). Finally, 1 drop of the reagent extracted from the sweet purple potatoes was added, and the solution was gently mixed, and the color change observed by comparing with a blank using 1 drop of DI water as the sample solution.

Optimization of reagent volume for determination of Cu^{2+} by naked-eye detection

Buffer solution pH 7 (0.01 M) in the volume of 500 μL was mixed with 200 μL of various concentrations of Cu^{2+} (0.00, 5×10^{-5} , 8×10^{-5} , 1×10^{-4} , 3×10^{-4} , 5×10^{-4} , 8×10^{-4} , 1×10^{-3} , 3×10^{-3} and 5×10^{-3} M). Four sets of each of these mixtures were prepared, and a reagent volume of 5, 10, 20 and 30 μL were individually added to each set, which were then mixed well and the color change of all solution observed by naked-eye comparison with a blank that did not contain Cu^{2+} .

Interfering effect

A 500 μL volume of buffer solution at pH 7 (0.01 M) was mixed with 200 μL of various Cu^{2+} concentrations (0.00, 5×10^{-5} , 8×10^{-5} , 1×10^{-4} , 3×10^{-4} , 5×10^{-4} , 8×10^{-4} , 1×10^{-3} , 3×10^{-3} and 5×10^{-3} M). Five sets of each of these mixtures were prepared, and 20 μL of 0.1 M $\text{Ca}(\text{NO}_3)_2$, MgCl_2 , KCl and Na_2SO_4 were individually added as interfering ions of each 4 sets; the 5th set was prepared as the control set which did not contain interfering ions. When 10 μL of reagent was dropped into each set, the color change was observed by comparison with the control set in the same Cu^{2+} concentrations.

Quantitative analysis of Cu^{2+} by naked eye detection

A 500 μL volume of buffer solution at pH 7 (0.01 M) was mixed with 200 μL of various Cu^{2+} concentrations (0.00, 5×10^{-5} , 8×10^{-5} , 1×10^{-4} , 3×10^{-4} , 5×10^{-4} , 8×10^{-4} , 1×10^{-3} , 3×10^{-3} and 5×10^{-3} M), and 10 μL of reagent was dropped into each individual sample solution. The color shades were grouped depending on the Cu^{2+} concentration range.

Application in real water sample

Reagent extracted from purple sweet potato was applied to determine Cu^{2+} in a real water sample. The water sample was wastewater from a laboratory, which was prepared in 3 different Cu^{2+} concentrations determined by the AAS technique. A 500 μL volume of buffer solution pH 7 (0.01 M) was mixed with 200 μL of the water sample, and 10 μL of reagent was added. The color of the solution was observed comparing with the color shades of standard Cu^{2+} showed as semiquantitative analysis.

Results and Discussion

Reagent Extracted from Purple Sweet Potato

Many solvents were used to extract pigment from purple sweet potato. It was found that ethanol was the most appropriate solvent. Deep red-violet solution could be stored in the refrigerator for a long time without physical properties change (Fig. 3 (a)). After removal of nonpolar molecules, using chloroform as a solvent, the reagent was characterized by UV-Visible spectrophotometry. The spectrum showed the absorption band at 230–280 nm and 360 nm in UV region, whereas the absorption band at 538.8 nm was found in the visible region (Fig. 3 (b)). These results were in agreement with Kidmose et al. (2002) Steed and Truong (2008) who had reported on the presence of anthocyanin in purple sweet potato.

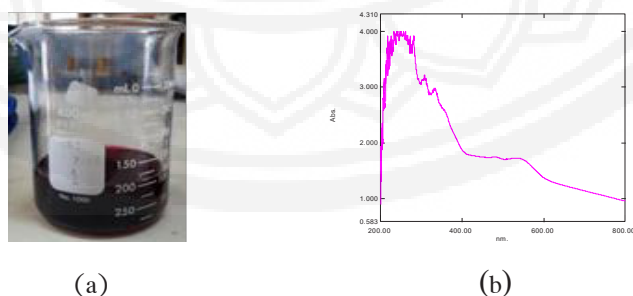


Figure 3 Reagent extracted using ethanol as solvent (a) and spectrum of reagent (b)



Qualitative determination of metal ions

After adding reagent into the mixture of metal ions (Co^{2+} , Cu^{2+} , Pb^{2+} , Ni^{2+} , Mn^{2+} , Cr^{3+} , Zn^{2+}) in buffer solution pH 7 it was found that Cu^{2+} and Pb^{2+} showed color differences from the blank. Cu^{2+} could be detected in the buffer solution in the pH range of 5–7, whereas Pb^{2+} responded at only to pH 6 (Fig 4.). The color change occurred because of complexation between cyanidin extracted from the purple sweet potatoes and the metal ions. However, in our study, we focused on detecting Cu^{2+} at pH 7 because Pb^{2+} would interfere at pH 6. At pH 7 the different colors of the complex were more clearly observed than at pH 5.

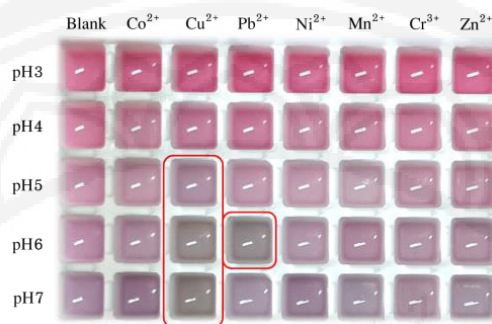


Figure 4 The appearance color of complex between reagent extracted from purple sweet potato and metal ions

Optimization of reagent volume for determination of Cu^{2+} by naked-eye detection

The color of reagent might affect the color of the complex molecules, so the reagent volume should be considered when attempting to achieve the lowest Cu^{2+} concentration detectable. Our results showed that the lowest concentration of Cu^{2+} was found at the reagent volume only 10 μL as presented in Fig 5. Optimum conditions for determination of Cu^{2+} by naked-eye detection are summarized in Table 1.

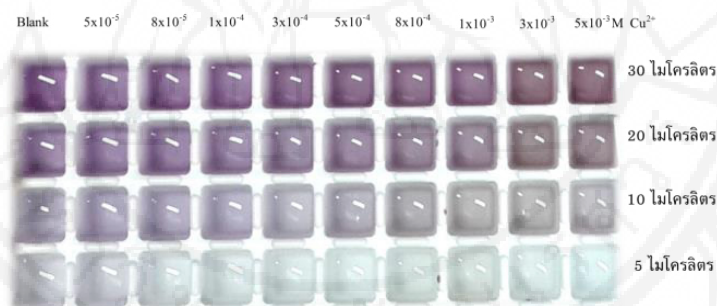


Figure 5 Reagent volume for determination of Cu^{2+} by naked-eye detection

Table 1 The optimum conditions for determination of Cu^{2+} by naked-eye detection

Solution	Volume (μL)
Buffer pH 7 (0.01 M)	500
Standard metal / water sample	200
Reagent	10

Interfering effect

Interfering ions used in this study are the major ions in natural water. Our results showed that after adding these to all chemicals ($\text{Ca}(\text{NO}_3)_2$, MgCl_2 , KCl และ Na_2SO_4) no interfering was found (Fig.6). This indicates that some cations (Ca^{2+} , Mg^{2+} , K^+ และ Na^+) and anions (NO_3^- , Cl^- and SO_4^{2-}) would not affect naked-eye detection of Cu^{2+} in a water sample.

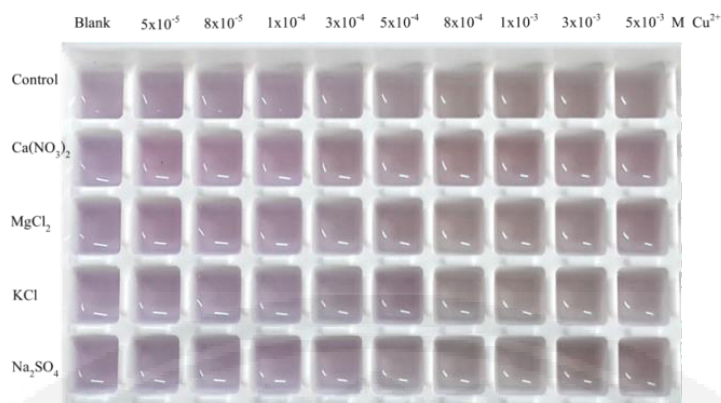


Figure 6 Interfering effect of naked-eye detection

Quantitative analysis of Cu²⁺ by naked-eye detection

Cu²⁺ could be determined as semiquantitative analysis using reagent from purple sweet potatoes via naked-eye detection. The concentration of Cu²⁺ could be separated into 3 color shades depended on Cu²⁺ concentration as shown in Fig. 7 and described in Table 2.

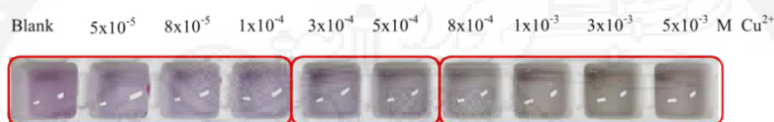


Figure 7 Semiquantitative analysis of Cu²⁺

Table 2 Color shade of Cu²⁺ determined as semiquantitative analysis via naked-eye detection

Cu ²⁺ concentration (M)	Color
< 3x10 ⁻⁴	Purple-Blue
3x10 ⁻⁴ to 5x10 ⁻⁴	Purple-Gray
> 5x10 ⁻⁴	Gray

Method validation

For the method validation, this method was applied for determination of Cu²⁺ in waste water sample from the laboratory. The accuracy of the results was compared with the AAS technique which is the standard method. It was found that Cu²⁺ concentrations obtained from both techniques were similar (Table 3). So this method could be simply, conveniently and rapidly used for Cu²⁺ detection in a real water sample.

Table 3 Cu²⁺ concentrations obtained from AAS and naked-eye detection using reagent from purple sweet potato

Unknown	Cu ²⁺ concentration (M)	
	AAS	naked-eye detection using reagent from purple sweet potato
1	1.2x10 ⁻³	> 5x10 ⁻⁴
2	8x10 ⁻⁵	< 3x10 ⁻⁴
3	5x10 ⁻⁴	3x10 ⁻⁴ to 5x10 ⁻⁴



Conclusion

Cyanidin, one type of anthocyanin, is a reagent extracted from purple sweet potato which interacts with Cu^{2+} in a water sample. Cu^{2+} was detected in terms of both qualitative and quantitative determination under the mixture solution of buffer pH 7 (0.01 M) 500 μL and reagent 10 μL . A small, 200 μL , sample was applied. The detection limit of this method was 3×10^{-4} M by naked-eye observation. However, the semiquantitative analysis of this method could be divided into 3 color shades depending on Cu^{2+} concentrations. In addition, some anions or cations including some transition metals did not show any effects. This method also showed the correlated results with AAS technique determining Cu^{2+} in wastewater from the laboratory. The research indicated that, this developed technique simply and safely to be applied for determining of Cu^{2+} in water sample and it also friendly for the environment according to using small volume of all reagents.

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