การตรวจวัดเชิงสีของไทไอโซยาเนตไอออนโดยใช้พอลิเมอร์เรซินดัดแปร

นางสาวสุจินดา ขาวสอาด

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2560

COLORIMETRIC DETERMINATION OF THIOCYANATE ION USING MODIFIED POLYMER RESINS

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2017

Thesis Title	COLORIMETRIC DETERMINATION OF THIOCYANATE ION USING MODIFIED POLYMER RESINS
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Field of Study	Chemistry
Thesis Advisor	Assistant Professor Fuangfa Unob, Ph.D.

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สุจินดา ขาวสอาด : การตรวจวัดเชิงสีของไทไอโซยาเนตไอออนโดยใช้พอลิเมอร์เรซินดัดแปร (COLORIMETRIC DETERMINATION OF THIOCYANATE ION USING MODIFIED POLYMER RESINS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.เฟื่องฟ้า อุ่นอบ, 60 หน้า.

งานวิจัยนี้ได้พัฒนาวิธีการใหม่ในการตรวจวัดไทโอไซยาเนตไอออนในตัวอย่างน้ำนมโดยอาศัยการเกิดสารประกอบเชิงซ้อนไดไซยานาโต ไดไพริดีน คอปเปอร์(II) [$Cu(SCN)_2(Py)_2$] บนผิวของพอลิเมอร์เรซิน โดยเรซินชนิดแอมเบอร์ไลท เอกซเอดี-7 (Amberlite XAD-7) ถูกดัดแปรผิวของด้วยสารเชิงซ้อนของคอปเปอร์-ไพริดีน และใช้เป็นวัสดุสำหรับการตรวจวัด การเปลี่ยนแปลงสีบนผิวของเรซินจากสีน้ำเงินเป็นสีเขียวเมื่อความเข้มข้นของไทโอไซยาเนตเพิ่มขึ้น ซึ่งสามารถสังเกตการเปลี่ยนแปลงสีได้ด้วยตาเปล่า และสามารถได้ข้อมูลความเข้มสีเพื่อในการวิเคราะห์เชิงปริมาณโดยใช้โปรแกรมอิมเมจเจ มีการศึกษาผลของตัวแปร ได้แก่ ความเข้มข้นของสารละลายคอปเปอร์ เวลาที่ใช้ในการดัดแปรผิวของเรซิน ปริมาณของสารตัวอย่าง เวลาที่ใช้ในการตรวจวัดการเปลี่ยนแปลงสีบนผิวเรซิน และปริมาณไพริดีน ภายใต้สภาวะที่เหมาะสมสามารถตรวจวัดไทโอไซยาเนตโดยมีช่วงความเป็นเส้นตรงของการตรวจวัด คือ 11.6 ถึง 116.0 มิลลิกรัมต่อลิตร ให้ค่าความเข้มข้นต่ำสุดที่ตรวจวัดได้คือ 3.6 มิลลิกรัมต่อลิตร และค่าความเข้มข้นต่ำสุดของไทโอไซยาเนตสำหรับการวิเคราะห์ปริมาณคือ 8.0 มิลลิกรัมต่อลิตร สำหรับการตรวจวัดไทโอไซยาเนตในตัวอย่างน้ำนม ใช้วิธีเติมสารมาตรฐานเพื่อลดการรบกวนจากผลของเมทริกซ์ ซึ่งค่าร้อยละการได้กลับคืนของไทโอไซยาเนตจากตัวอย่าง (%recovery) อยู่ในช่วง 93.4 – 104.6% และค่าความเที่ยงในการวิเคราะห์ (%RSD) อยู่ในช่วง 1.2 – 4.2% วิธีการวิเคราะห์ที่นำเสนอนี้สามารถใช้ในการตรวจวัดไทโอไซยาเนตในตัวอย่างน้ำนมและให้ผลการวิเคราะห์ที่มีความแม่นและความเที่ยงที่ยอมรับได้

ภาควิชา เคมี ลายมือชื่อนิสิต .....

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2560

5872073823 : MAJOR CHEMISTRY

KEYWORDS: THIOCYANATE, POLYMER RESINS, COMPLEXATION REACTION, COLORIMETRIC DETERMINATION

SUJINDA KHAOSAARD: COLORIMETRIC DETERMINATION OF THIOCYANATE ION USING MODIFIED POLYMER RESINS. ADVISOR: ASST. PROF. FUANGFA UNOB, Ph.D., 60 pp.

A new method for thiocyanate determination in milk samples based on the formation of dithiocyanato dipyridine copper(II) [$Cu(SCN)_2(Py)_2$] complex on polymer resin surface is proposed. Amberlite XAD-7 resin on was modified with copper-pyridine complex and used as the material for detection. The color of resin changed from blue to green with the increase of thiocyanate concentration. It could be observed by naked-eye and the color intensity used as quantitative data was measured *via* Image-J software. The effect of various parameters was investigated including Cu^{2+} concentration, time for resin modification with reagents, sample volume, detection time and pyridine volume. Under the optimal condition, the linear range from 11.6 – 116.0 mg/L was obtained with the limit of detection and quantification of 3.6 mg/L and 8.0 mg/L, respectively. To detect thiocyanate in milk samples, the standard addition method was performed to overcome the matrix effect. The recovery of thiocyanate in milk samples observed by proposed method was 93.4 – 104.6% with the relative standard deviations in the range of 1.2 – 4.2%. The proposed method was successfully applied to detect thiocyanate in milk samples with acceptable accuracy and precision.

Department: Chemistry Student's Signature .....

Field of Study: Chemistry Advisor's Signature .....

Academic Year: 2017

ACKNOWLEDGEMENTS

First of all, I would like to thank my thesis advisor, Assistant Professor Dr. Fuangfa Unob for suggestion, tremendous assistance and encouragement during my study and research. I would also like to extend my appreciation to thesis committees; Associate Professor Dr. Vudhichai Parasuk, Associate Professor Dr.Pakorn Varanusupakul, and Dr. Tinnakorn Tiensing for their valuable comment and suggestion.

My grateful thanks are also extended to Miss Retno Prasetia and Miss Patita Salee who helped me throughout my thesis, suggested me to maintain good attitudes in work and cheered me up every time. I also thank all of Enviromental Analysis Research Unit (EARU) members for their kind assistance, support, and friendship. Most importantly, I would like to express my gratitude to my parents for their love and always supporting me throughout writing this thesis and my older sister who was a big supporter when I was under pressure.

Finally, I would like to acknowledge the financial support from Petromat, the Center of Excellence on Petrochemical and Materials Technology, and the Department of chemistry, Faculty of Science, Chulalongkorn University.

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LIST OF ABBREVIATIONS

M	Molar
mM	Millimolar
ppm	Part per million
mg/L	Milligram per liter
μg/L	Microgram per liter
μL	Microliter
mL	Milliliter
g	gram
^{0}C	Degree celsius
LOD	Limit of detection
LOQ	Limit of quantitative
Min	Minute
rpm	Revolutions per minute
nm	Nanometer

CHAPTER I
INTRODUCTION

1.1 statement of purpose

Thiocyanate (SCN^-), as an important chemical, has been widely applied in various industrial processes such as metal separation, electroplating, fabric dyeing, pesticides and herbicide production [1-3]. Moreover, the low concentrations of thiocyanate can be found in biological fluids (blood, urine) through the digestion of cassava foods or by intake of thiocyanate-containing foods such as milk and cheese [4, 5].

The lactoperoxidases system or lactoperoxidases–thiocyanate–hydrogen peroxide system (LPS) is an antibacterial system in milk. The oxidation reaction of thiocyanate is occurred by hydrogen peroxide (H_2O_2) to produce intermediated products, which are quite powerful antibacterial agents. This reaction is catalyzed by lactoperoxidase (LP), which is an enzyme naturally present in milk [6]. Therefore, thiocyanate may be added into dairy products as preservative to extend the shelf life of dairy products [7, 8]. However, thiocyanate can interfere with iodide uptake at the sodium-iodide symporter of the human body and is a potential disruptor of thyroid hormone synthesis [9]. In addition, higher thiocyanate concentration in the human body will lead to vertigo or unconsciousness [10]. To protect the legitimate rights and

interests of consumers, the International Dairy Federation set the international norms of thiocyanate in milk at 14 mg/L [11, 12].

Several methods have been reported for determining thiocyanate such as spectrophotometry [13, 14] ion chromatography (IC) [5, 9, 15], high-performance liquid chromatography (HPLC) [16], gas chromatography (GC) [17], flow injection analysis [18], fluorescence spectroscopy [19], and surface enhanced Raman scattering (SERS) [20]. As mentioned above, most of these techniques require complex and expensive instruments, time-consuming and hardly portable. Naked-eye detection is a colorimetric method that allows on-site detection, rapid response time and real-time qualitative or semi-quantitative detection without the use of any complicated instruments or skillful technicians. Many colorimetric methods for thiocyanate detection are based on the formation of red-brown complex with Ferric ion [8, 13] or oxidation the thiocyanate to hydrogen cyanide (HCN) which further reacts with picric acid to give brown color product [21, 22]. However, the red-brown complex is unstable, and the presence of several ions (e.g. Cl^- and CN^-) can interfere the analysis [13, 23] and the oxidation reaction of thiocyanate generates highly very toxic product. Thiocyanate can form copper-pyridine complex, which can be extracted and observed the green color complex in organic solvent. However, the complex was analyzed by using atomic absorption spectrometer for quantitative analysis [24]. As described previously, the detection of thiocyanate despite some advantages, it still requires the

use of instrument such as a UV-Vis spectrophotometer, atomic absorption spectrometer or consumes some toxic reagent/solvent.

To overcome this drawback, a new approach to detect thiocyanate at low concentration is proposed in this research based on the extraction of dithiocyanato dipyridine copper(II), $[Cu(SCN)_2(Py)_2]$, complex onto solid to observe the color on solid by naked eyes without using toxic organic solvent. Amberlite XAD-7 polymer resin, a polyacrylic ester polymer, was selected as solid phase. It exhibits good physical properties such as porosity, uniform pore size distribution, and chemically homogeneous non-ionic structure. These properties have been shown to be good adsorbents for large number of uncharged compounds [25, 26]. Moreover, the use of amberlite XAD-7 also helps observation of a color change more obvious on a solid than that in the solution without interfering effect from sample color. The material color changed from blue to green with increasing concentration of thiocyanate. The color on the solid was then observed by naked eyes and the color intensity was measured using the Image-J software [27] to construct a calibration curve and obtain quantitative data. The method was applied to detect the level of thiocyanate in milk samples. Moreover, this approach would be useful for the farmers and manufacturer to screen the quality of milk before the production.

1.2 Research objectives

1.2.1 To develop a colorimetric method for determination of thiocyanate by naked-eye and Image-J software.

1.2.2 To apply the method to detect thiocyanate in milk samples.

1.3 Scope of the research

For thiocyanate detection, the concentration of thiocyanate is determined in the range of 0 – 116.0 mg/L. The parameters affecting on the detection of thiocyanate (i.e., the concentration of reagent, the volume of samples, detection times) were optimized. For the analysis of milk sample, the samples were purchased from retail shop. A sample pretreatment process was introduced before the detection. The effect of various parameters were investigated such as milk matrix and the presence of co-existing ions such as chloride, citrate, iodide, phosphate, sulfate, bromide and fluoride. The color of the material was observed by naked eyes and the color intensity was measured by ImageJ software using blue scale mode to construct a calibration curve and obtain quantitative data. The obtained results were compared to those obtained from an ion chromatography method.

1.4 The benefit of this research

To obtain a new colorimetric method for naked-eye detection of thiocyanate in milk sample using copper-pyridine reagent.

CHAPTER II
THEORY AND LITERATURE REVIEW

2.1 Relationship between thiocyanate and Lactoperoxidase system in milk

Thiocyanate (SCN^-) is a simple inorganic ion and a natural substance existing in animal tissue that can be found in secretion such as blood, saliva, and urine. It is mainly used as an anti-bacterial agent in daily product.

Milk and milk product have made significant contributions to human nutrition. Milk is a complete food, readily digested and absorbed and a source of good quality nutrients such as fat, proteins, minerals, and vitamins which, are present in highly desirable proportions [8, 28]. However, during transportation from the point of its production to the place of processing, bacterial growth leading to breakdown of milk components is inevitable. Cooling is the most commonly used method to stop or retard the deterioration of milk [29], but this is not always feasible for various reasons, such as lack of available capital, lack of electricity, less developed road systems, or high operational costs. To solve this problem, an alternative method to increase the storage stability of milk is known as Lactoperoxidase system (LPS) [6, 30-32]. The Lactoperoxidase system (LPS) consists of the production of an antibacterial compound as intermediate product from the reaction of thiocyanate ion catalyzed by Lactoperoxidase (LP) in the presence of hydrogen peroxide. Antimicrobial compound from the lactoperoxidase system (LPS) acts as a natural inhibitor in milk and the

intermediate products inhibit Gram-positive microorganisms and some Gram-negative microorganisms [33, 34]. To obtain such activity, thiocyanate is added into milk. However, the excessive use of thiocyanates (above 120 mg/L) can lead to human health hazards as it can disrupt thyroid function by competitively inhibiting iodide uptake at the sodium-iodide symporter (NIS), which transports iodide into thyroid follicular cells. Consequently, it can lead to decreased thyroid hormone production, followed by an increase in the thyroid stimulating hormone. Finally, thyroid gland enlargement (goiter) can occur in the human body [7, 8, 32, 35, 36]. The International Dairy Federation (IDF) recommended that the level of added of thiocyanate in milk should not exceed 14 mg/L [11-12].

2.2 Method for thiocyanate detection

Several methods for determining thiocyanate have been reported. These include colorimetric method using spectroscopic technique. The colorimetric methods are based on the complex formation or reaction to generate the color compounds which are described hereafter in more details.

2.2.1 König reaction

Generally, the König reaction was used for cyanide and thiocyanate determination. This method involved the conversion of cyanide to cyanogen chloride (ClCN) by using chloramine-T (Figure 2.1). Then cyanogen chloride further reacted with pyridine to form N-cyanopyridinium chloride which underwent hydrolysis to glutaconic aldehyde. The aldehyde was then coupled with barbituric acid (the coupling agent) to

form blue color product that was detected by a spectrophotometer in the wavelength range from 570 - 630 nm [37, 38].

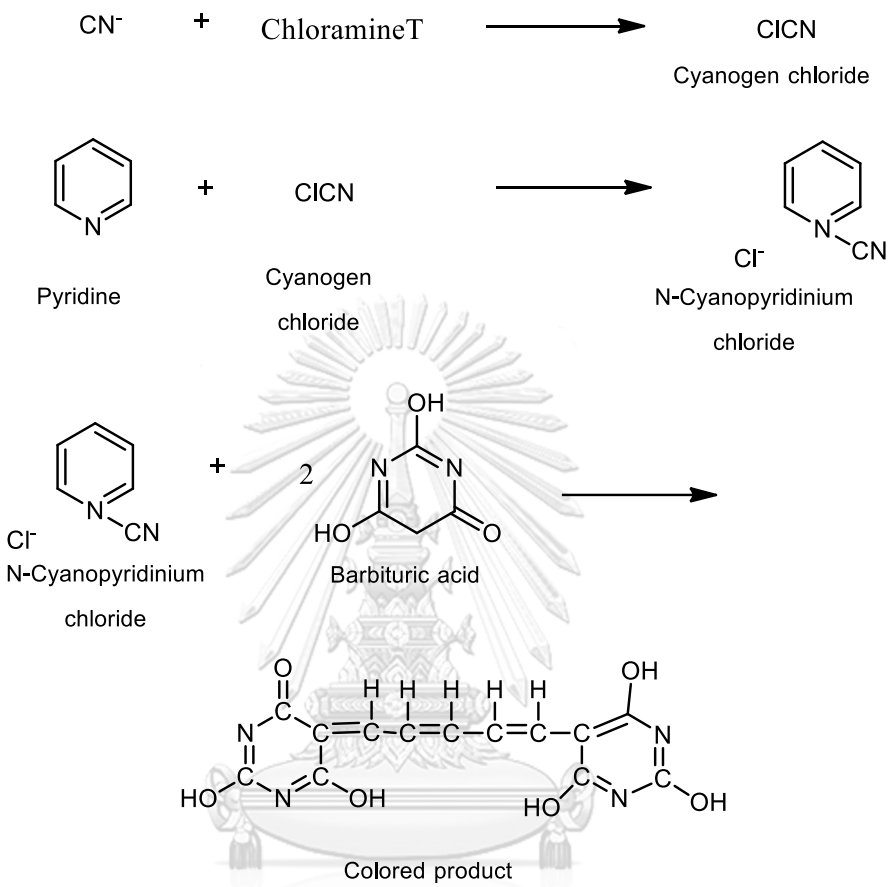


Figure 2.1 The König reaction

In addition, König method was modified as to use another oxidizing agent for conversion of cyanide to cyanogen chloride such as bromine in water. Other coupling reagents were also applied for cyanide and thiocyanate analysis such as isonicotinic acid-barbituric acid, pyridine-benzidine, and pyridine-*p*-phenyleneamine. The advantage of this reaction is its high selectivity. On the other hand, the major disadvantages are the use of toxic reagent and its complex procedure consisting of

many steps of reaction. The examples of thiocyanate detection by modified Konig reaction methods are given below.

Tanaka A. *et al.* [39] determined thiocyanate in solution by using isonicotinic acid-barbituric acid reaction (modified König method) with spectrofluorometric detection. The blue-purple compound was excited at 605 nm and the fluorescence was measured at 620 nm. The method had a linear range of 0 - 2.9 mg/L with reaction time of 30 minutes.

Aldridge W. N. [40] proposed a new approach for determination of cyanide and thiocyanate by using bromine water as an oxidizing agent to convert cyanide. The cyanogen bromide was formed and then the excess amount of bromide was removed with sodium arsenite. After that, it reacted with solution of benzidine in pyridine to give an orange to red color proportional to the quantity of cyanogen bromide compound with reaction time of 10 min. The linear dynamic range of thiocyanate detection was 1.24 - 6.20 μg/mL. This method was applied to detect thiocyanate in blood serum.

Botto R. I. *et al.* [41] modified the method developed by Aldridge by using *p*-phenylenediamine instead of benzidine because of its carcinogenicity. The cherry red color product in solution was obtained. The color change depended on the concentration of cyanide or thiocyanate and the absorbance was then measured at 508 nm. The calibration curve was constructed in the range of 0.01 - 0.2 μg/mL with a

detection limit of 0.0013 μg/mL. This method was applied to detect cyanide and thiocyanate in wastewater.

2.2.2 Picric acid method

Picric acid is an organic compound and explosive. In contrast, it is quite harmless (for an acid) in solution. Picric acid has a bright yellow color. When it reacted with cyanide, it is reduced to a more orange-brown compound called isopurpuric acid as shown in Figure 2.2.

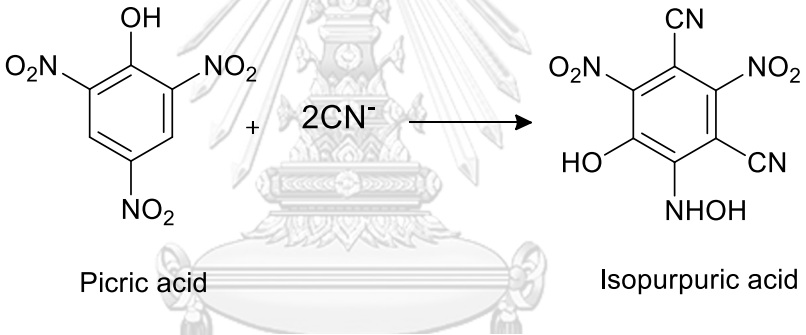


Figure 2.2 Reaction of cyanide with picric acid

In addition, by soaking filter paper in picric acid and the dried paper was then exposed with HCN vapor in closed system. The color of the paper changed from a bright yellow to an orange-brown and brown, respectively with increasing cyanide concentration. This method gave an intense color and more selective with cyanide or thiocyanate. However, the method required to convert cyanide to HCN gas which is highly toxic to human health [42].

Bradbury J. H. and Haque M. R. [21] developed a simple method for determination of thiocyanate in urine sample.

$$6MnO_4^- + 5SCN^- + 13H^+ \longrightarrow 6Mn^{2+} + 5HCN + 5SO_4^{2-} + 4H_2O \quad (2.1)$$

This method was based on the oxidation of thiocyanate by using permanganate (eq. 2.1) in closed vial to generate hydrogen cyanide (HCN) and the gas further reacted with a picrate paper. The paper was eluted for 30 minutes in 5.0 mL of water and the absorbance of the obtained solution was measured at 510 nm with a spectrophotometer. The linear range of thiocyanate concentration was 0 - 100 mg/L and the recovery was obtained in range of 103 - 105%.

Narongwanichgarn W. and Theeraphan A.[22] were developed a test kit for detection of thiocyanate in raw milk. The thiocyanate was oxidized to be hydrogen cyanide (HCN) by potassium permanganate and sulfuric acid in closed vial with a sheet of picrate paper attached inside the stopper. HCN reacted with yellow picric acid to form isopurpuric acid which is red-brown color. The color intensity depended on the amount of thiocyanate. Finally, the tested paper was dipped in water to elute isopurpuric acid, and the absorbance of the solution was determined at 510 nm. The developed test kit can detect thiocyanate in the range of 1 - 10 mg/L.

2.2.3 Complexation method

In complexation reaction, several ligands react with a metal atom to form coordination complex or coordination compound. A central metal atom or ion is usually transition metal and called the coordination center and a surrounding array of bound molecules or ions are known as ligand or complexing agent. Ligands are generally bound to the central atom by a coordinate covalent bond (donating electrons from a lone electron pair into an empty metal orbital). The number of ligands bound to the transition metal ion is called the coordination number [43].

2.2.3.1 Complexation reaction with Fe(III) method

The most common spectrophotometric method for thiocyanate detection is based on the formation of red complex between Fe(III) and thiocyanate ion, $[FeSCN]^{2+}$ (eq. 2.2). The method is simple but it lacks of sensitivity at very low level of thiocyanate. The color of complex is also unstable.

$$Fe^{3+} + SCN^{-} \longrightarrow [FeSCN]^{2+} \qquad (2.2)$$

Basova E. M. *et al.* [13] proposed the method for determination of thiocyanate in stratal waters using Fe(III) reagent to form $[FeSCN]^{2+}$ complex in sulfuric acid medium. The absorbance of the color compound was observed at 490 nm and the dyamic range of thiocynate determination was 400 - 3600 μg/L. They also studied the effect of various metal ions and anions which may interact with thiocyanate or ferric ion in this reaction. It was found that Cr(III), Cd(II), Cu(II) could form complexes with

thiocyanate ions as 1:1 complexes. The presence of F^-, $B_4O_7^{2-}$ and large amounts of Cl^- affected the determination due to the complexation with Fe(III) resulting in a decrease of $[FeSCN]^{2+}$ content.

Kanthale P. *et al.* [8] developed the qualitative method for determining thiocyanate in milk sample. Two procedures of qualitative analysis were performed; one used trichloroacetic acid (TCA) to precipitate protein prior to the analysis and the other applied the method directly to milk. In both systems, Fe(III) was used to form complex with thiocyanate and the color of solution changed from orange to orange-red color with increasing thiocyanate concentration. This developed method is simple and rapid. It requires no sample preparation and gave a distinct color in solution. However, the color of solution changed in a narrow range of thiocyanate concentration. The method was only used for qualitative analysis.

2.2.3.2 Complexation with metal pyridine method

Pyridine is a weakly basic heteroaromatic compound, which is one of benzene derivatives. It is miscible with water and almost all organic solvents. Moreover, pyridine is also a ligand that can form complexes with transition metal ions.

Several divalent cations can react with pyridine and thiocyanate to form water-insoluble compounds which can be extracted with organic solvent such as chloroform. The complexes in chloroform extracts can be analyzed by a spectrophotometer. This method was used for the spectrophotometric determination of several cations such

as Cu(II), Ni(II), Co(II), Fe(II), and Mg(II) [44]. Furthermore, a solution of copper-pyridine complex can be used to determine thiocyanate. Some literature review is given below.

Danchik R. S. and Boltz D. F. [25] proposed the indirect method for thiocyanate detection by determining the Cu(II) in complex using atomic absorption spectrometric method. This method was based on the formation of dithiocyanatodipyridine copper (II) complex (eq.2.3), which was extracted into chloroform solution.

$$Cu^{2+} + 2SCN^{-} + 2\text{pyridine} \longrightarrow Cu(SCN)_2(Py)_2 \qquad (2.3)$$

The green color of $Cu(SCN)_2(Py)_2$ complex in organic solution was aspirated into the atomic absorption spectrometer. The linear range of 7.0 – 18.0 ppm thiocyanate was obtained. However, the Ni(II), Hg(II), Fe(II), Ag(I), iodide, nitrite, permanganate, vanadate, and iodate had interfering effect in this reaction.

In addition, there are also other complexation reaction methods reported for determining thiocyanate concentration. For example, Das A. K. and Chattaraj S. [45] used Schiff base complex of $[Cu(BPTC)]^{+}$ to form Cu(BPTC)(SCN) complex. This method was more selective with thiocyanate but the synthesis of BPTC was complicated. Chow, A. and S.L. Ginsberg [46] developed method that Co(II) and Zn(II) were used as reagent to form metal-thiocyanate complex detected with X-ray fluorescence. Furthermore, Cu(II) and 2,2'-dipyridyl-2-pyridylhydrazone (DPPH) could be applied as reagent to form DPPH-Cu-SCN complex. The complex was extracted into methyl isobutyl ketone and

analyzed with an atomic absorption spectrophotometer. This research has been developed by Stratis J. A. and Vasilkiotis G. S. [47].

Spectrometric methods for determining thiocyanate as described previously provide several advantages including low detection limit, high sensitivity. However, many of these methods are complicated, time-consuming, costly, and hardly performed on-site.

2.2.2.4 Naked-eye detection

Colorimetric methods with naked-eye detection have drawn considerable attention with the advantage of on-site analysis, simplicity, no requirement for sophisticated instrument or skillful technicians. Naked-eye detection is to detect the color change of solution or material after the reaction of analytes with reagents. Furthermore, the color intensity could be measured by taking the photo of the material or scanning the color zone. The photo was further processed with specific program to determine color intensity. Many researchers reported the determination of thiocyanate in various samples and the examples are described in more detail.

Pena-Pereira F. *et al.* [48] developed the paper-based analytical device for salivary thiocyanate detection. The assay was based on the formation of Fe(III)-thiocyanate color complex on a paper-based sensing platform. The Fe(III) reagent was spotted in each detection zone on the paper-based device. Subsequently, the sample was dropped on the testing zone and the color on detection zone changed in the

presence of thiocyanate. The photo of the platform was obtained by using a scanner. The color intensity of the image was measured using Image-J software. The linear dynamic range of thiocyanate detection was 14.5 - 1160 mg/L with a detection limit of 3.48 mg/L.

From the literature review, many researchers reported the determination of thiocyanate by spectrophotometric method or paper-based platform while the colorimetric detection on solid has never been reported. In this work, we are interested in using the solid sorbent as a material for naked eye detection to overcome interfering effect of the sample solution color. A polymeric resin was used to extract and detect thiocyanate complex.

2.3 Amberite XAD-7 resin

Amberite XAD-7 is a polymer adsorbent with the chemical structure shown in Figure 2.3 [49].

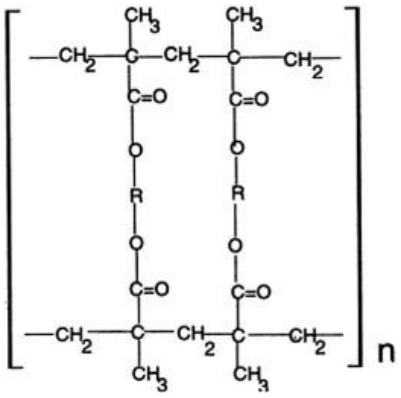


Figure 2.3 Chemical structure of Amberite XAD-7

XAD resins have excellent chemical stability and their physical and chemical properties are represented in Table 2.1 [25, 49].

Table 2.1 Physical and chemical properties of the polymeric adsorbent Amberlite XAD-7

Physical/chemical properties	values
BET surface area (m^2 g^{-1})	450
Particle size (mesh)	20-60
Average pore volume (cm^3 g^{-1})	1.14
Average pore diameter (A°)	90
Total porosity (cm^3 cm^{-3})	0.55
structure	Acrylic ester
Dipole moment	1.8
Dry/wet density (g cm^{-3})	1.24/1.05

Amberlite XAD-7 is an acrylic ester polymer having hydrophobic surface and moderate polarity (carbonyl groups). It is porous spherical polymer and has high average pore volume. In general, these resins are used for adsorption of organic substances or large number of uncharged compounds (hydrophobic compounds up to MW 20,000) from aqueous systems and it has been widely used to remove various pollutants or metal ions from aqueous wastes, and ground water [49, 50].

In this study, Amberlite XAD-7 resins was alternatively used as a material for detection of thiocyanate in milk sample based on the formation of Cu(II)-thiocyanate-pyridine [$Cu(SCN)_2(Py)_2$] complex and solid phase extraction. The color of the material was observed. The color intensity was proportional to the concentration of thiocyanate on the resin which can be determined by Image-J software.

CHAPTER III

EXPERIMENTAL

3.1 Chemicals

All of chemicals were of analytical reagent (AR) grade. The list of chemicals is shown in Table 3.1. De-ionized water was used as solvent to prepare all solutions in this study.

Table 3.1 List of chemicals

Chemicals	**Supplier**
Copper (II) nitrate	Baker analyzed
Potassium thiocyanate	CARLO ERBA
Potassium bromide	Sigma-Aldrich
Potassium fluoride	CARLO ERBA
Potassium Iodide	Sigma-Aldrich
Tri-sodium citrate	Fisher Scientific
Sodium sulfate	Fisher Scientific
Sodium chloride	Fisher Scientific
Sodium dihydrogenphosphate	Merck
Trichloroacetic acid	CARLO ERBA
Pyridine	Merck
Methanol	Merck
Amberlite XAD-7 resin	Sigma-Aldrich

3.2 Instruments

Table 3.2 List of instrument

Instrument	Model
Magnetic stirrer	Gem/MS 101
Centrifuge	Universal 320 HETTICH
Diffuse reflectance ultraviolet visible spectrophotometer	UV-2500 Shimadzu
Ion chromatography	Thermo scientific Dionex Integrion RFIC system
Camera	SONY a5100

3.3 Preparation of Amberlite resins

Amberlite XAD-7 resins (10 g) were stirred with methanol (10 mL) to eliminate some impurities for 15 minutes. The resin was filtered and de-ionized water (10 mL) was added. Then the mixture was continuously stirred for 5-10 minutes. Then the resins were filtered, dried at 80 ^{o}C, and kept for further use.

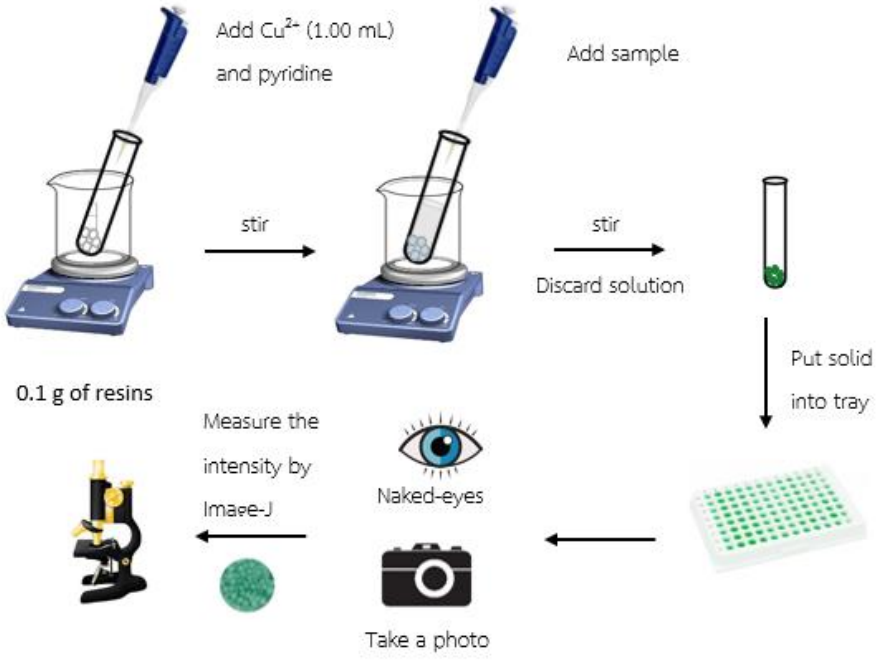
3.4 Characterization of the complex modified resin

To confirm that the copper–pyridine complex could be extracted onto the resin, the resin was characterized by a diffuse reflectance ultraviolet visible spectrophotometer (DR-UV-vis) in a wavelength range of 400 to 800 nm.

3.5 Colorimetric determination of thiocyanate

3.5.1 Thiocyanate detection

The resin surface was modified with copper-pyridine complex by adding Cu^{2+} solution (1.00 mL) and pyridine onto 0.1 g of resin. The mixture was stirred for a specific period of time. Thiocyanate standard solution or sample solution was subsequently added to copper-pyridine modified resins and stirred. After discarding the solution, the solid was filled in a well plate for color observation. The photo of the solid was taken in a lighting studio box and imported to Image-J program for measuring the color intensity. The method of thiocyanate determination is schematically shown in Scheme 3.1 and the parameters of camera for taking photo are shown in the Table 3.3.



Scheme 3.1 The analytical procedure for thiocyanate colorimetric detection

Table 3.3 The parameters of camera for taking photo

Parameters	Values
F-stop	f/7.1
Exposure time	1/640 sec.
ISO speed	ISO-160
Focal length	44 mm

3.5.1.1 Image-J program

Image-J program [27] is a free image processing and analysis software for measuring the color intensity. The measuring of color intensity from image or photo can be measured in gray mode or red, green, blue (RGB) channel. The formula of each of the R, G, B channels are shown below (eq.3.1-3.3) [51]. The spectral responsivity of each channel are roughly Gaussian functions with typical ranges of 400-500, 500-580, and 580-700 nm for the blue, green, and red channels, respectively. The blue channel was used to measure the color intensity in this work.

$$R = \int_{\lambda} P(\lambda) S_R \lambda \, d\lambda \quad (3.1)$$

$$G = \int_{\lambda} P(\lambda) S_G \lambda \, d\lambda \quad (3.2)$$

$$B = \int_{\lambda} P(\lambda) S_B \lambda \, d\lambda \quad (3.3)$$

where,

P is incident intensity

S is spectral responsivity for a particular channel

3.5.2 Optimization of thiocyanate determination method

To obtain the optimal condition for thiocyanate detection, the effect of several parameters (*i.e.*, Cu^{2+} concentration, pyridine volume, time for resin modification with copper-pyridine complex, sample volume, and detection time) were investigated. The range of values of the studied parameters is presented in Table 3.3.

Table 3.4 The range of values of studied parameters for thiocyanate detection

Parameters	**Range of values**
Cu^{2+} concentration	1 – 50 mM
Modification time	1- 30 minutes
Sample volume	1.00 – 20.00 mL
Detection time	1 – 10 minutes
Pyridine volume	0.10 – 0.50 mL

3.6 Method performance

The performance of the proposed method including limit of detection (LOD), limit of quantification (LOQ), linear working range, accuracy, and precision of the method were evaluated under optimal condition.

3.6.1 Linear working range

The calibration curve for thiocyanate detection was established under optimal condition obtained beforehand. The calibration curve was plotted between color intensity in blue value as y axis and the concentration of standard thiocyanate solutions in the range of 0 – 116.0 mg/L as x-axis. The linearity of proposed method was reported in terms of correlation coefficient value (R^2).

3.6.2 Limit of detection and limit of quantification

To obtain the limit of detection (LOD) and limit of quantification (LOQ) of this method, a blank solution containing 3% trichloroacetic acid was used as solution for the analysis by the method under the optimal condition. The experiment was performed in ten replicates. Based on color of the resin, the color intensity was measured by using ImageJ program. In this work, the color intensity of the solid was observed in RGB mode and the blue values were recorded (mean blue values). In the detection of lower thiocyanate concentration, the color of material was in blue tone and the mean blue values were high. In contrast, the blue-green or green tone of material used in solution containing high thiocyanate concentration had low mean

blue values. The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to Eq.3.4-3.5, respectively;

$$I_{LOD} = I_{blk} - 3SD_{blk} \quad (3.4)$$

$$I_{LOQ} = I_{blk} - 10SD_{blk} \quad (3.5)$$

where,

I_{LOD} = the color intensity of resin used in solution at LOD concentration

I_{LOQ} = the color intensity of resin used in solution at LOQ concentration

I_{blk} = the mean of color intensities of resin used in blank solution

SD_{blk} = the standard deviation of color intensities of resins used in blank solution

The level of LOD and LOQ were obtained by comparing I_{LOD} and I_{LOQ} values to the standard calibration curve. The standard curve was plotted between color intensity (mean blue values) and concentration of thiocyanate in range of 0 - 116.0 mg/L.

3.7 Effect of potential interfering species

The effect of interfering species in the determination of thiocyanate in milk sample with the purposed method was studied. Anionic species often found in milk were added in thiocyanate standard solutions (11.6 and 116.0 mg/L) to prepare binary mixtures. The level of these anionic species studied (Table 3.5) was their normal concentrations found in milk [52].

Table 3.5 Anions and their concentrations in milk

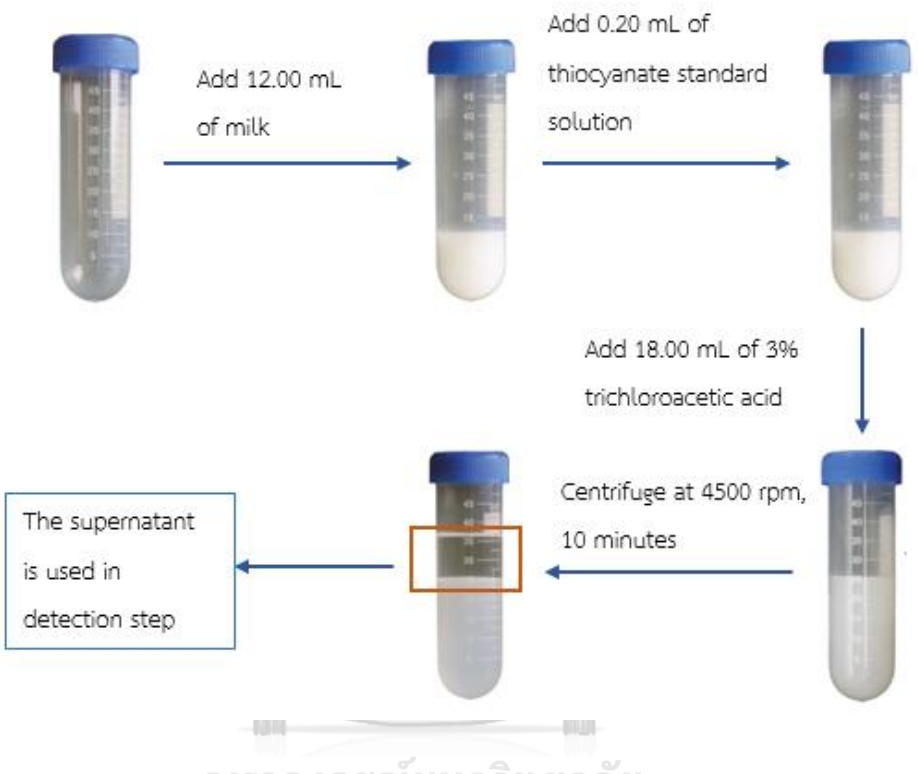
Constituents	Concentration (mg/L)
Chloride	1100
Phosphate	2300
Citrate	1750
Sulfate	100
Bromide	1
Fluoride	0.15
Iodide	0.06

3.8 Determination of thiocyanate in milk samples

3.8.1 Preparation and detection of thiocyanate in milk samples

For the analysis of thiocyanate in milk samples, since the samples contain various matrices, the external calibration curve was not suitable for the determination. Thus, the standard addition method was adopted to overcome the matrix effect from milk sample. The preparation of sample is shown in scheme 3.2. Milk samples were bought from local market. Thiocyanate standard solutions of different concentrations were added into a set of 12.00 mL of milk samples to construct a calibration curve. 18.00 mL of 3% of trichloroacetic acid (TCA) solution was added into each solution to precipitate protein in samples. Then the mixture was centrifuged at 4500 rpm/min for 10 min. 15.00 mL of supernatant of each sample was used for detection step according

to the proposed protocol (topic 3.5.1). The photo of solid was taken and the color intensity was determined by ImageJ software in blue scale mode to get data for constructing standard addition calibration curve.



Scheme 3.2 Preparation of milk sample for standard addition method

3.8.2 Effect of milk matrix

To analyze milk sample, the effect of matrix on thiocyanate detection was evaluated. The external calibration curve was constructed by using the results from the analysis of thiocyanate standard (11.6, 34.8, 58.0, 87.0, 116.0 mg/L) prepared in de-ionized water. In milk sample analysis, standard addition calibration curve was plotted. Thiocyanate standard solutions were added into 12.00 mL of milk to obtain the final spike concentration of 11.6, 34.8, 58.0, 87.0 or 116.0 mg/L in sample. Subsequently, a 3 % trichloroacetic acid solution (18.00 mL) was added into each sample to precipitate protein. After centrifugation, the supernatant of each sample was further used. Both the standard solutions and the pretreated milk samples were analyzed as described in the topic 3.5.1. The calibration curves were constructed from the obtained results and compared.

3.8.3 Accuracy and precision in milk sample analysis

To evaluate the accuracy of the method, the spiked sample method was performed under suitable condition by adding thiocyanate standard into samples to obtain the spike concentration of 11.6 mg/L and 29.0 mg/L in samples. Percent recovery can be calculated using Equation 3.3. Based on the AOAC International criteria [53], percent recovery of the spiked analyte should be in the range 90 - 107 % if the method has acceptable accuracy as shown in Table 3.6.

$$\% \text{ Recovery} = \frac{(Xs-Xb)}{S} \times 100 \qquad (3.3)$$

Xs is the concentration of thiocyanate found in the spiked sample

Xb is the concentration of thiocyanate found in non-spiked sample

S is the concentration of thiocyanate spiked in the sample

The precision of this method was presented in term of percentage of relative standard deviation (%RSD) which should not be higher than 5.3 % (Table 3.5) based on the AOAC International criteria.

Table 3.6 Percent recovery and percent RSD of analytical results obtained at different analyte concentrations according to AOAC international

Analyte (%)	Mass fraction	Unit	Recovery range (%)	RSD (%)
100	1	100%	98-102	1.3
10	10^{-1}	10%	98-102	1.9
1	10^{-2}	1%	97-103	2.7
0.1	10^{-3}	0.1%	95-105	3.7
0.01	10^{-4}	100 ppm	90-107	5.3
0.001	10^{-5}	10 ppm	80-110	7.3

Furthermore, the obtained analytical results were also compared to the results from ion chromatography. The same spiked samples and non-spiked samples were analyzed by an ion chromatography. The parameters for ion chromatography analysis are shown in Table 3.7. The standard addition method was also applied to determine thiocyanate by ion chromatography.

Table 3.7 List of parameters for ion chromatography method

Parameters	**Values**
Column	Dionex IonPac AG 19 (4 x 50 mm) guard column and Dionex IonPac AS 19 (4 x 250 mm) separation column, both which are packed with alkanol quaternary ammonium
Eluent	KOH (36 mM)
Eluent flow rate	1.0 mL/min
Injection volume	25 μL
Detector	Conductivity detector

CHAPTER IV
RESULTS AND DISCUSSION

In this work, the concept of the determination of thiocyanate was based on the reaction of thiocyanate with copper-pyridine complex (blue color) modified on polymer resin surface to form dithiocyanato dipyridine copper(II), $[Cu(SCN)_2(Py)_2]$ complex, (green color) as shown in Eq 4.1.

$$Cu^{2+} + 2Pyridine + 2SCN^- \longrightarrow Cu(SCN)_2(Py)_2 \quad (4.1)$$

Amberlite XAD-7 resin was modified by adding Cu^{2+} solution and pyridine onto the resin to form a copper-pyridine complex. After adding thiocyanate solution, $[Cu(SCN)_2(Py)_2]$ complex was formed and extracted on the resin. As Amberlite XAD-7 resin has hydrophobic surface, it could adsorb either copper-pyridine complex or neutral $[Cu(SCN)_2(Py)_2]$ complex and consequently, a change of material color could be observed. The color on material changed from blue in the absence of thiocyanate to green in the presence of thiocyanate.

4.1 Characterization of modified polymer resins

The presence of $[Cu(SCN)_2(Py)_2]$ complex on Amberlite XAD-7 resins used to test the solutions containing different amounts of thiocyanate was observed by a diffuse reflectance ultraviolet visible spectrophotometer (DR-UV-Vis). The spectra were recorded in the wavelength range from 400 to 800 nm as shown in Figure 4.1.

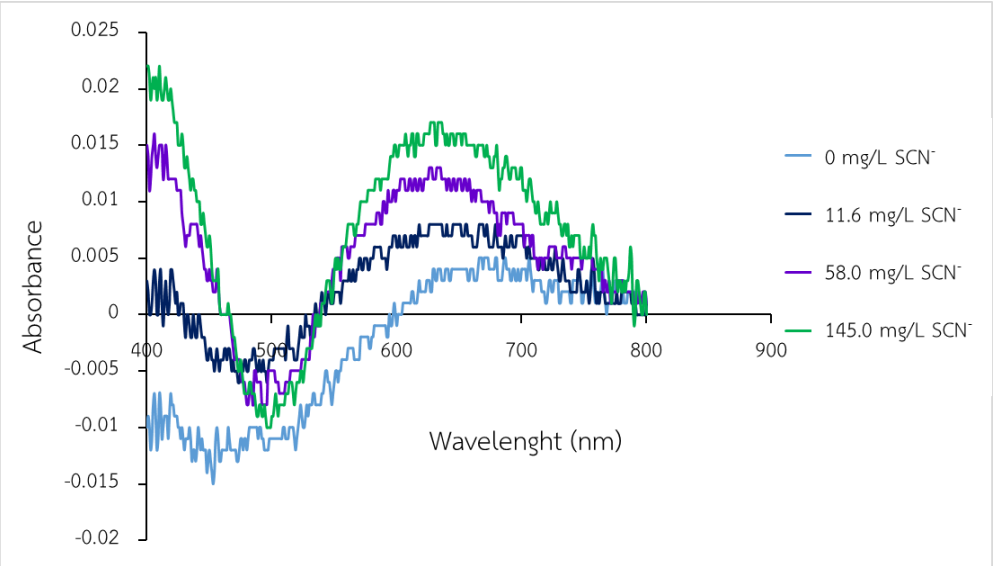


Figure 4.1 Absorbance spectrum of the resin obtained from testing solutions containing different concentrations of thiocyanate (0 – 145.0 mg/L)

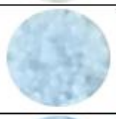
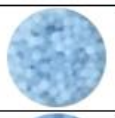
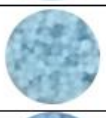
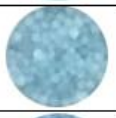
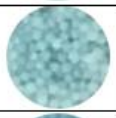
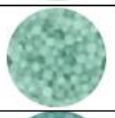
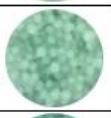
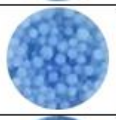
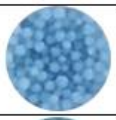
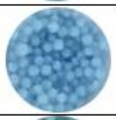
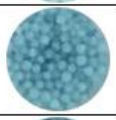
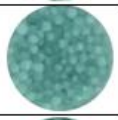
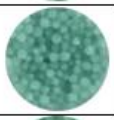
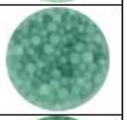
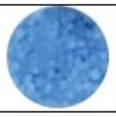
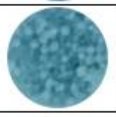
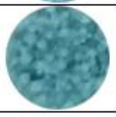
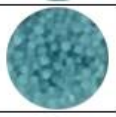
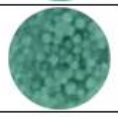
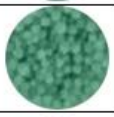
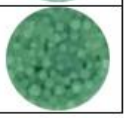
The absorbance spectrum of the materials used in the testing of thiocyanate solutions was shifted to shorter wavelength compared to that observed in the absence of thiocyanate. Moreover, the absorbance values increased when the concentration of thiocyanate increased. The results indicate that the extraction of $[Cu(SCN)_2(Py)_2]$ complex onto Amberlite XAD-7 resins was achieved probably due to the interaction between hydrophobic surface of resin and uncharged $[Cu(SCN)_2(Py)_2]$ complex.

4.2 Colorimetric detection of thiocyanate

4.2.1 Effect of Cu^{2+} concentration

The effect of Cu^{2+} concentration used to modify Amberite XAD-7 resin for thiocyanate detection was investigated in range of 1.00-50.00 mM. These Cu^{2+} solutions were mixed with 0.50 mL of pyridine and the obtained copper-pyridine complex modified resins were further used to detect thiocyanate standard solutions covering the range of 5.8 -174.0 mg/L. The suitable concentration of Cu^{2+} solution was selected based on color change on the material. The results are shown in Table 4.1.

Table 4.1 Color of Amberlite XAD-7 obtained by using different concentrations of Cu^{2+}

Concentration of Cu^{2+} (mM)	Concentration of thiocyanate (mg/L)						
	0	5.8	29.0	58.0	116.0	145.0	174.0
1							
5							
10							
30							
50							

(condition: resins 0.1 g, pyridine volume 0.5 mL, modification time 30 minutes, sample volume 10.00 mL, detection time 10 minutes)

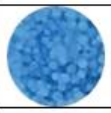
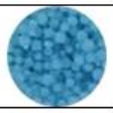
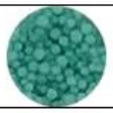
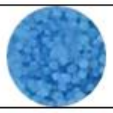
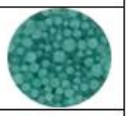
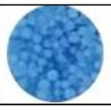
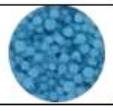
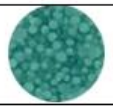
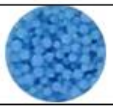
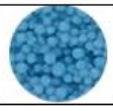
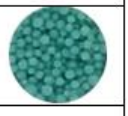
The color of resin was more intense in increasing Cu^{2+} concentration from 1.00 to 50.00 mM, revealing that there was higher content of complex on the resin. To form $[Cu(SCN)_2(Py)_2]$ complex, the mole ratio of Cu^{2+}: SCN^-: pyridine should be 1:2:2. At concentration of Cu^{2+} lower than 10 mM, the color of solid was pale and the color change was not clearly observed by naked-eye. Under this condition, the number of Cu^{2+} moles on resin was not sufficient to form complex with thiocyanate in particular at high concentration of thiocyanate. By using a Cu^{2+} solution concentration of 10 to 50 mM, it resulted in a more noticeable color change. However, only the use of 50 mM Cu^{2+} concentration could result in a color change according to thiocyanate concentration change from 5.8 to 174.0 mg/L. Thus, the Cu^{2+} concentration of 50 mM was chosen for further study.

4.2.2 Effect of time for resin modification with reagents

In this section, the effect of time for resin surface modification with reagents was studied in a range of 1 to 30 minutes. The resin (0.100 g) was modified with 50 mM Cu^{2+} solution (1.00 mL) and pyridine (0.50 mL) using different modification times. The obtained resin was subsequently tested with thiocyanate standard solution at the concentration of 5.8 mg/L and 145.0 mg/L (Table 4.2). The color of resins was not different either using the modification time of 1, 5, 10 or 30 minutes when observed by naked-eyes. Moreover, in order to obtain more reliable results than the naked-eye detection, the color intensity was measured by using Image-J program to analyse the

photo of resin in RGB color mode and determine the intensity in blue channels. The color intensities of solids obtained at different modification times were compared using one-way ANOVA. There was no significant difference in color intensities of the resins used in the determination of 5.8 mg/L thiocyanate (P = 0.976) or 145.0 mg/L thiocyanate (P = 0.922) at the 95% confidence level (α= 0.05). These results revealed that the formation and adsorption of copper-pyridine complex occurred rapidly on the resin surface. To reduce the analysis time, the time for modification of the reagent on the resins of 1 minute was selected.

Table 4.2 Color of Amberlite XAD-7 observed at the different time of modification (color intensity as blue values are given under each photo)

Time of modification (minutes)	Concentration of SCN^- (mg/L)			Time of modification (minutes)	Concentration of SCN^- (mg/L)		
	0	5.8	145.0		0	5.8	145.0
1				10			
	193.3	171.1	116.1		193.6	170.6	117.0
5				30			
	192.5	168.3	116.2		192.0	168.6	118.0

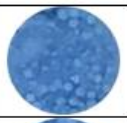
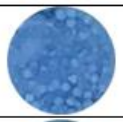
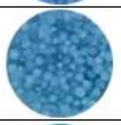
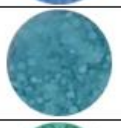
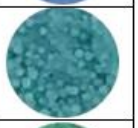
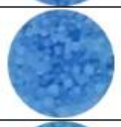
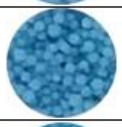
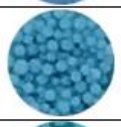
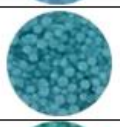
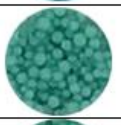
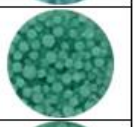
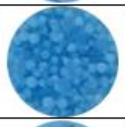
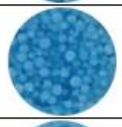
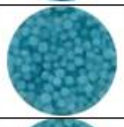
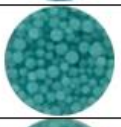
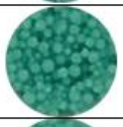
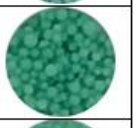
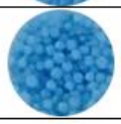
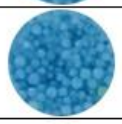
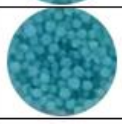
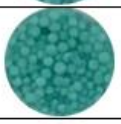
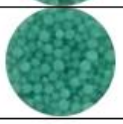
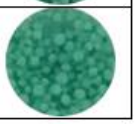
(condition: resins 0.1 g, 1.00 mL of 50 mM Cu^{2+}, Pyridine volume 0.5 mL, sample volume 10.00 mL, detection time 10 minutes)

4.2.3 Effect of sample volume

In an attempt to obtain a sensitive detection, a sample volume was one of parameters to be investigated. The sample volume was investigated at 1.00, 5.00, 10.00, 15.00 and 20.00 mL for the detection of thiocyanate with 0.1 g modified resin and the ratio of the resin and sample volume (solid: liquid ratio) was 1:10, 1:50, 1:100, 1:150, and 1:200 g/mL, respectively. Table 4.3 shows the color of the modified resin after the test with thiocyanate solutions of different volumes for 10 minutes. It was observed that the use of solid/sample volume ratio of 1:10 and 1:50 g/mL did not result in the color change on resin surface, except at very high concentration of thiocyanate (for solid/sample volume ratio of 1:50 g/mL). Under this condition, the amount of thiocyanate was low and the content of $[Cu(SCN)_2(Py)_2]$ complex was much less than the content of copper-pyridine complex on the resin. Consequently, the resin color did not obviously change. On the other hand, when the solid/sample volume ratio of 1:100, 1:150, and 1:200 g/mL were applied, it resulted in a noticeable change of color on the resin with different thiocyanate concentrations. The larger of the solid/solution volume ratio, the more obvious the color change was obtained due to higher amount of $[Cu(SCN)_2(Py)_2]$ complex. However, using the resins to sample volume ratio of 1:100 g/mL was not suitable for thiocyanate detection at lower concentration because the resins showed distinct colors at only high concentration of thiocyanate (58.0 – 145.0 mg/L). Furthermore, when increase the sample volume and hence the solid/liquid ratio to 1:150 and 1:200 g/mL, a similar color change at 29.0 – 145.0 mg/L

thiocyanate was observed due to the limited surface area of the resins. Therefore, the solid and sample volume ratio of 1:150 g/mL was chosen for further study.

Table 4.3 The effect of resins and sample volume ratio on the resin color

Resins/sample volumes ratio (g/mL)	Concentration of SCN^- (mg/L)					
	0	5.8	29.0	58.0	116.0	145.0
1 : 10						
1 : 50						
1 : 100						
1 : 150						
1 : 200						

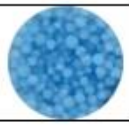
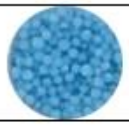
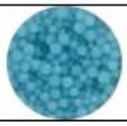
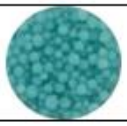
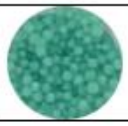
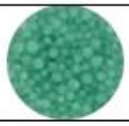
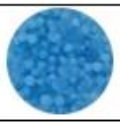
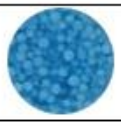
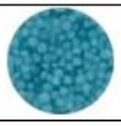
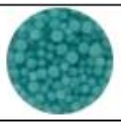
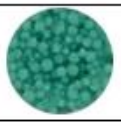
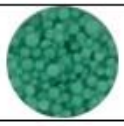
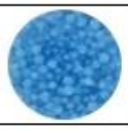
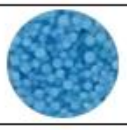
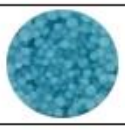
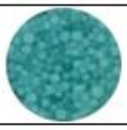
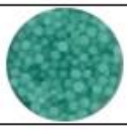
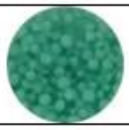
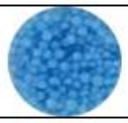
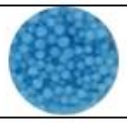
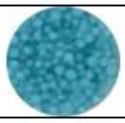
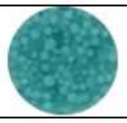
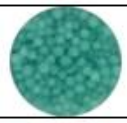
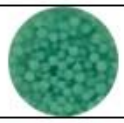
(condition: resins 0.1 g, 1.00 mL of 50 mM Cu^{2+}, pyridine volume 0.5 mL, modification time 1 minute, detection time 10 minutes)

4.2.4 Effect of detection time

The effect of detection time was examined in order to obtain a suitable time for the observation of color on resins after adding sample solution. The detection time was varied from 1 to 10 minutes. The thiocyanate concentration in the range 0 - 145.0 mg/L was chosen for this study. Table 4.4 shows similar color on resins obtained using different detection time. These results had been confirmed by statistical calculation

using one-way ANOVA and it showed that the color intensities of resins used to test same concentration of thiocyanate with detection time of 1, 2, 5, and 10 minutes were not significantly different (P = 0.547 (5.8 mg/L thiocyanate), P = 0.087 (29.0 mg/L thiocyanate), P = 0.784 (58.0 mg/L thiocyanate), P = 0.877 (116.0 mg/L thiocyanate), P = 0.301 (145.0 mg/L thiocyanate)) at the 95% confidence level (α= 0.05).

Table 4.4 Color of resin using different of detection time (mean color intensity as blue values are given under each photo)

Detection time (minutes)	Concentration of SCN^- (mg/L)					
	0	5.8	29.0	58.0	116.0	145.0
1						
	183.9	176.1	151.4	132.2	119.6	112.5
2						
	183.3	176.2	150.4	132.9	116.8	113.9
5						
	183.9	174.0	151.8	133.7	119.3	114.8
10						
	184.1	176.1	150.8	134.0	119.6	113.9

(condition: resins 0.1 g, 1.00 mL of 50 mM of Cu^{2+}, pyridine volume 0.5 mL, modification time 1 minute, sample volume 15.00 mL)

From the results of the mean blue values, it should be noticed that if the color of material is in blue tone, the mean blue values will be high. In contrast, a color of blue-green or green tone has low mean blue values. For quantitative analysis, calibration curves were constructed between the mean blue values against thiocyanate concentration. The results from using different detection time (Figure 4.2) indicate that the sensitivity of the detection was not remarkably increased in increasing the detection time. Therefore, 1 minute detection was selected in this work to shorten the analysis time. However, a good linearity was not obtained, and hence, the condition in the detection was reconsidered. The effect of pyridine amount as modifying reagent was eventually studied.

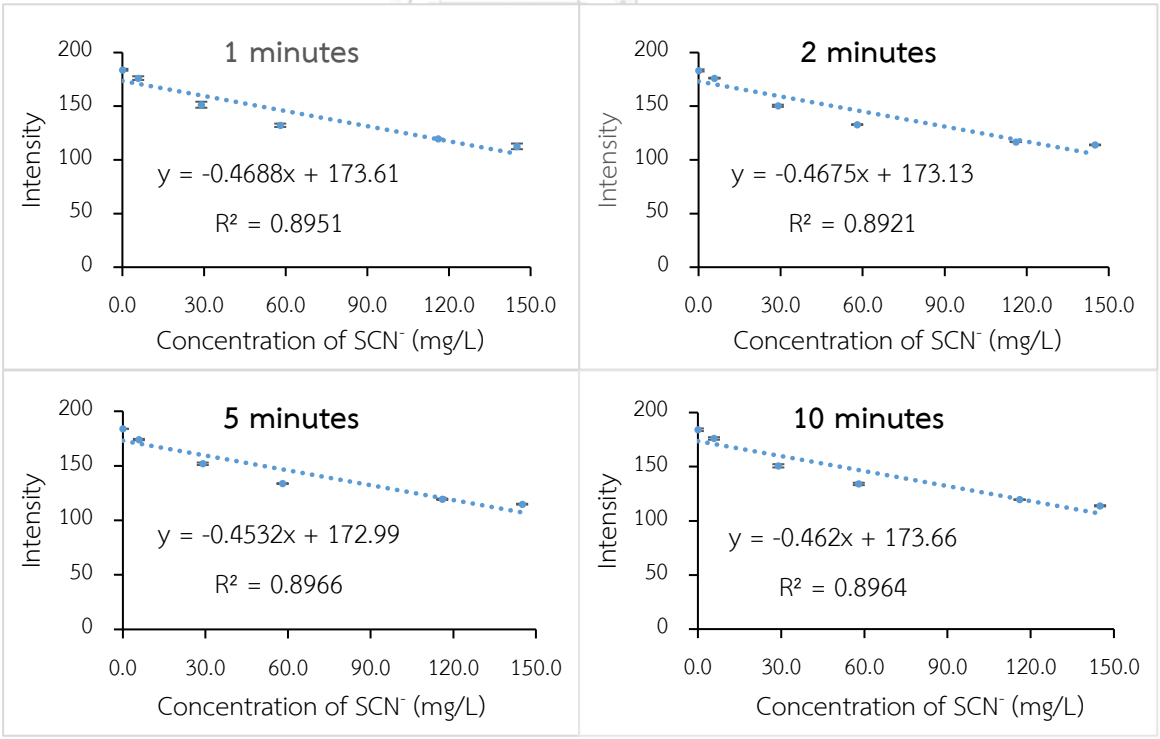


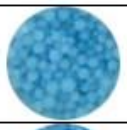
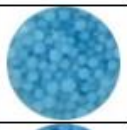
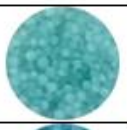
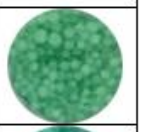
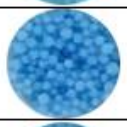
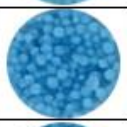
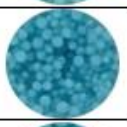
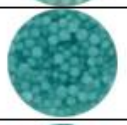
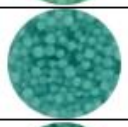
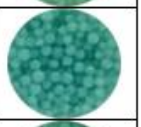
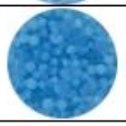
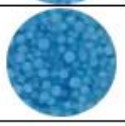
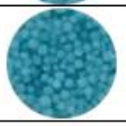
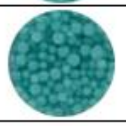
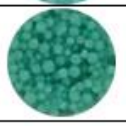
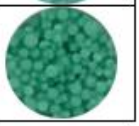
Figure 4.2 Calibration curves for determination of thiocyanate obtained by using different detection time.

4.2.5 Effect of pyridine volume

In this method, pyridine is a reagent to form complex with Cu^{2+} to produce copper-pyridine complex for a further use to react with thiocyanate. It can also affect the color change on resin. Thus, the amount of this reagent should be sufficient for the reaction to result in distinct color on resins when used in thiocyanate detection. In this experiment, pyridine volume used to modify 0.1 g of resin was varied from 0.10, 0.25 to 0.50 mL, and hence the solid/pyridine volume ratio was 1:1, 1:2.5, and 1:5.0 g/mL, respectively. The change of resin color using different ratio of solid/pyridine volume are shown in Table 4.5. By using the resins/pyridine volume ratio of 1:2.5 and 1:5.0 g/mL, the color was intense and the color changed from blue to green in increasing thiocyanate concentration. However, at low concentration of thiocyanate (29.0 mg/L), it was difficult to differentiate the color of thiocyanate complex from the blue color of copper-pyridine complex due to the presence of high content of free copper-pyridine complex on resin. On the other hand, at the lowest resin/pyridine volume ratio (1:1 g/mL), it gave a noticeable color change from blue to green in increasing thiocyanate concentration from 29.0 to 145.0 mg/L. However, at high concentration of thiocyanate (116.0 mg/L and 145.0 mg/L), the resins exhibited similar color probably due to the saturation of the complex on the resin having limited surface area.

Furthermore, in finding a suitable and more reliable detection than the naked-eye detection, the calibration curves were constructed between mean blue values against thiocyanate concentration as shown in Figure 4.3. The results obtained by using resin/pyridine volume ratio of 1:1 g/mL show a good linearity and sensitivity in the detection of thiocyanate. Therefore, this ratio was selected for this study.

Table 4.5 Color of resins obtained by using different resins/pyridine volumes ratio

Resins/pyridine volume ratio (g/mL)	Concentration of SCN^- (mg/L)					
	0	5.8	29.0	58.0	116.0	145.0
1 : 1						
1 : 2.5						
1 : 5.0						

(condition: resins 0.1 g, 1.00 mL of 50 mM of Cu^{2+}, modification time 1 minute, sample volume 15.00 mL, detection time 1) minute)

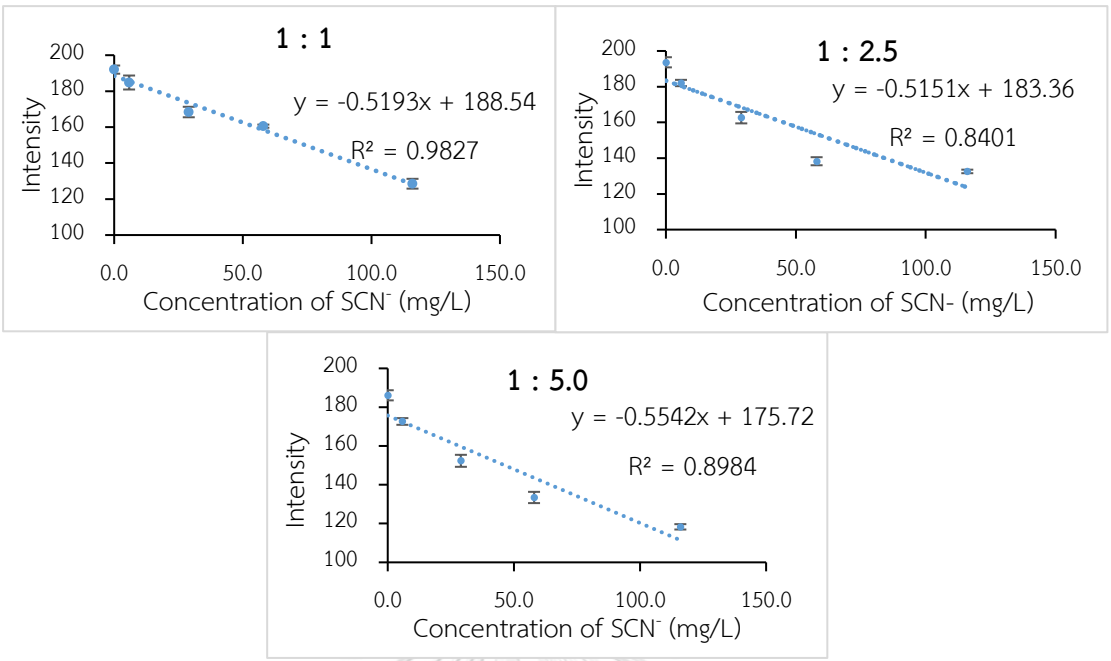


Figure 4.3 Calibration curves for the determination of thiocyanate obtained by using different resin/pyridine volumes ratio

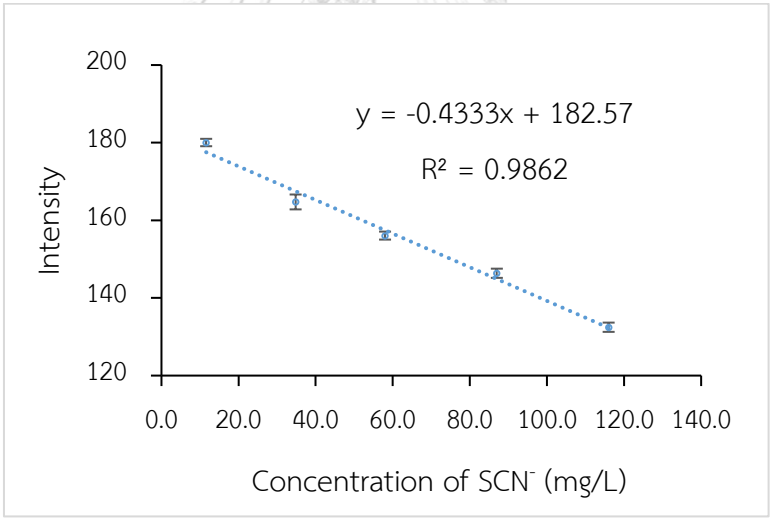
4.3 Method performance

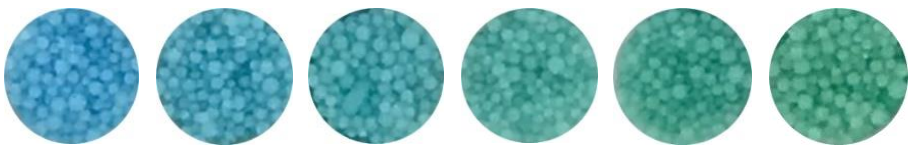
In this section, the performance of the proposed method was evaluated under the chosen condition as followed. A solution of 50 mM of Cu^{2+} (1.00 mL) and pyridine (0.1 mL) were used to modify the resin (0.1 g) for 1 minute to prepare the copper-pyridine complex containing resin for thiocyanate complexation. The obtained resins were further used to extract thiocyanate in solution with resins/solution volume ratio of 0.1 g :15 mL (1: 150 g/mL) for 1 minute. Then the resin color was observed by naked eyes and the photo of resin was taken and subjected to Image-J software to determine the color intensity in RGB mode (mean blue values). Under this condition, the linear

working range, the limit of detection (LOD), and limit of quantification (LOQ) were determined.

4.3.1 Linear working range

The linear relationship between color intensity (mean blue values) and thiocyanate concentration was obtained in the thiocyanate concentration range of 5.8-116.0 mg/L. The calibration curve is shown in Figure 4.4. The color chart observed at different thiocyanate concentrations is also presented. This linear working range covers the level of thiocyanate found in milk samples and the method was further applied to detect thiocyanate in different milk samples.





0 11.6 34.8 58.0 87.0 116.0 mg/L SCN^-

Figure 4.4 The calibration curve for thiocyanate determination and resin color chart.

4.3.2 Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the proposed method was achieved from the analysis of the blank solution. The procedure was performed as described in section 3.5.1 under the chosen condition. The signal of the LOD and LOQ concentration were calculated based on the signal obtained from the blank determination minus three and ten times of standard deviation (SD_{blk}) of the blank solution signal (n=10), respectively (Eq. 4.2, 4.3).

$$I_{LOD} = I_{blk} - 3SD_{blk} \quad (4.2)$$

$$I_{LOQ} = I_{blk} - 10SD_{blk} \quad (4.3)$$

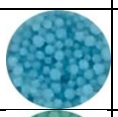
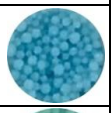
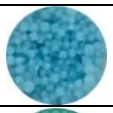
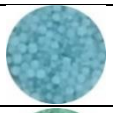
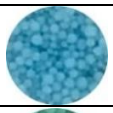
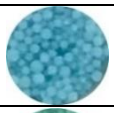
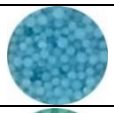
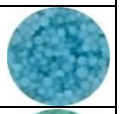
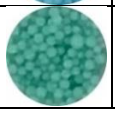
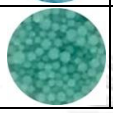
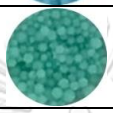
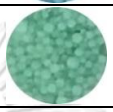
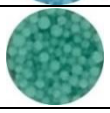
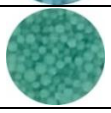
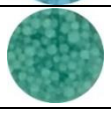
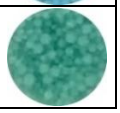
I_{LOD}, I_{LOQ}, and I_{blk} represent the color intensity of the solution of LOD concentration, LOQ concentration, and blank, respectively. The LOD and LOQ level were obtained by comparing the signal to the calibration curve and found to be 3.6 and 8.0 mg/L, respectively and the limit of detection by naked-eye was 11.6 mg/L.

4.4 Effect of potential interfering species

As the objective of this work is to apply the method to detect thiocyanate in milk samples, the effect of interfering species commonly found in milk sample (e.g. chloride ion, phosphate ion, citrate ion, sulfate ion, bromide ion, fluoride ion, and iodide ion) on the efficiency of thiocyanate detection was investigated. These interfering species may compete with thiocyanate in the complex formation with Cu^{2+} ion. Each of interferences having maximum concentration possibly found in milk was added into a thiocyanate standard solution (11.6 mg/L or 116.0 mg/L) to produce a binary mixture. In addition, these solutions were prepared using trichloroacetic acid (pH 5) as to simulate the reagent used in milk sample preparation. The solutions were further tested with the proposed method. The color of the resins and the color intensities obtained are shown in Table 4.6 and Figure 4.5, respectively.

It was found that the color of the resin used to detect thiocyanate in the absence and in the presence of interfering species was not significantly different at 95% confidence level by t-test, except for the presence of citrate ions. Citrate ion was the only one substance that strongly affected the analysis of thiocyanate at both low and high concentration. In solution of pH 5, citrate is in its deprotonated form having one carboxylate group and hence, it could competitively react with Cu^{2+} (K_f = 3.98 x 10^5) [53]. To solve this problem, pH of sample solutions should be lowered to pH 3 to keep it in protonated form (pKa_1 =3.13) [54].

Table 4.6 Color of resin observed in the detection of thiocyanate in the absence and in the presence of different foreign anions

Concentration of SCN^- (mg/L)	solution *							
	SCN^-	SCN^- + Cl^- (1100)	SCN^- + PO_4^{3-} (2300)	SCN^- + citrate (1750)	SCN^- + SO_4^{2-} (100)	SCN^- + Br^- (1)	SCN^- + F^- (0.15)	SCN^- + I^- (0.06)
11.6								
116.0								

* (concentration of foreign anions in mg/L is given in parentheses)

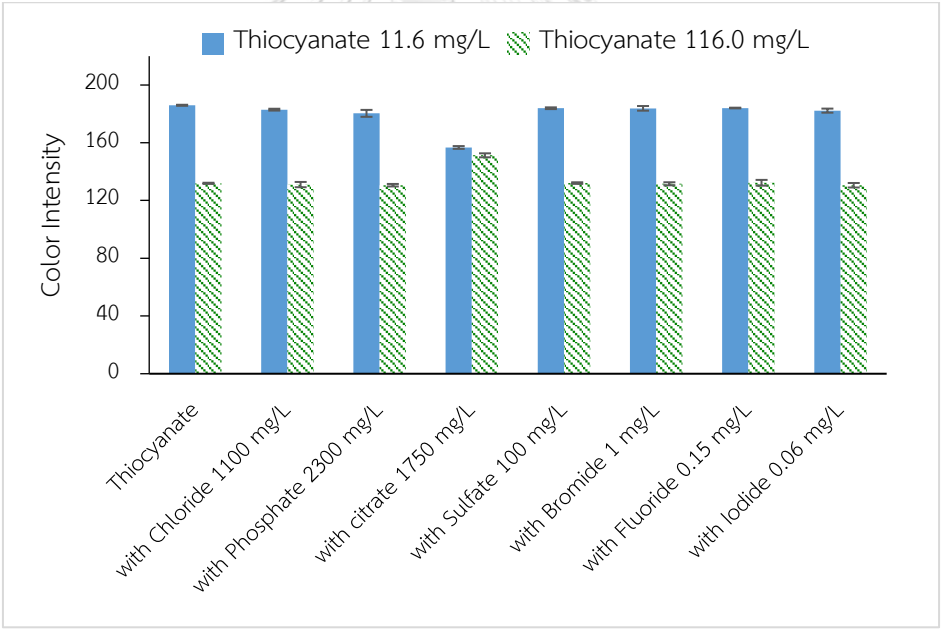


Figure 4.5 The color intensity observed in the detection of thiocyanate in standard solution in the absence (thiocyanate blank) and in the presence of various foreign anions

4.5 Determination of thiocyanate in milk samples

In analysis of thiocyanate in milk sample, sample preparation is required by using 3% trichloroacetic acid (TCA) solution for protein precipitation. After centrifuging, supernatant was used for detection step as described in topic 3.5.1.

4.5.1 Effect of milk matrix

The effect of milk sample matrix was investigated by comparing the external standard calibration curve constructed by using thiocyanate standard solutions (0 – 116.0 mg/L) and standard addition calibration curve using spiked milk sample (Figure 4.6). The same concentration of thiocyanate was spiked in a milk sample before performing sample pretreatment and thiocyanate detection by the proposed method. The calibration curve in this part was constructed by using delta color intensity which was the difference between color intensity of reagent blank solution and standard solution or milk sample. The delta color intensity would represent more accurately the level of thiocyanate complex formed.

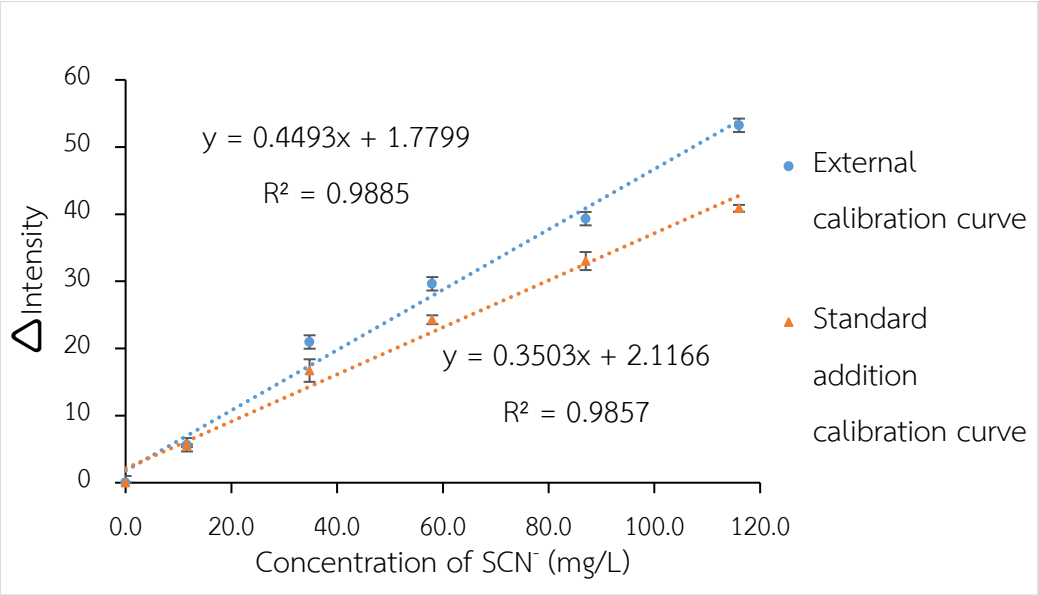


Figure 4.6 External calibration curve and standard addition calibration curve for thiocyanate determination in milk

The results display that in both matrix, a linear relationship between resin color intensities and thiocyanate concentration was obtained in this concentration range. However, the slopes of these calibration curves were different. The slope of the standard addition calibration curve was much less than that of external calibration curve, revealing a lower sensitivity in the detection of thiocyanate in milk matrix. The color of resins used to detect thiocyanate in spiked milk sample was paler than that observed in standard solution. Despite the sample preparation, the residual matrix probably affected the detection by competing with thiocyanate complex formation. Moreover, the matrix of milk may vary from sample to sample; thus, standard addition method was adopted for thiocyanate detection in milk sample to overcome the matrix effect.

4.5.2 Accuracy and precision in milk sample analysis

The proposed method was validated and the accuracy and precision were evaluated under the selected condition by using spiked method. The analytical results obtained by the proposed method were compared to the results from the analysis by using an ion chromatography. The accuracy and precision of these methods are presented in term of %recovery and %RSD, respectively. Standard addition method was adopted to determine thiocyanate in milk samples by the proposed method and ion chromatography method.

Milk samples of different brands and flavors were purchased from retail shops. Thiocyanate solution with a concentration of 11.6 mg/L or 29.0 mg/L was spiked into milk samples prior to the sample preparation process and then the samples were analyzed using both methods. The results obtained from all methods are compared in Table 4.7 and 4.8. The concentration of thiocyanate in milk sample determined by the proposed method and ion chromatography method were not significantly different at 95% confident level by pair t-test, indicating that the results from the proposed method was comparable to those from instrument. The percent recovery obtained by the proposed method was in the range of 93.4 – 104.6 %, compared to 98.7 – 101.0 % by ion chromatography method. The relative standard deviation (%RSD) of the results obtained was found in the range of 1.2 – 4.2 %, compared to 0.1-2.1% by ion chromatography method. These results demonstrate that the proposed method can

be used to detect thiocyanate in milk samples with acceptable accuracy and precision according to the criteria of AOAC International [52].

Table 4.7 Determination of thiocyanate in milk samples (different brand) by the proposed method and ion chromatography method

Sample	Amount added (mg/L)	Proposed method			Ion chromatography method		
		Found* (mg/L)	Recovery (%)	RSD (%)	Found* (mg/L)	Recovery (%)	RSD (%)
Pasteurized milk 1	0.00	8.6±0.2	-	-	7.4±0.1	-	-
	11.6	19.8±0.3	96.5	2.8	18.9±0.1	99.5	0.9
	29.0	38.1±0.9	101.5	3.2	36.6±0.1	100.9	0.2
Pasteurized milk 2	0.00	8.7±0.4	-	-	7.6±0.1	-	-
	11.6	20.0±0.3	97.4	1.2	19.2±0.1	100.1	0.4
	29.0	38.4±0.4	102.5	2.7	36.9±0.3	100.8	0.8
Pasteurized milk 3	0.00	9.5±0.4	-	-	7.3±0.1	-	-
	11.6	20.8±0.3	98.0	2.7	18.7±0.1	98.7	0.2
	29.0	39.8±1.1	104.4	3.6	36.3±0.2	99.9	0.8

*mean ± SD (n=3)

Table 4.8 Determination of thiocyanate in milk samples (different flavors) by the proposed method and ion chromatography method

Sample	Amount added (mg/L)	Proposed method			Ion chromatography method		
		Found* (mg/L)	Recovery (%)	RSD (%)	Found* (mg/L)	Recovery (%)	RSD (%)
Banana milk	0.00	7.2±0.1	-	-	6.1±0.1	-	-
	11.6	18.4±0.2	96.3	2.0	17.6±0.1	99.0	1.6
	29.0	36.8±1.2	102.1	4.2	35.4±0.5	101.0	2.1
Strawberry milk	0.00	7.5±0.1	-	-	7.3±0.1	-	-
	11.6	18.5±0.2	95.0	1.9	18.9±0.1	99.4	0.4
	0.50	37.2±0.7	102.4	2.4	36.6±0.2	101.0	0.8
Japanese melon milk	0.00	6.34±0.2	-	-	5.8±0.1	-	-
	11.6	17.1±0.2	93.4	1.6	17.4±0.1	99.5	0.2
	29.0	36.4±1.3	104.6	4.2	35.1±0.3	100.9	0.9

*mean ± SD (n=3)

CHEAPTER V
CONCLUSION

5.1 Conclusion

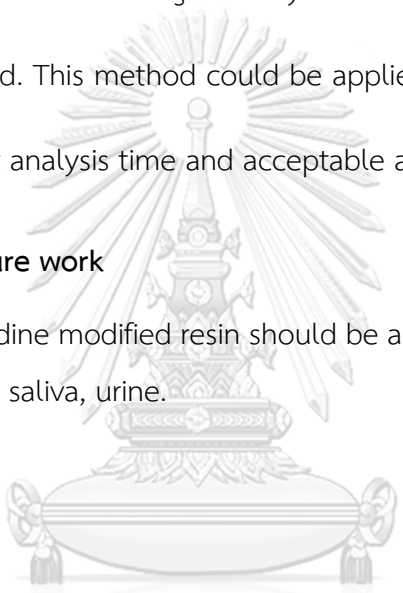
In this work, a new approach has been developed for thiocyanate determination based on the the reaction of thiocyanate on polymer resin surface modified with copper-pyridine complex to form dithiocyanato dipyridine copper(II), $[Cu(SCN)_2(Py)_2]$, complex.

The optimal condition for the determination of thiocyanate was obtained by studying the effect of various parameters. The reagent solution containing 1.00 mL of 50 mM of copper(II) solution and 0.1 mL pyridine was used to modify 0.1 g of polymer resin. The modified resin could successfully detect thiocyanate in a concentration range of 11.6 – 116.0 mg/L by using a resins/sample solutions volume ratio of 1:150 g/mL for 1 minute. When increased thiocyanate concentration, the color on resins changed from blue to blue-green and green, respectively. The color could be observed by naked eyes and the quantitative data was obtained *via* Image-J software used to measure the color intensity (blue scale mode). Under these conditions, the working range of this method covered 11.6 to 116.0 mg/L with a correlation coefficient (R^2) of 0.9862, and a detection limit and a limit of quantification of 3.6 mg/L and 8.0 mg/L, respectively.

The study of effect of interfering ions in milk sample did not affect the analysis of thiocyanate except the citrate ion. In order to overcome the effect of milk matrix, the standard addition method was required for detecting thiocyanate level in milk samples. The recoveries were in the range 93.4 - 104.6% and the percent relative standard deviation was less than 4.2%. The obtained results of thiocyanate analysis by the proposed method were not significantly different from the results from ion chromatography method. This method could be applied to determine thiocyanate in milk samples with short analysis time and acceptable accuracy and precision.

5.2 Suggestion for future work

The copper-pyridine modified resin should be applied to detect thiocyanate in biological fluids such as saliva, urine.



REFERENCES

[1] Song, J., Wu, F. Y., Wan, Y. Q., and Ma, L. H., Ultrasensitive turn-on fluorescent detection of trace thiocyanate based on fluorescence resonance energy transfer. Talanta 132 (2015): 619-24.

[2] Naik, R. M., Kumar, B., and Asthana, A., Kinetic spectrophotometric method for trace determination of thiocyanate based on its inhibitory effect. Spectrochimica acta. Part A, Molecular and biomolecular spectroscopy 75(3) (2010): 1152-8.

[3] Deng, H.-H., Wu, C.-L., Liu, A.-L., Li, G.-W., Chen, W., and Lin, X.-H., Colorimetric sensor for thiocyanate based on anti-aggregation of citrate-capped gold nanoparticles. Sensors and Actuators B: Chemical 191 (2014): 479-484.

[4] Bendtsen, A. B., and Hansen, E. H., Spectrophotometric flow injection determination of trace amounts of thiocyanate based on its reaction with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol and dichromate: assay of the thiocyanate level in saliva from smokers and non-smokers. The Analyst 116(6) (1991): 647-51.

[5] Demkowska, I., Polkowska, Z., and Namiesnik, J., Application of ion chromatography for the determination of inorganic ions, especially thiocyanates in human saliva samples as biomarkers of environmental tobacco smoke exposure. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences 875(2) (2008): 419-26.

[6] BORCH, E., WALLENTIN, C., ROSÉN, M., and BJÖRCK, L., Antibacterial Effect of the Lactoperoxidase/Thiocyanate/Hydrogen Peroxide System Against Strains of Campylobacter Isolated from Poultry. Journal of Food Protection 52(9) (1989): 638-641.

[7] Hamid, O. I. A., and Musa, Z. A. B., Effect of Different Levels of Sodium Thiocyanate and Percarbonate for Activation of Lactoperoxidase on the Keeping Quality of Raw Milk. Journal of Advanced Scientific Research 4(1) (2013): 27-30.

[8] Kanthale, P., Kumar, A., Upadhyay, N., Lal, D., Rathod, G., and Sharma, V., Qualitative test for the detection of extraneous thiocyanate in milk. Journal of food science and technology 52(3) (2015): 1698-704.

[9] Niemann, R. A., and Anderson, D. L., Determination of iodide and thiocyanate in powdered milk and infant formula by on-line enrichment ion chromatography with photodiode array detection. Journal of chromatography. A 1200(2) (2008): 193-7.

[10] Ghasemi, J., Amini, R., and Afkhami, A., Kinetic Spectrophotometric Determination of Thiocyanate Based on Its Inhibitory Effect on the Oxidation of Methyl Red by Bromate. Analytical Sciences 17(3) (2001): 435-437.

[11] Yang, Q., Liang, F., Wang, D., Ma, P., Gao, D., Han, J., Li, Y., Yu, A., Song, D., and Wang, X., Simultaneous determination of thiocyanate ion and melamine in milk and milk powder using surface-enhanced Raman spectroscopy. Anal. Methods 6(20) (2014): 8388-8395.

[12] Ponce, P., Thiocyanate content in raw milk under the american tropic conditions in relation to the activation of the lactoperoxidase system. Rev. Salud Anim. 34(2) (2012): 115-119.

[13] Basova, E. M., Ivanov, V. M., and Apendeeva, O. K., Spectrophotometric determination of thiocyanate ions in stratal waters. Moscow University Chemistry Bulletin 69(1) (2014): 12-19.

[14] van Staden, J. F., and Botha, A., Spectrophotometric determination of thiocyanate by sequential injection analysis. Analytica Chimica Acta 403(1) (2000): 279-286.

[15] Destanoğlu, O., and Gümüş Yılmaz, G., Determination of cyanide, thiocyanate, cyanate, hexavalent chromium, and metal cyanide complexes in various mixtures by ion chromatography with conductivity detection. Journal of Liquid Chromatography & Related Technologies 39(9) (2016): 465-474.

[16] Chen, S. H., Yang, Z. Y., Wu, H. L., Kou, H. S., and Lin, S. J., Determination of thiocyanate anion by high-performance liquid chromatography with fluorimetric detection. Journal of analytical toxicology 20(1) (1996): 38-42.

[17] Ammazzini, S., Onor, M., Pagliano, E., Mester, Z., Campanella, B., Pitzalis, E., Bramanti, E., and D'Ulivo, A., Determination of thiocyanate in saliva by headspace gas chromatography-mass spectrometry, following a single-step aqueous derivatization with triethyloxonium tetrafluoroborate. Journal of chromatography. A 1400 (2015): 124-30.

[18] Keyvanfard, M., Alizad, K., and Elahian, P., Determination of Thiocyanate by Kinetic Spectrophotometric Flow Injection Analysis. Journal of Chemistry 2013 (2013): 1-5.

[19] Banerjee, A., Sahana, A., Lohar, S., Hauli, I., Mukhopadhyay, S. K., Safin, D. A., Babashkina, M. G., Bolte, M., Garcia, Y., and Das, D., A rhodamine derivative as a "lock" and SCN- as a "key": visible light excitable SCN- sensing in living cells. Chemical communications 49(25) (2013): 2527-9.

[20] Zhang, Z.-Y., Liu, J., and Wang, H.-Y., Microchip-Based Surface Enhanced Raman Spectroscopy for the Determination of Sodium Thiocyanate in Milk. Analytical Letters 48(12) (2015): 1930-1940.

[21] Haque, M. R., and Bradbury, J. H., Simple method for determination of thiocyanate in urine. Clinical chemistry 45(9) (1999): 1459-64.

[22] Narongwanichgarn, W., and Theeraphan, A., Development of rapid test kit for detection of thiocyanate in raw milk. Thai-NIAH eJournal (2006).

[23] Patel, R., and Patel, K. S., Flow-injection analysis determination of thiocyanate in industrial waste water. 44 (1999): 917-923.

[24] Danchick, R. S., and Boltz, D. F., Indirect spectrophotometric and atomic absorption spectrometric methods for determination of thiocyanate. Analytical Chemistry 40(14) (1968): 2215-2216.

[25] Woon Lee, D., Eum, C., Ho Lee, I., and Joo Jeon, S., Adsorption behavior of 8-hydroxyquinoline and its derivatives on Amberlite XAD resins, and adsorption of metal ions by using chelating agent-impregnated resins. 4 (1988): 505-510.

[26] Tewari, P. K., and Singh, A. K., Amberlite XAD-7 impregnated with Xylenol Orange: a chelating collector for preconcentration of Cd(II), Co(II), Cu(II), Ni(II), Zn(II) and Fe(III) ions prior to their determination by flame AAS. Fresenius' journal of analytical chemistry 367(6) (2000): 562-7.

[27] Schneider, C. A., Rasband, W. S., and Eliceiri, K. W., NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9(7) (2012): 671-675.

[28] Zamberlin, Š., Antunac, N., Havranek, J., and Samaržija, D., Mineral elements in milk and dairy products. Mineralni sastav mlijeka i mliječnih proizvoda. 62(2) (2012): 111-125.

[29] Njage, P., and Wangoh, J., Use of the lactoperoxidase system to enhance keeping quality of pasteurised camel milk. 4 (2010): 61-63.

[30] CACGL, Guidelines for the Preservation of Raw Milk by Use of the Lactoperoxidase System. Conference Proceedings (2011).

[31] Haddadin, M. S., Ibrahim, S. A., and Robinson, R. K., Preservation of raw milk by activation of the natural lactoperoxidase systems. Food Control 7(3) (1996): 149-152.

[32] Banerjee, K. K., Marimuthu, P., Bhattacharyya, P., and Chatterjee, M., Effect of thiocyanate ingestion through milk on thyroid hormone homeostasis in women. The British journal of nutrition 78(5) (1997): 679-81.

[33] Sørhaug, T., and Stepaniak, L., Psychrotrophs and their enzymes in milk and dairy products: Quality aspects. Trends in Food Science & Technology 8(2) (1997): 35-41.

[34] Oram, J. D., and Reiter, B., The inhibition of streptococci by lactoperoxidase, thiocyanate and hydrogen peroxide. The oxidation of thiocyanate and the nature of the inhibitory compound. The Biochemical journal 100(2) (1966): 382-8.

[35] Seifu, E., Buys, E. M., and Donkin, E. F., Significance of the lactoperoxidase system in the dairy industry and its potential applications: a review. Trends in Food Science & Technology 16(4) (2005): 137-154.

[36] Cengiz, M. F., and Bilgin, A. K., Determination of major sodium iodide symporter (NIS) inhibitors in drinking waters using ion chromatography with conductivity detector. Journal of pharmaceutical and biomedical analysis 120 (2016): 190-7.

[37] Salimon, J., Abdullah, B. M., and Salih, N., Rubber (Hevea brasiliensis) seed oil toxicity effect and Linamarin compound analysis. Lipids in health and disease 11 (2012): 74.

[38] Mak, K. K., Yanase, H., and Renneberg, R., Cyanide fishing and cyanide detection in coral reef fish using chemical tests and biosensors. Biosensors & bioelectronics 20(12) (2005): 2581-93.

[39] Tanaka, A., Deguchi, K., and Deguchi, T., Spectrofluorimetric determination of cyanide and thiocyanate based on a modified König reaction in a flow-injection system. Analytica Chimica Acta 261(1) (1992): 281-286.

[40] Aldridge, W. N., A new method for the estimation of micro quantities of cyanide and thiocyanate. The Analyst 69(822) (1944): 262-265.

[41] Botto, R. I., Karchmer, J. H., and Eastwood, M. W., Spectrophotometric determination of uncomplexed cyanide and thiocyanate in wastewater with p-phenylenediamine. Analytical Chemistry 53(14) (1981): 2375-2376.

[42] Analyctical®, M. I. a. A., The Picric Acid Method for Determining Weak Acid Dissociable (WAD) Cyanide.

[43] Lawrance, G. A., *Introduction to Coordination Chemistry*. John Wiley & Sons, Ltd, 2009.

[44] Kirkbright, G. F., and Johnson, H. N., Application of indirect methods in analysis by atomic-absorption spectrometry. Talanta 20(5) (1973): 433-451.

[45] Chattaraj, S., and Das, A. K., Indirect determination of thiocyanate in biological fluids using atomic absorption spectrometry. Spectrochimica Acta Part B: Atomic Spectroscopy 47(5) (1992): 675-680.

[46] Chow, A., and Ginsberg, S. L., The extraction and determination of thiocyanate complexes by use of polyurethane foam. Talanta 30(8) (1983): 620-622.

[47] Stratis, J. A., and Vasilikiotis, G. S., Indirect determination of thiocyanate and selenocyanate by atomic absorption spectrometry and solution spectrophotometry. Microchemical Journal 32(1) (1985): 1-7.

[48] Pena-Pereira, F., Lavilla, I., and Bendicho, C., Paper-based analytical device for instrumental-free detection of thiocyanate in saliva as a biomarker of tobacco smoke exposure. Talanta 147 (2016): 390-6.

[49] Domínguez, J. R., González, T., Palo, P., and Cuerda-Correa, E. M., Removal of common pharmaceuticals present in surface waters by Amberlite XAD-7 acrylic-

ester-resin: Influence of pH and presence of other drugs. Desalination 269(1-3) (2011): 231-238.

[50] Hosseini, M. S., Hosseini-Bandegharaei, A., Raissi, H., and Belador, F., Sorption of Cr(VI) by Amberlite XAD-7 resin impregnated with brilliant green and its determination by quercetin as a selective spectrophotometric reagent. Journal of hazardous materials 169(1-3) (2009): 52-7.

[51] Cantrell, K., Erenas, M. M., de Orbe-Payá, I., and Capitán-Vallvey, L. F., Use of the Hue Parameter of the Hue, Saturation, Value Color Space As a Quantitative Analytical Parameter for Bitonal Optical Sensors. Analytical Chemistry 82(2) (2010): 531-542.

[52] Mehta, B. M., Chemical Composition of Milk and Milk Products. (2015): 1-34.

[53] AOAC Official Methods of Analysis, Appendix F: Guidelines for standard method performance requirements, 2016. [cited May 5, 2018]; http://www.eoma.aoac.org/app_f.pdf.

[54] Olin, Å., and Wallién, B., Determination of citrate by potentiometric titration with copper(II) and a copper ion-selective electrode. Analytica Chimica Acta 151 (1983): 65-75.

[55] Heller, A., Barkleit, A., Foerstendorf, H., Tsushima, S., Heim, K., and Bernhard, G., Curium(III) citrate speciation in biological systems: a europium(III) assisted spectroscopic and quantum chemical study. Dalton transactions (Cambridge, England : 2003) 41(45) (2012): 13969-83.

APPENDIX

VITA

Miss Sujinda Khaosaard was born on October 29, 1992 in Chonburi, Thailand. She graduated with a Bachelor's degree of Science from Burapha University in 2014. After that she becomes a graduate student at Environmental Analysis Research Unit (EARU), Department of Chemistry, Faculty of Science, Chulalongkorn University. She finished her Master's degree of Science in 2018.