Method Development for Copper Determination in Natral Rubber (*Hevea Brasilliensis*) Latex

Khwannapha Rattanadaecha

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Thesis Title : Method Development for Copper Determination in Natural Rubber (*Hevea Brasilliensis*) Latex
Author : Miss Khwannapha Rattanadaecha
Major Program: Analytical Chemistry

Major Advisor

…………………………….
(Dr. Supunnee Duangthong)

Co-advisor

…………………………………………………
(Dr. Puchong Wararatananuruk)

…………………………………………………
(Asst. Prof. Dr. Wilairat Cheewasedtham)

Examining Committee:

…………………………………Chairperson
(Dr. Tinnakorn Tiensing)

…………………………………………….…
(Mrs. Varaporn Kajornchaiyakul)

………………………………………………
(Dr. Thitima Rujiralai)

……………………………………………..
(Dr. Supunnee Duangthong)

………………………………………………
(Dr. Puchong Wararatananuruk)

………………………………………………
(Asst. Prof. Dr. Wilairat Cheewasedtham)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Science Degree in Analytical Chemistry

……………………..………………

(Prof. Dr. Amornrat Phongdara)

Dean of Graduate School

ชื่อวิทยานิพนธ์ การพัฒนาวิธีสำหรับการวิเคราะห์หาปริมาณทองแดงในน้ำยางพารา (ฮีเวียบราซิลเลียนซิส)
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สาขาวิชา เคมีวิเคราะห์
ปีการศึกษา 2555

บทคัดย่อ

ปริมาณทองแดงที่มีอยู่ในน้ำยางเป็นปัจจัยหนึ่งซึ่งมีผลต่อคุณภาพของผลิตภัณฑ์ยาง เนื่องจากทองแดงจะทำหน้าที่เป็นตัวเร่งปฏิกิริยาออกซิเดชันในโมเลกุลยาง ดังนั้นการวิเคราะห์หาปริมาณทองแดงจึงเป็นสิ่งจำเป็น โดยงานวิจัยชิ้นนี้สนใจที่วิเคราะห์หาปริมาณทองแดงในตัวอย่างน้ำยางพาราข้น ซึ่งวิธีการอย่างง่ายที่พัฒนาขึ้นมานั้น อาศัยการเกิดสารประกอบเชิงซ้อนระหว่างทองแดงกับซิงค์ไดเอทิลไดไทโอคาร์บาเมต และสกัดในไดคลอโรมีเทน สารประกอบเชิงซ้อนสีเหลืองที่เกิดขึ้นถูกนำไปตรวจวัดที่ความยาวคลื่น 435 นาโนเมตร โดยวิธีการที่ได้พัฒนาขึ้นมานั้นจะรวมไปถึงการพัฒนาวิธีการย่อยตัวอย่างน้ำยางพารา ให้มีความง่ายและมีราคา ถูกสำหรับการย่อยตัวอย่างด้วยวิธีการที่พัฒนาขึ้นมานั้นอาศัยการย่อยตัวอย่างน้ำยางในหลอดแก้ว โดยย่อยตัวอย่างที่ศึกษาด้วยกรดซัลฟิวริกและกรดไนตริกเข้มข้น ที่อุณหภูมิ 180 องศาเซลเซียส และนำไปวิเคราะห์ภายใต้สภาวะที่เหมาะสม จากผลการทดลองพบว่า วิธีการวิเคราะห์ทองแดงที่พัฒนาขึ้นนั้นให้ช่วงความเป็นเส้นตรงอยู่ในช่วง 0.2-4.0 มิลลิกรัมต่อลิตร โดยให้ค่าสัมประสิทธิ์สหสัมพันธ์ที่มากกว่า 0.99 ขีดจำกัดการตรวจวัดต่ำสุดและปริมาณการตรวจวัดต่ำสุดเท่ากับ 0.0004 และ 0.0014 มิลลิกรัมต่อลิตร ตามลำดับ ผลการศึกษาความเที่ยงของวิธีจากการวิเคราะห์ภายในวันเดียวกันและระหว่างวันนั้นพบว่า ค่าเบี่ยงเบนมาตรฐานอยู่ในช่วงร้อยละ 0.4-3.2 และ 0.8-3.4 ตามลำดับ นอกจากนี้ค่าการได้กลับคืนของปริมาณทองแดงในตัวอย่างน้ำยางพาราอยู่ในช่วงร้อยละ 92-101 สำหรับการศึกษาผลของตัวรบกวน ของไอออนบวกบางตัว เช่น เหล็ก แมงกานีส และสังกะสี พบว่า ไออนเหล่านี้ไม่ส่งผลรบกวนต่อการวิเคราะห์หาปริมาณทองแดง ในตัวอย่างน้ำยางพาราข้น โดยที่ความเข้มข้นที่มากที่สุดที่จะไม่รบกวนการวิเคราะห์ทองแดงมีค่า 9.0, 9.0 และมากกว่า 500 มิลลิกรัมต่อลิตร สำหรับไอออนเหล็ก แมงกานีส และสังกะสี ตามลำดับ และปริมาณของไอออนบวกเหล่านี้ที่พบในน้ำยางข้นนั้นมีปริมาณน้อยกว่าระดับ ที่รบกวน ดังนั้น วิธีการที่พัฒนาขึ้นจะนำไปประยุกต์ใช้กับการวิเคราะห์หาปริมาณทองแดงในตัวอย่างน้ำยางพาราข้นซึ่ง

เตรียมจากน้ำยางพาราสด โดยตัวอย่างน้ำยางพาราสดนั้น ได้จาก ผู้ประกอบการขายน้ำยางสด ใน 5 อำเภอ อำเภอละหนึ่งราย ในจังหวัดสงขลา ได้แก่ อำเภอนาหม่อม อำเภอหาดใหญ่ อำเภอเมืองสงขลา อำเภอบางกล่ำ และอำเภอนาทวี โดยน้ำยางสดเหล่านี้จะถูกนำมาเติมไดแอมโมเนียมไฮโดรเจนออโทฟอสเฟต เพื่อทำการตกตะกอนแมกนีเซียมก่อนนำไปปั่นเหวี่ยงเป็นน้ำยางข้น ซึ่งปริมาณไดแอมโมเนียมไฮโดรเจนออโทฟอสเฟตที่เติมลงไปนั้นขึ้นกับจำนวนของปริมาณแมกนีเซียมที่มีอยู่ในน้ำยาง โดยปริมาณแมกนีเซียมนั้นทำการวิเคราะห์โดยการไทเทรตกับอีดีทีเอ จากนั้นจึงนำตัวอย่างน้ำยางพาราทั้งสดและข้นมาทำการวิเคราะห์ทองแดง จากผลการวิเคราะห์พบว่าปริมาณทองแดงในน้ำยางข้นอยู่ในช่วง 6.7-7.0 มิลลิกรัมต่อกิโลกรัม ของของแข็งทั้งหมดในยาง นอกจากนี้วิธีการที่พัฒนาขึ้นนี้ถูกนำไปเปรียบเทียบการวิเคราะห์หาปริมาณทองแดงกับวิธีอินดัคทิฟลีคัพเพิล พลาสมา จากการวิเคราะห์พบว่าปริมาณทองแดงที่ได้จากทั้งสองวิธีไม่มีความแตกต่างกันอย่างมีนัยสำคัญที่ช่วงความเชื่อมั่นร้อยละ 95 โดยใช้สถิติในการวิเคราะห์คือการทดสอบแบบทีและแบบอโนวา นอกจากนี้แล้วก็ได้ทำการเปรียบเทียบวิธีการย่อยตัวอย่างตามวิธีที่พัฒนาขึ้นกับวิธีแบบเจลดาห์ล ผลการศึกษาพบว่าปริมาณทองแดงที่ได้ไม่มีความแตกต่างกันอย่างมีนัยสำคัญเช่นเดียวกัน

สำหรับปริมาณทองแดงที่พบในตัวอย่างน้ำยางพาราสดนั้นอยู่ในช่วง 16.6-23.8 มิลลิกรัมต่อกิโลกรัมของของแข็งทั้งหมด ในยาง และได้ทำการเปรียบเทียบกับปริมาณทองแดงที่พบในน้ำยางข้น พบว่า ปริมาณทองแดงในน้ำยางสดสูงกว่าในน้ำยางข้นทั้งนี้เนื่องจาก ไดแอมโมเนียมไฮโดรเจนออโทฟอสเฟตที่เติมลงไปนั้นจะตกตะกอนทองแดงด้วย

วิธีการที่พัฒนาขึ้นมานั้นมีความง่ายและถูกต้องเหมาะสมที่จะนำไปประยุกต์ใช้กับการวิเคราะห์หาปริมาณทองแดงในตัวอย่างน้ำยางข้นในโรงงานอุตสาหกรรมน้ำยางข้นต่อไป

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Author	Miss Khwannapha Rattanadaecha
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ABSTRACT

Copper content is one of factors which affects on rubber product quality due to its catalytic function on the oxidation reaction in rubber molecule. Therefore, the determination of copper contents in NR latex is required. This research was focused on the method development for copper determination in concentrated natural rubber (NR) latex samples. The simple method based on the complex formation between Cu^{2+} and zinc diethyl dithiocarbamate (ZDEC) and extraction with dichloromethane was developed. The yellow complex formed was detected by vis-spectrophotometry at 435 nm. The method development included the development of simple and low cost wet digestion system for NR latex. The sample was digested in Pyrex tube with the acid mixture of conc. H_2SO_4 and conc. HNO_3 at 180°C in the developed digestion system. Under the optimum conditions, the linear was in the range of 0.2-4.0 mg L^{-1}, with a good correlation coefficient ($R^2 > 0.99$). The limit of detection and limit of quantification of copper were 0.0004 and 0.0014 mg L^{-1}, respectively. The intra-day (n=10) and inter-day (n=6) precision were 0.4-3.2 and 0.8-3.4%, respectively. The accuracy with good recoveries of 92-101% was provided. Potential interfering cations such as Fe^{3+}, Mn^{2+} and Zn^{2+} were investigated. They did not interfere seriously with high tolerance limit of 9.0, 9.0 and more than 500 mg L^{-1} for Fe^{3+}, Mn^{2+} and Zn^{2+}, respectively. This is due to the concentration of these interfering cations present in latex is usually lower than their interfering levels. The method was applied for copper determination in concentrated latex samples which were prepared from field latex samples by centrifugation method. Field latex samples were purchased from 5 different latex traders in following 5 different districts; Na Mom, Hat Yai, Muang Songkhla, Bang Klam and Na Thawi, located in Songkhla

Province. In the process of concentrated latex preparation, diammonium hydrogen orthophosphate (DAHP) was added in field latex to precipitate magnesium. The amount of DAHP added into field latex is depending on magnesium content. Magnesium content was determined by titration with EDTA. Then, the method was applied for copper determination in field and concentrated latex. The copper contents were found in the range of 6.8-7.0 mg kg^{-1} (on %TSC). Furthermore, the developed method was compared with Inductively Coupled Plasma - Optical Emission Spectrophotometry (ICP-OES) to confirm accuracy. No significant difference was observed (at 95% confident limit; t-test and ANOVA). In addition, the sample digestions with the developed and kjeldahl digestion system were performed. There was no significant difference of copper contents in the NR latex samples digested by both methods.

Copper content in field NR latex samples were determined. The copper content in field NR latex samples were found in the range of 16.6-23.8 mg kg^{-1} (on %TSC). The copper content in field and concentrated latex samples were also compared. It was observed that copper content in field NR latex was higher than copper content in concentrated NR latex, due to the precipitation of DAHP with copper.

This simple and accurate method will be further applied for copper determination in concentrated latex industries.

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LISTS OF ABBREVIATIONS

ASTM = American Standard Test Method
CPS = Count per second
DAHP = Diammonium hydrogen phosphate
DCM = Dichloromethane
DPASV = Differential Pulse Anodic Stripping Voltammetry
DRC = Dry Rubber Content
FAAS = Flame Atomic Absorption Spectrophotometry
GFAAS = Graphite Furnace Atomic Absorption Spectrophotometry
ICP-OES = Inductively Coupled Plasma Optical Emission Spectrophotometry
ISO = International Organization for Standardization
LLE = Liquid-Liquid extraction
NR = Natural Rubber
RRIT = Rubber Research Institute of Thailand
RSD = Relative Standard Deviation
TIS = Thai Industrial Standards
TSC = Total Solids Content
VFA = Volatile Fatty Acid
ZDEC = Zinc diethyldithiocarbamate

CHAPTER 1

INTRODUCTION

1.1 Introduction

Natural rubber is an economic plant and plays an important role in Thai economy. Rubber trees have been planted in various countries worldwide, especially in Asian countries (Sangkhawatin, 2008). Recently, Thailand is the world's largest producer and exporter of natural rubber such as technically specified natural rubber, smoke sheet rubber, and concentrated latex (RRIT, 2011).

Field natural rubber latex derived from rubber tree is applied as a raw material for various rubber products including sheet rubber, crepe rubber, and concentrated latex (Fisher, 1931). Normally, the concentrated latex is more popular than field latex due to the better uniform in quality, richer rubber content and the higher degree of purity. Furthermore, the field NR latex contains excessive water, which is costly for transportation (Blackley, 1997, volume 1, p. 10-11). Generally, characteristics of concentrated latex must be analyzed in a testing laboratory to assure quality of the latex which should be met the specifications before applying for the manufacturing processes. Parameters indicating the latex properties including total solids content (%TSC), dry rubber content (%DRC), magnesium content, copper and manganese content, and alkalinity are controlled by TIS 980 (2009).

Copper is one of metals which has a catalytic effect on the degradation of latex and rubber. Regarding to the Notification of Thai Industrial Standards TIS 980 (2009), copper content in concentrated latex must not exceed 8 mg kg^{-1} (on %TSC). The copper determination, therefore, is sometimes required to assure the latex quality.

Various methods can be applied for copper determination. Analysis of copper content in industrial laboratory is followed by the method of International Organization for Standardization (ISO). The ISO announces the photometric method for determination of copper content in rubber and latex (ISO 8053 (1995)). However, 1,1,1-trichloroethane, the solvent used in photometric method is not commercially available. Furthermore, the copper content containing in solid natural rubber can be determined by the American Standard Test Method (ASTM (1982)). The reaction of ASTM is based on copper with zinc dibenzyldithiocarbamate (ZDBC) in CCl_4. For the sample preparation, ASTM recommends the ashing method but this method presents some drawbacks such as the attachment of metal on the crucible wall, the attachment of metal with ash residue that cannot dissolve by acid. Consequently, it affects on the low recovery. From these reasons, the wet digestion is chosen for sample preparation in this work. The wet digestion recommended by ISO 8053 (1995) method implements kjeldahl system but there are some drawbacks including costly apparatus and time consumption. Hence, this research focuses on the development of a simple and rapid method for copper analysis which is suitable for routine analysis in industrial laboratories.

1.2 Background and Rationale

1.2.1 Natural rubber

Natural rubber is an elastomer composing of macromolecule obtained from latex. For many years, natural rubber has been a commercial plant of several countries. Thailand is one of the largest natural rubber plantations. Currently, Thailand remains the largest country with a large rubber plantation in the world (Sangkhawathin, 2008).

The natural rubber was originated from the Amazon River in Brazil to South America. Natural rubber has been known to the natives for ages. It was widely used for making erasers, balls and was more recognizable in Europe and South America. Then it has been widely spread to England, Sri Lanka, Singapore, Indonesia, Malaysia, and Thailand. For Thailand, the natural rubber was first cultivated in Trang province. The biggest rubber production area is the South of Thailand (Sangkhawatin, 2008). After that rubber plantations have been spread to Northeast, East, North and Central region. Thailand has been world’s major supplier of natural rubber compared with other producer countries. (Phongpiya, 2005).

The main source of natural rubber latex is **Para rubber** (*Hevea Brasilliensis*). Other plants, for examples, Gutta-percha, Balata, Guayule, and Ficus elestica, can be used for producing latex as well.

Para rubber (*Hevea Brasilliensis*)**:** It is a plant in the family of **"Euphorbiaceae"** and is a primary source of natural rubber widely used in various industries (Kajornchaiyakul, 2006). The chemical structure of the rubber is *cis* 1,4-polyisoprene. The Para rubber provides large quantity and superb quality of latex. There are various clones of *Hevea Brasilliensis*, for instance, RRIM 600, RRIT 251, RRIT 226, RRIT 209, BPM 24, PB 235, PB 86 and Tjir 1. Among these clones, RRIM 600 is the most widely grown. It is able to survive in different climates and provides the greatest latex production (Sangkhawatin, 2008).

Gutta-percha and Balata: Gutta-percha latex is obtained from trees in the family of *Sapotacae*. These trees are propagated by seeds or cuttings, and Balata latex is derived from trees in the species of *Mimusops balata* (Blackley, 1997, volume 2, p. 115). The chemical structure of these rubbers, unlike the **Para rubber**, is *trans* 1,4-polyisoprene. The latex contains high resin providing rubber in elastic. Moreover, when temperature is accelerating from 70-100°C, the rubber property is changed from hard material to a plastic-like one. Gutta-percha and Balata were earlier used as cable corridors and transmission belt, respectively (Kajornchaiyakul, 2006). Nowadays, Gutta rubber is used as a raw material for manufacturing golf balls and Balata is used for the denture devices (Phatthanakul, 2011).

Guayule (Parthenium argentatum): It is a perennial low shrub widely planted in Mexico. The plant provides the resin similar to rubber and the content of this rubber specie is about 70% of hydrocarbons, 20% of resin and the part of substance about 10% being non-dissolve in benzene. The structure of Guayule is *cis*-polyisoprene and tackier than the **Para rubber**. The Guayule is short-lived and provides lower latex production. Therefore, Guayule is not suitable for producing the rubber products and mostly grown by the locals. The guayule rubber is used for producing fabric fiber and ball rubber (Lloyd, 1942).

Rubber fig (Ficus elastica): It is a large tree of banyan group and produces the milky latex as the same as Para rubber. Since the latex extracted from rubber fig is irritant to human's skin and eyes and causes the fatal if consumption, it is rarely used to produce gloves and other products (Kang *et al*., 2000).

1.2.2 Natural rubber (*Hevea Brasiliensis*) latex

Natural rubber is a hydrocarbon compound, which each monomer consists of 5 carbons and 8 hydrogens in one chain. The chemical structure is C_5H_8 which is called as isoprene. The bonding of rubber structure is linear chain and identified as *cis* 1,4 - polyisoprene structure. The chain of natural rubber presents the double bond and α-methylene group (Fisher, 1931). Actually, the double bond of this chain is sensitive to oxygen or ozone, yielding the oxidation reaction of the natural rubber. This result brings the natural rubber being degraded (Saeoui, 2006). The structure of natural rubber (*cis* 1,4 - polyisoprene) is illustrated in **Figure 1-1**.

Figure 1-1 Monomer of rubber structure (*cis* 1,4-isoprene).

The rubber derived from a tapped tree is collected in three forms; latex, cup lump, and tree lace. The fresh milky latex which is the principal form is called “field natural rubber (NR) latex”, and the latex obtained from centrifugation of field NR latex is called “concentrated latex”.

1.2.3 Field natural rubber latex

1.2.3.1 Properties of field NR latex

Field natural rubber latex is milky fluid tapped from rubber tree. The character of field natural rubber latex is a small particle that disperses in water. It is a white fluid, colloidal substance, and the dry rubber content in field latex is 33%, and the total solids content is 36% (Fisher, 1931). The main composition of field natural rubber latex can be divided into the importance elements as shown in **Table 1-1.** The typical properties of field natural rubber latex are illustrated in **Table 1-2**.

Table 1-1 Composition of field natural rubber latex

Constituents	**Content % w/w**
1. Water	55-60
2. Poly (cis-1,4-isoprene)	30-45
3. Non isoprene component	5-6
- Protein	2.0
- Lipids	1.6
- Carbohydrates	1.3
- Inorganic salts	0.5
4. Ash	1.0

(Fisher, 1931; Kajornchaiyakul, 2006).

Table 1-2 Typical properties of field natural rubber latex

Property	**Content**
Density (g mL^{-1}) *	0.975-0.980
pH*	6.5-7.0
Viscosity (cps) **	12-15
Total Solids Content (%TSC) **	36
Dry Rubber Content (%DRC) **	33
Ash content (%w/w) **	0.5-1.0
Magnesium content (mg kg^{-1}) ***	587

(*Kajornchaiyakul, 2006; **Phatthanakul, 2011;***Kang *et al.*, 2000)

1.2.3.2 Rubber preservation

After tapping, the stability of fresh latex is gradually decreased by the organic acids produced from the microorganism activities and latex eventually becomes coagulation. The non-rubber compositions including proteins and carbohydrates in the latex are the good nutrients for microorganism growth (Fisher, 1931). After tapping for a few hours, the spontaneous latex coagulation can occur. The preservation of field latex, therefore, is necessary to avoid the coagulation and preservative should be added as soon as possible after collecting latex. The preservatives including ammonia, formaldehyde, sodium sulfite, and potassium hydroxide are often used (Kajornchaiyakul, 2006). Ammonia is the most common preservative owing to advantages of its low price and the ease of its removal. Furthermore, ammonia enhances colloidal stability of latex. About 0.2% (w/w) of ammonia is enough for short-period (2-3 days) preservation of field latex. In case a longer storage period (one month) of field latex is required, higher ammonia content up to 0.7% (w/w) must be added (Phatthanakul, 2011).

1.2.4 Concentrated latex

1.2.4.1 Specifications of concentrated latex

The concentrated latex means the latex that is prepared from the field latex by various methods such as evaporation, creaming, centrifugation and electrodecantation and it can be applied for many industrial rubber products, for example, rubber gloves, condoms, and latex foam. The quality of concentrated latex must be verified to meet the latex specification. According to the Thai Industrial Standard (TIS 980 (2009)), the specifications of concentrated latex are listed in **Table 1-3**.

Table 1-3 Specification of concentrated latex by TIS 980 (2009)

Characteristics	Limits					Test method
	HA	LA	MA	HA cream	LA cream	
Total Solids Content (%w/w); minimum	61.0 or pursuant to an agreement the buyer and seller.			65.0	65.0	ISO 124
Dry Rubber Content (%w/w); maximum	60.0	60.0	60.0	64.0	64.0	ISO 126
Non rubber solid (%w/w;) minimum	1.7	1.7	1.7	1.7	1.7	-
Alkalinity (NH_3), (% w/w of concentrated latex)	minimum 0.6	maximum 0.29	0.30-0.59	minimum 0.55	maximum 0.35	ISO 125
Mechanical stability (second); maximum	650	650	650	650	650	ISO 35
Coagulum (%w/w); minimum	0.03	0.03	0.03	0.03	0.03	ISO 706
Copper (mg kg^{-1} on TSC); minimum	8	8	8	8	8	ISO 8053
Manganese (mg kg^{-1} on TSC); minimum	8	8	8	8	8	ISO 7780
Magnesium (mg kg^{-1} on TSC); minimum	40.0 or pursuant to an agreement the buyer and seller					RRIT
Sludge (%w/w); minimum	0.10	0.10	0.10	0.10	0.10	ISO 2005
Volatile Fatty Acid Number (VFA number); minimum	0.06 or pursuant to an agreement the buyer and seller					ISO 506
KOH number; minimum	0.7 or pursuant to an agreement the buyer and seller					ISO 127

(Thai Industrial Standard: 980-2009)

1.2.4.2 Process of concentrated latex

The concentrated latex is obtained from the field NR latex using various processes as follows:

(1) Evaporation is the reversible process involving the evaporation of water off the field latex. The stabilizer such as potassium soap is added to prevent skin formation on the latex. The evaporated latex of TSC of 75% or higher is provided. All non-rubber constituents present in the original field NR latex are still remained in evaporated latex.

(2) Creaming is the chemical process related to the addition of creaming agents, for example, sodium alginate, locust bean gum and karaya into the field latex. Subsequently, rubber particles, having density lower than dispersion medium, float to the top of the surface and separate to the serum part. This process can be used for concentrating from 30% DRC field latex to 60% DRC concentrated latex.

(3) Centrifugation is a mechanical process for concentrating field latex. The field NR latex is centrifuged to separate the rubber hydrocarbon from water. This process yields the concentrated latex of 60% DRC.

(4) Electrodecantation is based on the electricity for separating the rubber hydrocarbon from serum. The apparatus comprises of electrodes (anode and cathode) and vertical semi-permeable membranes. Rubber particles carry a negative charge migrated through the membranes towards a positive electrode (anode). This migration results in a rubber–rich layer which is separated from rubber-impoverished layer. However, cost economics does not flavor this process to be utilized in industry.

Currently, centrifugation method is the most often used (Kajornchai-yakul, 2006). This method is rapid and provides the pure concentrated latex. Concentrated latex is classified into 3 grades; low ammonia (LA), medium ammonia (MA) and high ammonia (HA). Before centrifuging, about 0.3 and 0.7% (w/w) of ammonia are added into field latex for LA and HA, respectively.

The concentrated latex is used as a raw material for the manufacture of various products, especially dipped products, i.e., gloves, condoms, nipples, and balloons. Skim latex, a by-product during the preparation of concentrated latex, contains 3-5% of dry rubber content. The skim latex is utilized for manufacturing the skim rubber (block or crepe rubber).

Normally, the field natural rubber latex is determined the quantification of some chemical properties before centrifuging to concentrated latex such as dry rubber content (%DRC), volatile fatty acid (VFA) and magnesium content. Magnesium content presented in field latex before centrifugation must less than 100 mg kg^{-1} on %TSC. In case magnesium content exceeds 100 mg kg^{-1} on %TSC, diammonium hydrogen phosphate (DAHP) is added into field latex for magnesium precipitation. The amount of magnesium affects to mechanical stability time (MST). Higher amount of magnesium relatively results in less MST. Consequently, the magnesium determination in field and concentrated latex is required for controlling the quality of latex. The amount of DAHP added into field latex depends on magnesium content. Normally, DAHP 5.4 g is added into field latex to precipitate 1 g of magnesium (Kajornchaiyakul, 2006). The DAHP is added into the field latex and left overnight, then the magnesium precipitation similar to sludge is obtained. Furthermore, DAHP can precipitate other metals such as copper. Therefore, less amount of copper in concentrated latex is provided. The chemical reaction of magnesium and DAHP is provided in **equation 1.1**.

$$Mg^{2+} + (NH_4)_2HPO_4 \longrightarrow Mg(NH_4)PO_4 + H^+ + NH_4^+$$ ….**equation 1.1**

(Kajornchaiyakul, 2006)

From the mention about the reaction of DAHP with magnesium, the magnesium content, therefore, is determined for calculating the amount of DAHP added in field latex before centrifugation. Furthermore, this work requires to study whether, the rest of the precipitation with magnesium will affect the copper precipitation and the magnesium content in field and concentrated latex is determined to examine the hypothesis.

1.2.4.3 Application of concentrated latex

Generally, many rubber latex products are produced from the concentrated latex, for instance, nipples, gloves, shoes and condoms. Concentrated latex is a form of latex providing high dry rubber content (DRC) of about 60%. About 10% of the global production of NR is processed as preserved and concentrated latex. The concentrated latex is more interested than the field latex due to the better uniform in quality, richer rubber content and higher degree of purity. Furthermore, the field NR latex contains excessive water, causing costly transportation (Blackley, 1997, volume 1, p. 10-11).

1.2.5 Rubber Degradation

Rubber degradation is the oxidation reaction of rubber chain with oxygen in the air. An autoxidation of the rubber occurs when rubber is exposed to the oxygen in the air. This reaction causes the chemical structure of rubber which consists of several minor molecules connecting to the chain cracks (chain scission). The oxygen in the air reacts with rubber molecule, causing the hardness and the stiffness of rubber. It causes the crosslinking of chemical structure (raw rubber) in rubber. The mechanism of the oxidation can be described in **equation 1.2**.

a.) $RH + O_2 \longrightarrow R\bullet + HOO\bullet$

b.) $R\bullet + O_2 \longrightarrow ROO\bullet$

c.) $ROO\bullet + RH \longrightarrow ROOH + R\bullet$

d.) $ROOH \longrightarrow RO\bullet + OH\bullet$

e.) $RO\bullet + R'H \longrightarrow ROH + R'\bullet$

f.) $OH\bullet + R'H \longrightarrow H_2O + R'\bullet$ ….**equation 1.2**

(Rajan *et al*., 2006; Kajornchaiyakul, 2009(a), Saeoui, 2005)

The mechanism of oxidation reaction can be described that double bond presenting in rubber hydrocarbon (RH) molecule reacts with oxygen in air that obtains in R• and HOO• in (a). The free radical (R•) reacts with O_2 obtains ROO• (peroxide) in (b) and this process converts the double bond to single bond in rubber hydrocarbon, causing the rubber molecule structure cracks easily. Peroxide radical continues to react with another rubber hydrocarbon which changes to hydroperoxide (ROOH) and free radical (R•) in (c). Since hydroperoxide presents oxygen bond which bond is easy to break, consequently, it can dissociate to RO• and OH• form in (d). Then RO• and OH• will react with another rubber hydrocarbon (R´H) in (e) and (f) obtains R´• that will continue to react with another rubber hydrocarbons rubber structure (Saeoui, 2006).

Following factors including temperature and some metal ions affect the reaction:

-Temperature; the elevated temperature increases the rate of oxidation. Consequently, it activates the increasing of rubber degradation.

-Metal ions; some metal ions such as Cu^{2+}, Fe^{2+}, $Mn^{2+}$and Co^{2+} present in rubber latex are one of the factors affecting to rubber degradation. These metal ions serve as catalysts promoting the oxidation. Previous work found the copper content in NR latex presented higher than other metals except for iron (Scott *et al.*, 2003). Consequently, this work focuses on copper determination in NR latex samples.

Besides the rubber degradation, latex may be degraded due to field natural rubber latex tapped from the rubber tree maintains status of latex for 3-6 hours. The latex will be contaminated from bacteria in the air and peel of rubber tree, while the rubber tree is tapped. Bacteria destroy the nutrients containing in latex, for example, protein and phospholipid saccharide. Bacteria can grow up rapidly and produces various gases such as CO_2 and CH_4. This causes latex spoil, resulting in bad smell (Fisher, 1931). Furthermore, the addition of sufficient quantities of water-soluble salts of divalent cations, such as calcium, zinc, manganese, magnesium, copper to carboxylate-stabilized anionic lattices tends to cause gross colloidal destabilization with rapid particle agglomeration of the latex and separation of the polymer (Blackley, 1997, volume 1, p. 259). The latex degradation can be separated into three types:

Flocculation degradation: A large number of small aggregation of polymer particles are formed as separate particles. This degradation can be regarded as micro coagulation.

Coagulation degradation: Latex becomes to a lump and can be separated into two parts, rubber and serum.

Gelation degradation: Latex is changed from a fluid to a semi-rigid gel displaying some elasticity. The gel has remained the same shape and size as the initial latex.

(Blackley, 1997, volume 1, p. 148-149)

1.2.6 Effect of copper on natural rubber

Copper is recognized as one of essential nutrient elements required by rubber plant (Staden *et al*., 1999). Copper plays an important role on the growth and the composition of the rubber plant. Copper is presented in latex because of rubber tree's absorption of copper from soil and fertilization (Jankiewicz *et al*., 1998).

From the specifications of latex shown in **Table 1-3,** copper content is one characteristics to be considered in the specifications of the latex. Generally, the copper found in natural rubber latex presents in the forms of CuO and Cu-oleate ($C_{36}H_{66}CuO_4$) (Kajornchaiyakul, 2009(a)). The copper can catalyse oxidative degradation of olefinically-unsaturated rubber (Blackley, 1997, volume 1, p.483). Generally, the solubility of Cu-oleate in natural rubber latex is higher than CuO. Therefore, copper that acts as catalyse obtains from Cu-oleate. According to the previous reports, the copper content of 0.27 mg L^{-1} reported by Kang *et al.*, (2000), 1.65 mg L^{-1} reported by Scott *et al.*, (2003) and 1.34-2.26 mg kg^{-1} (on %TSC) reported by Ng and Lai (1972) were found in field NR latex.

Moreover, the copper contamination may cause discoloration of rubber products during aging (Blackley, 1997, volume 1, p. 483). Furthermore, it is found that only 0.001% of Cu^{2+} and Mn^{2+} content can act as catalysts for the autoxidation of raw rubber and vulcanized rubber. Consequently, these metal ions can be called "rubber poisons" and the autoxidation effects on both natural rubber and synthesis rubber (Kajornchaiyakul, 2009(a)). Moreover, darken spots on rubber glove can be observed if high amount of copper contaminates in the container that contained concentrated latex during dipping process (Kajornchaiyakul, 2009(b)). According to the Thai Industrial Standards (TIS), copper concentrated content in latex must not exceed 8 mg kg^{-1} (on %TSC) (TIS 980 (2009)).

1.3. Analytical procedure for copper determination

1.3.1 Technique for copper determination

Trace copper content (mg kg^{-1}) in NR latex affects on rubber degradation, therefore, it is necessary to use a sensitive and accurate method for determination copper in real samples. Various techniques are employed for copper determination in NR latex samples. Visible-spectrophotometry is the most inexpensive and simple method (Shishehbore *et al.,* 2005). Furthermore, the alternative techniques for the copper determination in NR latex samples are Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS), Flame Atomic Absorption Spectrophotometry (FAAS) and Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP-OES).

1.3.1.1 Atomic Absorption Spectrophotometry (AAS)

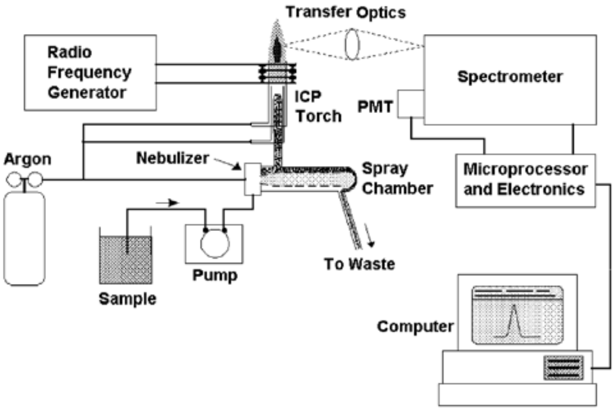
AAS has been the most widely used single-element technique for the metals determination. The principle of this technique is based on the absorption of light by atom in the ground state. Atom is measured when the sample is converted to atom by atomizer and irradiated by light source (Summerfield, 2010). Atom that obtains from the dissociation by flame can be called as Flame Atomic Absorption Spectrophotometry (FAAS) and atom that is dissociated by electrothermal can be called as Graphite Furnace Atomic Absorption Spectrophotometry (Amornsit and Petchsom, 1992).

Ng and Lai (1972) reported the determination of Cu, Mn, Fe, and Ca in NR latex analysed by GFAAS and compared results with FAAS. In the previous work, a cathode layer dc arc method was applied for calcium in NR latex and this technique was extended for trace metals in rubber. Thus, the estimation of these metals in field latex would give some guidances to the quality of the raw product. Until 1970, the Cu and Mn contents were used as indicators of rubber quality in Malaysia. Consequently, the method that is capable of simultaneous determination of copper, manganese, iron and calcium were required and this work used GFAAS instead of the layer dc arc method. Furthermore, this work illustrated the comparison of GFAAS and FAAS to determine trace metals. Sample preparation of this method was the ashing method and the calibration curve was divided into two ranges of calibration curve. One was for Cu, Mn, Fe and Ca which covered the low concentration (0.005-1.0%) and the other is for Fe and Ca which covered the high concentration (0.025-10%). From the results of several raw rubbers, copper content in field latex, cup lump and tree lace by GFAAS were in the range of 1.74-2.26, 2.02-3.58 and 0.76-1.52 mg kg^{-1} on TSC, respectively. For FAAS, copper content in field latex, cup lump and tree lace were in the range of 1.34-1.79, 1.78-3.22 and 0.98-3.08 mg kg^{-1} on TSC, respectively. The results showed no significant differences between two methods.

1.3.1.2 Inductively Coupled Plasma-Optical Emission Spectrophotometry (ICP-OES)

Process of ICP-OES

The principle of ICP-OES can be explained as followed; normally, the sample is passed through the instrument as a stream of liquid sample by pump. The liquid is exchanged to an aerosol through a procedure known as a neubulization by nebulizer and remove the large droplet from the aerosol by spray chamber and drain. The aerosol of sample is then delivered to the plasma where it is desolvated, vaporized, atomized and excited and/or ionized by plasma that consists of torch and radio frequency generator. The excited atoms and ions emit their quality of radiation which is accumulated by an equipment (wavelength dispersive device) that sorts the radiation by wavelength. The radiation is measured and turned into electronic signals that are changed into concentration data for the analyst by detector (Boss *et al.*, 1997). The components for ICP-OES are shown in **Figure 1-2**.



(Boss *et al*., 1997)

Figure 1-2 Components of ICP-OES.

Kang *et al.* (2000) analyzed the metal ion contents in latex of *F. benghalensis (F. elastica), F. carica and Hevea brasiliensis* by ICP-OES. These metal ions such as Mg^{2+}, K^{+}, Ca^{2+}, Cu^{2+}, Fe^{2+} and Mn^{2+} affect on the physiological factor of rubber controlling in biosynthetic activities of latex that produce from plant species. From the result, concentration of copper in *F. benghalensis (F. elastica), F. carica and Hevea brasiliensis* were 0.34, 1.15 and 0.27 mg L^{-1}, respectively.

Scott *et al.* (2003) analyzed the effect of activated the interaction of metal ions in field NR latex. Na, Mg, K, Ca, Mn, Fe, Cu, Zn and pyrophosphate substance were taken in this experiment. Metals are essential activators for the catalytic activity of prenyl transferases. The researcher evaluated that the concentration of metal ions in 2 species latex were *Hevea brasiliensis* and *Ficus elastic* which were detected by ICP-OES. The results were found that the content of Na, K, Mn, Fe, Cu and Zn in *Hevea brasiliensis* were higher than those in *Ficus elastic*, while Mg and Ca in *Ficus elastica* were higher than those in *Hevea brasiliensis*. Copper content in *Hevea brasiliensis* and *Ficus elastica* were 1.65 and 0.60 mg L^{-1}, respectively.

1.3.1.3 UV-Visible Spectrophotometry

Principle of UV-Visible Absorption Spectrophotometry

The principle of this technique is based on measuring the absorption of UV (190-390 nm) or visible (400-790 nm) radiation by molecules. Radiation in this wavelength region causes electronic transitions at the specific wavelength of the molecular structure. The absorption of the organic substances relate to the groups that are responsible for absorption and can be called “chromophores”. Whereas, the absorption by inorganic molecules often involves transitions correlating unfilled d and f orbitals of metals or charge-transfer transitions. In transition metal complexes, the bonding between d orbitals of the metal and orbitals from the ligand wrecks the degeneracy of the five d orbitals. Therefore, the transitions are between lower-energy filled d orbitals and higher-energy unfilled d orbitals (Ingle and Crouch, 1988). In this work, vis- spectrophotometry was applied for copper determination. Because, it is specific for the colored complex.

The absorption of copper complex involves the charge transfer between copper and ZDEC. This reaction based on electron transfer between electron acceptor (Cu) and electron donor (ZDEC), which, this reaction acts as a redox reaction. The reaction of copper and ZDEC is presented in **Figure 1-3**.

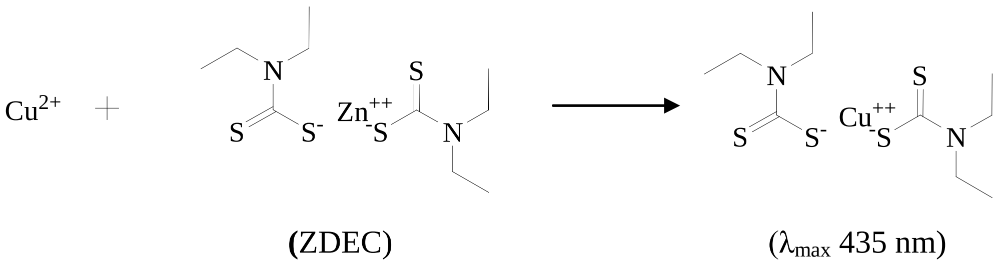


Figure 1-3 The reaction of copper and ZDEC.

For the absorption measurement, radiation from light source passes the wavelength selector for the required wavelength. When the radiation passes through sample that contains in sample container, radiation is absorbed and detected by a detector. Then signal processing and recorder are evaluated by signal processor and readout unit.

A copper solution is colorless and non absorption, therefore, complexing agent is important for copper determination. Various complexing agents for copper such as 4-(2-pyridylazo)-Resorcinol (PAR) (Araujo *et al.,* 1999), 1,5-bis (di-2-pyridylmethylene) thiocarbonohydrazide (DTPH) (Rodriguez *et al*., 1998), thiomichlersketone (TMK) (Fu *et al*., 2007), poly[allylamine-co-N-4-(8-aminoquinolyl-5-azo) benzylideneallylamine] (Ma *et al*., 1996) and diethyldithiocarbamate (DDTC) (Staden *et al*., 1999) were applied for copper determination. Among these complexing agents, DDTC was chosen for complexation with copper because of its high sensitivity (Yang *et al.,* 1996) and high formation constant (K_f) of Cu and ZDEC (4.05×10^{11}) (Qiang *et al.,* 2008), specific for copper (Staden *et al.,* 1999) and comercial availability. Various salts of DDTC such as Na and Zn were applied for complexation with copper. For this work, zinc diethyldithiocarbamate (ZDEC) has been used a complexing agent because it can react directly with copper (Cesur, 2004) and directly dissolve in an organic solvent. Whereas, Na(DDTC) must be dissolved in water before complex is formed with copper in an organic solvent, resulting in less convenient than Zn(DDTC). Generally, the ISO 8053 (1995) method used zinc diethyldithiocarbamate (ZDEC) as a complexing agent in 1,1,1-trichloroethane. However, 1,1,1-trichloroethane is a hazardous substance (class 3) listed by Annex of the Notification of the Ministry of Industry and is permitted under the responsibility of the Department of Industrial Works. Therefore, 1,1,1-trichloroethane is not commercially available in Thailand. Thereby, other organic solvents, carbon tetrachloride (CCl_4), chloroform ($CHCl_3$) and dichloromethane ($CHCl_2$) were considered. Then this research has been used dichloromethane as a solvent, since it is less toxic than CCl_4 and $CHCl_3$ and commercially available. Therefore, the complexing agent for copper determination is ZDEC prepared in DCM. ZDEC has the N, N- substituted derivatives dithiocarbamic acid and the reagent coordinates with copper through the two sulfur atoms to form a water-insoluble chelate. The spectrophotometric determination can be carried out in the aqueous phase after stabilization of the pseudo-solution with a protective colloid. Copper-dithiocarbamate is insoluble in water but soluble in organic solvents, therefore, the solvent extraction (liquid-liquid extraction, LLE) is required for copper extractrion. Liquid-liquid extraction is the separation procedure of a chemical species from a matrix. The principle of this technique is based on the transferring of the solute between the two involved phases. These phases composites of organic phase and inorganic phase. Specifically, solvent extraction is based on the principle of unique solute distribution ratios between two immiscible solvents. The factors of LLE for extraction of copper from the aqueous phase are number of extraction, volume of solvent and time for extraction (Anthemidis and Ioannou, 2009). The zinc diethyldithiocarbamate and copper ions form a yellow-brown neutral chelate, $Cu(DEC)_2$, at pH 5.5-10 (Staden *et al.,* 1999). The summarization of complexing agent for copper determination by vis-spectrophotometry are shown in **Table 1-4**.

Table 1-4 Complexing agent for copper determination in various samples

Sample	**Complexing agent**	**λ_{max} (nm)**	**Linearity range**	**Molar absorptivity (L mol $^{-1}$ cm^{-1})**	**Interference**	**Reference**
River and sea water	Acetylsalicyl Hydroxamic acid (Ac-SHA) in ethanol	700	5×10^{-6}-120×10^{-6} mg L^{-1}	1.42×10^{4}	Fe(II), Fe(III) and Cr(III)	Asan *et al.* (2003)
Sea water	4-(2-pyridylazo)-Resorcinol (PAR) in ethanol	517	0-1.27 mg L^{-1}	3.2×10^{4}	Zn(II), Pb(II), Fe(II) and Fe(III), etc.	Araujo *et al.* (1999)
Alloy and bio logical material	1,5-bis(di-2-pyridylmethy-lene) thiocarbonhydrazide (DTPH) in IBMK	420	0.2-1.2 mg L^{-1}	0.42×10^{5}	Hg(I),Hg(II),Ag (I),Pb(II),Mn(II) and Cd(II)	Rodriguez *et al.* (1998)
Water sample	Thiomichlersketone (TMK) in propanol	500	0-0.51 mg L^{-1}	1.10×10^{5}	-	Fu *et al.* (2007)

Table 1-4 Complexing agent for copper determination in various samples (continued)

Sample	**Complexing agent**	**Wavelength (nm)**	**Linearity range**	**Molar absorptivity (L mol $^{-1}$ cm^{-1})**	**Interference**	**Reference**
Alloy	Poly[allylamine-co-N-4-(8-aminoquinolyl-5-azo)benzylideneallylamine] in DI water	590	0-1 mg L^{-1}	4.1×10^{4}	-	Ma *et al*. (1996)
Plant food	N-ethyl-2-naphthylamine in acetronitrile	533	0-0.24 mg L^{-1}	1.72×10^{5}	Fe(III),Co(II),Ni(II), SO_4^{2-},NO_3^{2-}	Mor *et al*. (1995)
Water sample	Neocuproine in ethanol	456	0.8-2.0 mg L^{-1}	3.6×10^{3}	Fe(III)	Prokurnin *et al*. (2002)
Water sample	Sodium diethyldithiocarbamate (Na(DDTC)) in water and $CHCl_3$	460	0.5-5.0 mg L^{-1}	1.4×10^{4}	Fe(II)	Staden *et al.* (1999)

Beer and Lambert's law

Beer's law mentions that "light that is absorption depends on the concentration of substance" and Lambert's law mentions that "light that is passed through the cell absorbed in the equal ratio". Hence, it can be summarized Beer and Lambert's law as **equation 1.3**

$$A = \log (P_0/P)$$

$$A = \varepsilon b C$$

$$\% T = (P/P_0)\times 100 \qquad \textbf{....equation 1.3}$$

Where

P means to radiant power transmitted through an analyte in sample solution

P_0 means to radiant power transmitted through a blank solution

C means to the concentration of substance (mol L^{-1})

b means to thickness of cell (cm)

ε means to molar absorptivity (L mol^{-1} cm^{-1})

A means to absorbance value

T means to transmittance (%)

From this equation, the transmittance (T), is used to calculate the absorbance. Under suitable conditions, the absorbance is directly proportional to the analyte concentration.

ASTM (1982) is the standard method for copper determination in solid natural rubber. Ashing method was applied for sample preparation followed by nitric digestion. Then the solution was formed with zinc dibenzyldithiocarbamate (ZDBC) in CCl_4. The solution was transferred in a separatory funnel to separate layer. The organic layer was taken to absorbance measurement at 435 nm.

ISO 8053 (1995) is the standard method for copper determination in raw rubber. Zinc diethyldithiocarbamate (ZDEC) was used as a complexing agent. This technique was similar to **ASTM (1982)** except the complexing agent and solvent used. This method involved the ashing and wet digestion method for sample preparation. This method was applied for the method development of copper determination in this study.

The developed method will be applied for copper determination in natural rubber latex. In order to validate the developed method, the standard method, ICP-OES was employed. The results obtained from the developed method and those by ICP-OES were compared. **Table 1-5** summarizes the technique for copper determination in various samples.

Table 1-5 Technique for copper determination in various samples

Techniques	**Samples**	**Linearity**	**LOD/LOQ**	**Copper content**	**Interference**	**Reference**
Visible-Spectophotometry	Aluminium alloy	0.04-5.0 µg mL^{-1}	LOD = 0.03 µg mL^{-1}	0.26-2.68 µg mL^{-1}	Al(III) and Fe(III)	Shishehbore *et al*. (2005)
ICP-OES	Dogfish muscle	0.25-2.50 mg L^{-1}	LOD < 1.5 µg L^{-1}	2.28-2.32 mg kg^{-1}	-	Raessler *et al.* (2004)
FAAS	Peanut	0-5 µg L^{-1}	-	10 µg g^{-1}	-	Anzano and Gonzalez (1999)
ICP-OES	Sugar-can spirit	-	LOD = 3.4 µg L^{-1}, LOQ = 11 µg L^{-1}	0.5-14.7 mg L^{-1}	-	Caldas *et al*. (2011)
Visible-Spectrophotometry	Alloys	20-70 µg L^{-1}	-	30.1-81.1%	-	Kamble *et al.* (2011)
FAAS	Sugar-can spirit	0.10-4.0 mg L^{-1}	LOD = 15 µg L^{-1}	0.66-6.64 mg L^{-1}	-	Miranda *et al.* (2010)
Electrochemistry (DPASV)	Ethanol fuel	0.1-1.2 µmol L^{-1}	LOD = 3.9×10^{-8} mol L^{-1}	1.33-3.67 $(\times10^{-7})$ mol L^{-1}	Pb^{2+}, Fe^{3+}, Cd^{2+} Zn^{2+} and Mn^{2+}	Cesarino *et al.* (2010)
Visible spectrophotomety	Food and water samples	>50 µg L^{-1}	LOD = 0.4 µg L^{-1},	5.2-7.68 µg L^{-1}	-	Wen *et al.* (2011)

This research was focused on the development of simple and rapid method for copper analysis which was suitable for the industrial laboratory. Among those techniques, titrimetry is insufficiently sensitive and could not detect the copper content due to its low level in NR latex. Cost effective and low cost maintenance techniques are required for the industrial laboratory. The drawbacks of ICP-OES and AAS are expensive instruments and required high instrument maintenance cost, thus, they are not preferred to utilize in the industrial laboratory. Normally, the copper determination performed in the industrial laboratory is followed as The International Organization for Standardization (ISO 8053 (1995)). The ISO has published the method for determination of copper content in rubber and latex by photometric method (ISO 8053:1995).

1.3.2 Sample preparation

Sample preparation for trace analysis is necessary step prior to determination. Sample matrices must be destroyed or removed to avoid the interference effect. Normally, dry ashing and wet digestion procedure are subjected for the sample decomposition method.

-Ashing method; the principle of this technique is based on burning the sample at high temperature between 500-600ºC. Volatile water are vaporized and the inorganic substance remains in the crucible. Then the inorganic residue is dissolved by an appropriate acid and heated prior to filtration and analysis by an appropriate technique. The ashing method being suitable for determination of involatile analyte and the sample must be dried prior to ash. Advantages of this technique are safe, less reagent consumption and it can analyze the specific mineral content. However, the analyte from the ashing method may loss, causing the less recovery than wet digestion. The analyte will react with organic substance and is not stable at the temperature lower than 500ºC such as copper oleate (boiling point: 360ºC), and copper attachs beside the crucible. Furthermore, the ashing method is not suitable for liquid sample, and copper may attach with ash residue that is not dissolve with acid (Balcerzak, 2002).

Xiang *et al.* (2010) reported the trace copper content in food sample that detected by Flame Atomic Absorption Spectrophotometry (FAAS). Dried sweet potato presented 46.9 µg g^{-1} of copper content and limit of detection was 0.8 ng mL^{-1}. Ashing method was applied for the sample preparation by weighing the sample into the 100 mL crucible and heating until no smoking from the sample. Then crucible was heated in a muffle furnace with temperature of 500±25ºC and the residue was dissolved with 500 mL of 1% (v/v) nitric acid after cooling.

Saracoglu *et al*. (2006) determined trace element contents of baby foods from Turkey, and the ashing method was applied for sample digestion. Samples were dry-ashed in a furnace at a temperature at 450-500ºC for 16 hrs. Then they were treated with 1 mL conc. nitric acid for ash whitening and ashed again for 6 hrs. The residue was dissolved with 1-2 mL conc. nitric acid, filtered through the paper and diluted to 10 mL with DI water. Content of trace elements in baby food such as Cu, Mn, Fe, Zn, Se, Cr, Al, Ni and Co were 3.97, 2.90, 33.2, 31.2, 0.04 µg g^{-1}, 26.5, 10.9, 4.21 and 11.5 µg kg^{-1}, respectively. % Recovery obtained from this method is less than that obtained from the wet digestion.

-Wet digestion; The principle of this technique is based on the boiling the sample by concentrated acid such as single acid or acid mixture. The acid that used for the wet digestion are HNO_3, H_2SO_4 and $HClO_4$. The wet digestion is usually carried out under the temperature of 100-200ºC (Balcerzak, 2002). Acids, such as HNO_3, $HClO_4$ and H_2SO_4, are the oxidizing agent whereas HCl and HF are non oxidizing agent. Normally, the single acid is applied for the digestion, however, for some cases, the performance of single acid is not enough. Therefore, the acid mixture is required for the digestion. The oxidizing power of nitric acid is insufficient at low temperature, thus, sulfuric acid is added in the sample for increasing the temperature of a digestion solution. Furthermore, the microwave assisted digestion is the one technique of wet digestion. The principle of this technique is placing a sample in an acid solution and heating to high temperature and pressure. Although, this method can dissolve the material but it is quite dangerous and must pay attention when use this method (Gonzales *et al*., 2009).

Saracoglu *et al*. (2006) determined trace element contents of baby foods from Turkey, and wet digestion was applied for sample digestion. Samples were digested by using a mixture of HNO_3:H_2O_2 (6:2, v/v) and heated up to 130ºC for 4 hrs on the hotplate. Then 5 mL of DI water was added to the sample and made volume to 10 mL. Content of trace elements in baby food such as Cu, Mn, Fe, Zn, Se, Cr, Al, Ni and Co were 4.10, 2.96, 32.1, 31.6, 0.10 µg g^{-1}, 27.7, 10.7, 4.63 and 11.8 µg kg^{-1}, respectively.

Karimi *et al.* (2007) reported the content of Co, Ni, Cu and Fe in environmental samples by FAAS. Samples were performed by wet digestion (2 mL conc. HNO_3 and 2 mL of 70% $HClO_4$) and heated for 1 hrs. Then the solution was made volume to 250 mL with DI water, and pH was adjusted to 8.5 prior to determination by FAAS. Limit of determination of Co, Ni, Cu and Fe were 0.7, 0.7, 0.8 and 0.7 ng mL^{-1}, respectively.

Saracoglu *et al*. (2006) determined trace element contents of baby foods from Turkey and microwave digestion was applied for the sample digestion. Samples were performed by transferring to 100 mL teflon vessels. Six millitres of HNO_3 and 2 mL of H_2O_2 were added into the sample which then is digested by microwave system (2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W and vent 8 min). The solution was diluted to 10 mL with DI water. Content of trace elements in baby food such as Cu, Mn, Fe, Zn, Se, Cr, Al, Ni and Co were 4.38, 3.01, 34.2, 32.4, 0.13 µg g^{-1}, 29.2, 11.4, 4.98 and 12.0 µg kg^{-1}, respectively.

Dantas *et al.* (2010) applied the microwave-assisted digestion method and used ICP-OES for measuring the gum deposits of internal combustion engine. Sample digestion was performed by adding 1 mL of 30% H_2O_2 and 2.5 mL of 68% HNO_3 into the vessel. Then the solution was heated by microwave (300 W for 4 min, 300-600 W for 6 min and vent 15 min) and the solution was made volume to 25 mL with DI water.

This research is focused on wet digestion by acid to treat the NR latex sample. Kjeldahl digestion system recommended by **ISO 8053 (1995)** was used for NR latex digestion and based on heating the substance by sulfuric acid. However, this method is time consuming. Moreover, the kjeldahl apparatus that consists of block digester for heating and kjeldhal tube for the solution (as shown in **Figure 1-3**) are cost expensive. Hence, a part of this research was focused on the development of simple digestion system for NR latex digestion prior to determination.

Figure 1-4 Digestion system for kjeldahl method.

The simple wet digestion system was proposed. It consists of digestion tube (Pyrex; 3×25cm). NR latex sample was accurately weighed into the tube. The digestion tube was placed in a paraffin liquid oil bath since this digestion system requires temperature control at 180ºC instead of hotplate. Furthermore, the heating can be transferred across the digestion tube by paraffin liquid. The sample was heated by hotplate connected with thermostat for keeping constant digestion temperature. Parameters such as type of acid, digestion temperature and sample weight were optimized in order to achieve good digestion efficiency. The proposed digestion system is depicted in **Figure 1-5**.

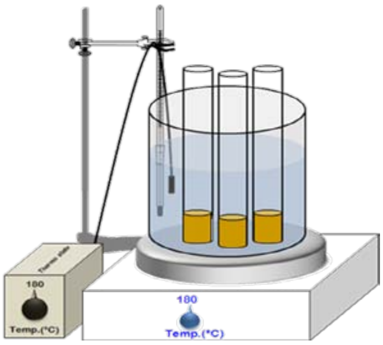


Figure 1-5 The proposed digestion system.

1.4 The developed simple vis-spectrophotometry in this research

In this work, the developed method can be separated in 2 parts, the first part is the developed vis-spectrophotometry. For this part, the complexing agent used as a complexing agent is ZDEC and the organic solvent for the extraction is dichloromethane. Moreover, the absorption spectra of copper complex, concentration of ZDEC and number of extraction were studied for this part. The second part is the developed digestion system for NR latex digestion. For this part, the wet digestion was applied in this work by the developed digestion system that shows in **Figure 1-5**. The acid type, digestion temperature and amount of concentrated latex were studied for this part.

1.5 Objectives

1. To develop the simple visible spectrophotometric method for copper analysis in natural rubber latex
2. To develop the simple acid digestion method for natural rubber latex sample and optimize the conditions
3. To apply the method for determination of copper content in field and concentrated natural rubber latex samples
4. To compare the copper content in field and concentrated natural rubber latex samples

1.6 Scopes

1. To investigate the simple and rapid method for copper determination in natural rubber latex
2. To develop acid digestion system and study the optimum conditions of the system for natural rubber latex samples and compared with kjeldahl digestion system
3. To prepare the concentrated latex from field latex by centrifugation
4. To apply the method for copper determination in natural rubber latex samples and compared with ICP-OES
5. To determine magnesium content in field and concentrated natural rubber latex samples by titrimetric method for calculating the amount of DAHP
6. To determine some properties such as %TSC, %DRC of field and concentrated NR latex samples

1.7 Benefits of the research

1. The simple visible-spectrophotometric technique for copper determination in natural rubber latex
2. The simple method for natural rubber latex digestion

1.8 Place for research

Department of Chemistry, Faculty of Science, Prince of Songkla University

CHAPTER 2
EXPERIMENTAL

2.1 Chemicals and preparation

2.1.1 Standard and Chemicals

- $CuSO_4.5H_2O$ (Carlo Erba, USA)
- $ZnSO_4.7H_2O$ (Fluka Chemika, Switzerland)
- $MnCl_2.4H_2O$ (APS Finechem, Australia)
- $Fe(NO_3)_3.9H_2O$ (Fluka Chemika, Switzerland)
- $MgSO_4.7H_2O$ (Carlo Erba, USA)
- Nitric acid (HNO_3) 65%, AR grade (RCL Labscan, Thailand)
- Sulfuric acid (H_2SO_4) 96%, AR grade (Labscan, Ireland)
- Hydrogen peroxide (H_2O_2) 30% (Merck, USA)
- Ammonium hydroxide (NH_4OH) 28-30%, A.C.S. reagent (J.T. Baker, USA)
- Citric acid ($C_6H_{10}O_8$) (Ajax Finechem, Australia)
- Dichloromethane (CH_2Cl_2) AR grade (Fisher chemical, UK)
- Zinc diethyldithiocarbamate $[(C_2H_5)_2NCS_2]_2Zn$ 97%, (SIGMA ALDRICH)
- Paraffin liquid (Ajax Finechem Pty Ltd)
- Glacial acetic, AR grade (B.D.H., England)
- Potassium cyanide (KCN)
- Ethylenediamminetetraacetic acid (EDTA) ($C_2H_8N_2$) (Carlo Erba, USA)
- Eriochrome black T ($C_{20}H_{12}N_3O_7SNa$) (J.T. Baker, USA)
- Anhydrous sodium sulphate (Na_2SO_4) (RANKEM)
- Ammonium chloride (NH_4Cl) (Ajax Finechem, Australia)
- Di-ammonium hydrogen orthophosphate $(NH_4)_2$ HPO_4 (Ajax Fine Chem, Australia)

2.1.2 Preparation of standards and reagents

2.1.2.1 Stock solution (100 mg L^{-1}) of copper (II)

This stock solution of Cu(II) was prepared by weighing 0.393g of $CuSO_4.5H_2O$ (Carlo Erba, USA) into a beaker and dissolved with DI water. Three milliliters of concentrate H_2SO_4 was added into the beaker. Then the solution was transferred to a 1000 mL volumetric flask and the volume was adjusted to the mark with DI water. Standard working solutions of Cu(II) were prepared by properly dilution of 100 mg L^{-1} standard Cu(II) with DI water just before use. Standard working solutions of Cu(II) were prepared in the range of 0.1-6.0 and 0.005-1.0 mg L^{-1} for vis-spectrophotometry and inductively coupled plasma-optical emission spectrophotometry (ICP-OES), respectively.

2.1.2.2 Stock solution (1000 mg L^{-1}) of zinc (II)

This stock solution of Zn(II) was prepared by weighing 2.1980 g of $ZnSO_4.7H_2O$ (Fluka Chemika, Switzerland) into a beaker and dissolved with DI water. Then the solution was transferred to a 500 mL volumetric flask and the volume was adjusted to the mark with DI water.

2.1.2.3 Stock solution (1000 mg L^{-1}) of manganese (II)

This stock solution of Mn(II) was prepared by weighing 3.6000 g of $MnCl_2.4H_2O$ (APS Finechem, Australia) into a beaker and dissolved with DI water. Then the solution was transferred to a 1000 mL volumetric flask and the volume was adjusted to the mark with DI water.

2.1.2.4 Stock solution (1000 mg L^{-1}) of iron (III)

This stock solution of Fe(III) was prepared by weighing 2.4891 g of $Fe(NO_3)_3 \bullet 9H_2O$ (Fluka Chemika, Switzerland) into a beaker and dissolved with DI water. Then the solution was transferred to a 1000 mL volumetric flask and the volume was adjusted to the mark with DI water.

2.1.2.5 Stock solution (0.02 M) of magnesium sulfate

$MgSO_4$ 0.02 M was prepared by weighing 0.5915 g of $MgSO_4•7H_2O$ (Carlo Erba, USA) into a beaker and dissolved with DI water. Then the solution was transferred to a 100 mL volumetric flask and the volume was adjusted to the mark with DI water.

2.1.2.6 Zinc diethylditiocarbamate (ZDEC) 200 mg L^{-1}

This complexing agent was prepared by weighing 0.2000 g of zinc diethyldithiocarbamate (ZDEC) and dissolved with 1000 mL of dichloromethane.

2.1.2.7 Ammonium/Ammonium chloride buffer (pH 10.5)

Ammonium/Ammonium chloride buffer solution was prepared by dissolving 5.35 g of ammonium chloride with 7.43 mL of 28% ammonium hydroxide and diluting to 1000 mL with DI water. The buffer pH was adjusted to pH 10.5 by adding 1 M sodium hydroxide or 1 M nitric acid.

2.1.2.8 Nitric solution (0.5 M)

0.5 M nitric acid was prepared by pipetting 50 mL of nitric acid and the solution was adjusted with DI water to 500 mL.

2.1.2.9 Citric acid (50%, w/v)

Citric acid solution was prepared by weighing 50.00 g of citric acid and diluting to 100 mL with DI water.

2.1.2.10 EDTA (0.005 M)

0.005 M of EDTA was prepared by weighing 1.8612 g of EDTA. Then EDTA was dissolved and adjusted to 1000 mL by DI water. This reagent was standardized with 0.02 M $MgSO_4$ by titrimetric method.

2.1.2.11 KCN (0.6 M)

0.6 M KCN was prepared by weighing 19.53 g of KCN and dissolving with DI water. Then the reagent was adjusted to 500 mL by DI water.

2.1.2.12 Acetic acid solution (2 and 25%,v/v)

For 2 % (v/v) acetic acid, 10.0 mL of glacial acetic acid was transferred to a 500 mL volumetric flask and the volume was adjusted to the mark with DI water.

For 25% (v/v) acetic acid, 125.0 mL of glacial acetic acid was transferred to a 500 mL volumetric flask and the volume was adjusted to the mark with DI water.

2.1.2.13 Eriochrome Black T

Eriochrome Black T was prepared by grinding 0.2 g of eriochrome Black T with 300 g NaCl until it becomes homogeneous and kept in the bottle.

2.2 Instruments, apparatus and material/glassware

2.2.1 Instruments

2.2.1.1 Ultraviolet (UV) – Visible (Vis) Spectrophotometer

- Spectrophotometer Model Spectronic 21 (Italy) (wavelength 380-800 nm) for measurement of the absorbance of Cu(II) complex.
- UV-Vis Spectrophotometer with Diode Array Detector (Shimadzu UV160-U, wavelength 200-1100 nm) for scanning absorption spectra of Cu(II) complex.
- UV-Vis Spectrophotometer with Diode Array Detector (Hewlett-Packard 8453) for scanning absorption spectra of the interference study.

2.2.1.2 Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES)

- ICP-OES model Optima 4300 DV (Perkin Elmer, America) for copper determination in NR latex sample. The experimental ICP-OES conditions are shown in **Table 2-1**.

Table 2-1 ICP-OES conditions for copper determination in NR latex sample

Parameter	Optimum condition
Plasma gas	15 L min^{-1}
Auxiliary gas	0.2 L min^{-1}
Nebulizer gas	0.8 L min^{-1}
RF power	1150 W
Plasma view	Axial view
Signal processing	Intensity
Point per intensity	3
Wavelength (nm)	327.393

2.2.2 Apparatus

- Oven (Memmert) for total solids content (%) and dry rubber content (%) determination
- pH meter (Sartorious UK, Docu-pH)
- Water bath (Memmert, Germany) for %DRC determination
- Balance 2 digits (TE, Sartorius Germany)
- Balance 3 digits (OHAUS, America)
- Balance 4 digits (TE, Sartorius Germany)
- Centrifugal separator 8000 rpm (Westfalia separator AG, Singapore)
- Rotary evaporator (Buchi, Germany)
- Kjeldahl apparatus (Buchi, Germany) consists of kjeldahl block digester containing 20 holds for 500 mL kjeldahl tube

2.2.3 Materials/Glasswares

- Aluminum cup (11 cm-wide) for % TSC determination
- Crucible (Pyrex) for ash determination
- Whatman filter paper No. 42
- Universal indicator paper
- Litmus paper
- Separatory funnel, 250 mL (Pyrex)
- Cornical flask, 250 mL (Pyrex)

- Round bottom flask, 100 mL (Pyrex)
- Polyethylene bottle (1000 mL) for sample collection

All glasswares such as volumetric flask, beaker, cylinder, pipette and burette are Pyrex. These glasswares were cleaned with detergent and soaked in 10% (v/v) nitric acid (HNO_3) for 24 hours and then rinsed with de-ionized water.

De-ionized (DI) water was used throughout as a matrix for reagent and standard solutions.

2.3 System of digestion

For the ISO 8053 (1995) method, sample is digested by kjeldahl apparatus but this method is time consuming and its apparatus is expensive. Thus, a rapid and simple method for sample digestion is required. A part of this work is to develop the system for latex digestion, as depicted in **Figure 2-1**.

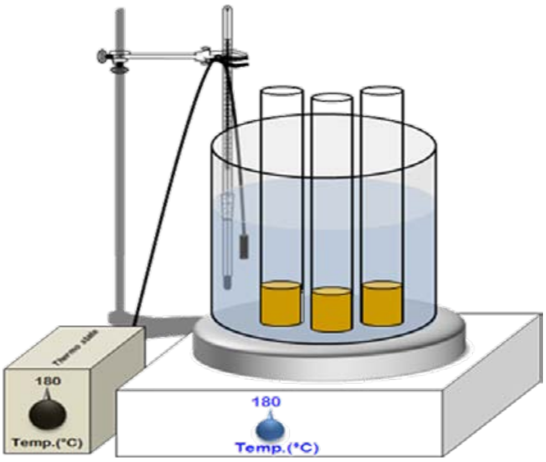


Figure 2-1 Digestion system for NR latex sample.

The simple wet digestion system was proposed. It consists of digestion tube (Pyrex; 3×25 cm). NR latex sample was accurately weighed in the digestion tube. The digestion tube was placed in paraffin liquid oil bath (boiling point: 300-500ºC). The sample was heated on hotplate connected with thermostat for keeping constant digestion temperature. Glass bead was added in the digestion tube during sample digestion to avoid bumping of the solution when it was heated at high temperature. Parameters such as type of acid, digestion temperature and sample weight were optimized in order to achieve good digestion efficiency.

2.4 Determination of copper content by the developed method

2.4.1 Choice of solvent

According to the ISO 8053 (1995) method, 1,1,1- trichloroethane was used as an organic solvent. However, 1,1,1-trichloroethane is hazardous substance and is not commercially available in Thailand. Thereby, other organic solvents, carbon tetrachloride (CCl_4), chloroform ($CHCl_3$) and dichloromethane ($CHCl_2$), for example, were investigated.

2.4.2 Procedure of copper determination

The procedure for copper determination was modified from ISO 8053 (1995) method. Latex samples must be digested by wet digestion before determining copper content. Five milliliters of 50% (w/v) citric acid was added in the solution of standard or sample in a beaker. The pH of the solution was adjusted by adding 5 mL of 28% NH_4OH and then adding drop by drop until the solution was just basic (≈ pH 8). Litmus paper was used as an indicator. After that, the solution was left to cool and transferred into the separatory funnel. Two milliliters of 28% ammonium hydroxide was used for rinsing the beaker. A portion of 25.00 mL of ZDEC in dichloromethane was added into the separatory funnel to form complex with copper in sample/standard solution. The solution was shaken for 2 min and left until aqueous and organic layer separated. The organic layer was drawn into a test tube and 0.1 g of anhydrous sodium sulphate was added in order to remove water. Then the organic layer was filtered through the Whatman filter paper No. 42 and measured absorbance at 435 nm.

2.4.3 Absorption spectra of copper complex

The wavelength providing the highest absorbance value (λ_{max}) of copper complex was investigated by scanning the absorption spectra of 4.0 mg L^{-1} Cu(II) standard solution against blank (200 mg L^{-1} ZDEC in DCM). The standard of 4.0 mg L^{-1} of Cu(II) was prepared by adding 5 mL of DI water and followed by adding 5 mL of conc. H_2SO_4 into a beaker. Then 1 mL of standard 100 mg L^{-1} Cu(II) was added. Blank solution can be prepared by adding 5 mL of DI water and followed by adding 5 mL of conc. H_2SO_4 into a beaker. Then copper determination was carried out as mentioned in **section 2.4.2**. The wavelength of organic layer was scanned in the range of 300-800 nm by UV-Vis spectrophotometer. The wavelength giving the maximum absorbance (λ_{max}) was selected for copper determination.

2.4.4 Optimization of ZDEC concentration

To optimize the concentration of ZDEC, determination of 2.0 mg L^{-1} Cu(II) standard solution with 25 mL ZDEC at following various concentrations; 50, 100, 150, 200 and 300 mg L^{-1} were conducted as mentioned procedure in the **section 2.4.2**. Triplicate portions of the 2.0 mg L^{-1} Cu(II) standard solution were extracted. Triplicate determinations were performed for each portion. The highest absorbance was considered as an optimum concentration of ZDEC.

2.4.5 Number of extraction

To obtain much copper from the extraction step, therefore, the number of copper extraction was optimized. The effects of extraction number at 1, 2 and 3 times on amount of copper from the extraction as evaluated from absorbance value were investigated. A 2.0 mg L^{-1} of standard Cu(II) was added into the solution in a beaker. The copper extraction procedure for 1, 2 and 3 times were carried out as mentioned in **section 2.4.2.** The extraction was repeated by pipetting 25 mL of 200 ppm ZDEC into the extract and followed by the procedure as mentioned in **section 2.4.2.** This step was carried out 2 and 3 times for duplicate and triplicate extractions, respectively. The solution was shaken for 2 min for each extraction. The organic layer obtained from each extraction was taken to evaporate by rotary evaporator until 5 mL of the extract remaining and adjusted the volume to 25 mL with DCM. The organic layer was then measured by vis-spectrophometry at 435 nm. Triplicate determinations were performed for each portion.

2.5 Sample digestion by the developed digestion system

Concentrated latex sample was digested by wet digestion. The developed digestion system (as shown in **Figure 2-1**) was utilized. Precise amount of concentrated NR latex sample was weighed into the digestion tube and digested with appropriate acid until the sample was totally dissolved. The digestion efficiency of concentrated NR latex depends on various factors such as the acid type, digestion temperature and amount of concentrated latex sample.

2.5.1 Type of acid

Influence of acid type to concentrated latex digestion efficiency was studied. The acids such as conc. HNO_3, conc. H_2SO_4 and mixture of conc. H_2SO_4 and conc. HNO_3 were studied by using the developed digestion system.

2.5.1.1 Conc. HNO_3 or conc. H_2SO_4

Amount of 1.00 g concentrated latex sample was weighed into the digestion tube. Fifteen milliliters of conc. HNO_3 or conc. H_2SO_4 was added and heated at 180°C. The sample digestion was carried out until the clear solution was observed.

2.5.1.2 Acid mixture of conc. HNO_3 and conc. H_2SO_4

Amount of 1.00 g concentrated latex sample was weighed into the digestion tube. Four milliliters of conc. H_2SO_4 was added into sample and followed by adding of 3 mL of conc. HNO_3. The digestion temperature was fixed at 180ºC. As soon as the vigorous reaction has ceased, 1 mL of conc. HNO_3 portion was then added. The addition of 1 mL conc. HNO_3 portions was continued until the colorless or pale yellow solution was observed. Then 0.5 mL of 30% H_2O_2 and 2 drops of conc. HNO_3 were added and repeated until no reduction in the color of the solution was observed. After that, the solution was left to cool at room temperature.

2.5.2 Digestion temperature

The influence of the digestion temperatures at 130, 160, 180 and 200ºC on digestion efficiency as evaluated from recovery (%) and digestion time was investigated. Amount of 1.00 g concentrated latex sample was weighed into the digestion tube for non-spiked sample. A 2.0 mg L^{-1} of standard Cu(II) was added into latex for spiked sample. The digestion procedure was carried out as mentioned in **section 2.5.1.2.** The sample solutions were then measured by developed vis-spectrophotometry. Triplicate portions of spiked and non-spiked samples were prepared. Triplicate determinations were performed for each portion.

2.5.3 Amount of concentrated latex

The amount of 1.00, 1.50 and 2.00 g of concentrated latex were optimized for concentrated latex digestion. Concentrated latex sample was weighed into the digestion tube for non-spiked sample. A 2.0 mg L^{-1} of standard Cu(II) was added into latex for spiked sample. The digestion procedure was carried out as mentioned in **section 2.5.1.2.** The sample solutions were then measured by developed vis-spectrophotometry. Triplicate portions of spiked and non-spiked samples were prepared. Triplicate determinations were performed for each portion. Recovery (%) was used to evaluate the digestion efficiency.

2.6 Sample digestion by kjeldahl digestion system

Kjeldahl digestion system is the procedure for NR latex digestion utilized by ISO 8053 (1995). The procedure can be performed by weighing amount 2 g of latex sample into kjeldahl tube. Four milliliters of conc. H_2SO_4 was added into a solution, and then 3 mL of conc. HNO_3 was added. The sample was placed in a kjedahl digestion block and the digestion temperature was initiated at 90ºC and was gradually increased until 270ºC. As soon as the vigorous reaction was ceased, 1 mL conc. HNO_3 portion was then added. The addition of 1 mL of conc. HNO_3 portions were continued until the colorless or pale yellow solution was observed. Then 0.5 mL of 30% H_2O_2 and 2 drops of conc. HNO_3 were added with repeating the addition until no reduction of color of the solution. After that, the solution was left to cool at room temperature.

2.7 Analytical performances of the developed method

2.7.1 Linearity range

Concentrations in the range of 0.1-6.0 mg L^{-1} of standard working solution Cu(II) were studied for evaluating the linear dynamic range. Then Cu(II) determination at each concentration was carried out as mentioned in **section 2.4.2**. The correlation coefficient and linear regression equation of the linear curve were considered as the response of linearity. Furthermore, the linear regression equation was taken to calculate the copper content in mg L^{-1}.

2.7.2 Limit of detection and limit of quantitation

The limit of detection (LOD) of an analyte can be described as the concentration which gives the signal of instrument significantly different from the 'blank' or 'background' signals. Limit of quantitation (LOQ) is regarded as the lower limit for precise quantitative measurement (Miller, 1993). LOD and LOQ were defined as $3S_{y/x}/b$ and $10S_{y/x}/b$, respectively (Tateo *et al.*, 2010) where

$S_{y/x}$ = blank signal standard deviation

b = slope of the calibration graph

Note; $S_{y/x}$ was calculated as **equation 2.1**

$$S_{y/x} = \sqrt{\left[\left(\frac{\sum(Y_i - Y_{ii})^2}{(n-2)}\right)\right]} \qquad \text{....}\textbf{equation 2.1}$$

Where

Y_i = absorbance from measurement

Y_{ii} = absorbance from calibration curve calculation

n = number of standard Cu(II), mg L^{-1} constructed in calibration curve

2.7.3 Accuracy and Precision

Accuracy of the developed method can be evaluated by recovery (%). The term of recovery (%) is used to detect yield of spiked standard. For this work, recovery (%) was performed by adding 1.0 and 2.0 mg L^{-1} of Cu(II) standard solution into the sample. Recovery (%) was obtained from the copper content that recovered for the analytical method and can be calculated from the **equation 2.2** as follows

$$\text{Recovery (\%)} = \frac{\text{copper content}_{\text{spiked}} - \text{copper content}_{\text{unspiked}}}{\text{concentration of std. Cu(II) that spiked}} \times 100 \quad \text{….}\textbf{equation 2.2}$$

Field NR latex sample from Namom district was selected for recovery study. Ten replicate portions of spiked and non-spiked (1.0 and 2.0 mg L^{-1} Cu(II)) samples were determined. Triplicate determinations were performed for each portion.

Precision is the level measurement of analytical method at the same conditions. Usually, precision is demonstrated as the relative standard deviation (% RSD) for a statistically significant amount of sample. Field NR latex sample from Namom district was also selected for precision study. The intra-day and inter-day precisions were investigated as follows.

- **Intra-day precision**

Intra-day precision is the precision of the method that perform the same procedure under the same experimental conditions on the same day in different time intervals. Ten replicate portions for non-spiked and spiked (1.0 and 2.0 mg L^{-1} Cu(II) NR latex samples were determined. Triplicate determinations were performed for each portion.

- **Inter-day precision**

Inter-day precision is the precision of the method that perform the same procedure under the same experimental conditions on different days. Ten replicate portions for non-spiked and spiked (1.0 and 2.0 mg L^{-1} Cu(II)) NR latex samples were determined. Triplicate determinations were performed for each portion. Determination of copper was repeated for 6 days in every second day.

The precision is evaluated as relative standard deviation (%RSD) which can be calculated as described in the **equation 2.3**

$$\%\text{RSD} = \frac{\text{S}}{\overline{\text{X}}} \times 100 \qquad \text{….}\textbf{equation 2.3}$$

$$\text{S} = \sqrt{\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{(n-1)}} \qquad \text{….}\textbf{equation 2.4}$$

Where

S = standard deviation calculated from **equation 2.4**

n = total number of replication

X_i = each individual copper content (mg kg^{-1})

$\overline{X}$ = average copper content (mg kg^{-1}) for n measurements

2.8 Interference study

The objective for this study was to investigate the effect of interfering ions especially, cations such as Fe(III), Zn(II) and Mn(II) on Cu(II) determination. These metal ions are normally present in NR latex and can form complex with ZDEC. Interference study was performed by adding standard solution of 1000 mg L^{-1} Fe(III) and Mn(II) of 25, 125, 225, 250 and 275 µL to obtain the final concentrations of 1, 5, 9, 10 and 11 mg L^{-1}, respectively, into the 2.0 mg L^{-1} Cu(II) standard solution and determining by the developed method. The concentration of Zn(II) at 50, 100 and 500 mg L^{-1} were performed by adding standard solution of 1000 mg L^{-1} Zn(II) at 1.25, 2.5 and 12.5 mL, respectively, into the 2.0 mg L^{-1} Cu(II) standard solution. Three replicates were carried out for each concentration.

The interfering level of interfering ions can be considered as tolerance limit. The tolerance limit is the maximum concentration of the interfering ions that does not interfere for copper determination significantly. The tolerance limit can be present as the maximum concentration of the interfering ions produced % deviation not greater than 5%. The % deviation of signal for Cu(II) determination in case with interfering ions present was calculated from **equation 2.5**.

$$\%\text{Deviation} = \frac{A-B}{A}\times 100 \qquad \textbf{….equation 2.5}$$

Where

A = Absorbance of copper determination without interfering ion

B = Absorbance of copper determination with interfering ion

2.9 Samples

2.9.1 Field NR latex sample

Field NR latex samples (*Hevea Brasiliensis)* were obtained from five different sources. They were purchased from five latex traders in 5 different districts (Na Mom, Hat Yai, Muang Songkhla, Bang Klam and Na thawi) in Songkhla Province. Field sample was preserved with 0.7% NH_3 and can be kept stable at least a month.

2.9.2 Concentrated NR latex

The concentrated NR latex was prepared from field NR latex sample by centrifugation method. The field NR latex sample was preserved with 0.7% ammonia and treated by adding diammonium hydrogen phosphate (DAHP) for Mg^{2+} precipitation. Then field NR latex was centrifuged by centrifugal separator at 8000 rpm (Westfalia, Singapore). After centrifugation, latex sample can be separated into 2 parts; concentrated latex and skim latex. The principle of centrifugal separator is based on the separation between rubber particle and serum due to the different of rubber particle and serum mass. While the field latex was centrifuged, the part that presents heavy weight will be attracted by gravity. This part will flow through the lower-part and can be called “skim latex” whereas the rubber particle that presents low weight will float to the latex surface. Then this part will flow through the upper-part and can be called “concentrated latex”.

The procedure for concentrated latex preparation can be performed by taking at least 6 L of field NR latex sample, then amount of DAHP was added in field latex and shaken. Then field latex was stood overnight and taken to centrifuge by centrifugal separator at 8000 rpm for 2.5 hrs and then concentrated latex was obtained.

The amount of DAHP being added in field latex to precipitate Mg^{2+} before centrifugation was calculated from the reaction between Mg^{2+} and DAHP as mentioned in **Appendix D**. It is stated that 5.43g of DAHP is added per 1 g of magnesium present in field NR latex. The magnesium content was determined by the titrimetric method.

2.10 Application to real samples

2.10.1 Determination of copper content in latex samples by vis-spectrophotometry

The latex sample was first digested with the developed digestion system prior to analysis by the developed method with optimal conditions. Triplicate portions of samples were prepared. Triplicate determinations were performed for each portion. Calibration curve method was used to calculate copper content (mg L^{-1}). Copper content (on %TSC) was expressed as mg kg^{-1}. It can be calculated as **equation 2.6**.

$$\text{Copper content (mg kg}^{-1}\text{)} = \frac{2500 \times A}{B \times C} \qquad \text{....equation 2.6}$$

Where

A = copper concentration (mg L^{-1})

B = weight of NR latex (g)

C = total solids content (%TSC)

Note; The calculation of 2500 can be seen in **appendix A**.

2.10.2 Determination of copper content in NR latex samples by ICP-OES

To investigate the accuracy of the proposed method, the standard method (ICP-OES) was employed for copper determination. The procedure of latex digestion was performed as mentioned in **section 2.5.1.2.** The sample solution was diluted with DI water to 50 mL and measured by ICP-OES. Working standard solutions of Cu(II) in the range of 0.005-1.000 mg L^{-1} were prepared by diluting appropriate volume of 100 mg L^{-1} standard Cu(II) stock solution with 0.5 M of nitric acid. Triplicate portions of samples were prepared. Triplicate determinations were performed for each portion. Calibration curve method was used to calculate copper content (mg L^{-1}). Copper content (on %TSC) was expressed as mg kg^{-1}. It can be calculated as **equation 2.7**.

$$\text{Copper content (mg kg}^{-1}\text{)} = \frac{5000 \times A}{B \times C}$$ **….equation 2.7**

Where

A = copper concentration (mg L^{-1})

B = weight of NR latex (g)

C = total solids content (%TSC)

Note; The calculation of 5000 can be seen in **appendix A**.

Copper contents obtained from the developed method and those obtained by ICP-OES were compared. The t-test and ANOVA at 95% limit confidence were applied for statistical analysis.

2.11 Statistical Analysis

T-test and ANOVA were used as the indicator showing the difference of the experiment value from two method analysis.

(a) t-test is the statistical analysis for comparing the results of a developed method with the results obtained by a standard method or other methods. t value from the calculation called $t_{calculated}$ and t value from the table at various confident limits called $t_{critical}$. If $t_{calculated} < t_{critical}$, it indicates no significant differences of the results from two methods and if $t_{calculated} > t_{critical}$, it indicates significant differences of results. In this work, the 95% confident limit was applied.

(b) ANOVA is the analysis of variance and can be applied to separate and evaluate the different causes of variation. In this work, the ANOVA single factor and 95% confident limit were applied. F value from the calculation called $F_{calculated}$ and F value from the table at various confident limits called $F_{critical}$. If $F_{calculated} < F_{critical}$, it indicates no significant differences of the results from two methods and if $F_{calculated} > F_{critical}$, it indicates significant differences of results.

2.12 Determination of magnesium in field and concentrated NR latex samples

The aims of magnesium determination are to examine the amount of DAHP being added into field NR latex sample and to study the accordance of copper with magnesium content before and after adding DAHP.

Determination of magnesium by the titrimetric method was based on complex formation between Mg(II) and EDTA. Eriochrome Black T was used as an indicator. The magnesium contents before and after precipitation by DAHP were determined and compared.

Magnesium content in field NR latex can be determined by weighing amount of 2.00 g of field latex sample into the conical flask. One hundred milliliters of DI water was added, and pH of the solution was adjusted to 10.3-10.8 by adding NH_4Cl/NH_4OH buffer pH 10.5. Then 5 mL of 0.6 M KCN was added as a masking agent and amount of 0.1 g of eriochrome black T was added. The solution was titrated with 0.005 M EDTA until the end point (blue color) was reached. For the concentrated NR latex, magnesium content can be determined by weighing amount 10.00 g of concentrated NR latex into 100 mL beaker. Then 10 mL of DI water was added and followed by adding 5 mL of 25% acetic acid for coagulation. Ten milliliters of serum from the rubber coagulant was pipetted into the conical flask and the procedure from the pH adjustment to the end of experimental was the same as field latex procedure (TIS 4033: 2009). Concentration of magnesium, mg kg^{-1} (%TSC) was calculated as **equation 2.8**.

$$\text{Magnesium content (mg kg}^{-1}\text{)} = \frac{C_{EDTA} \times V_{EDTA}}{A \times B} \times 2,431,000 \qquad \text{….equation 2.8}$$

Where C_{EDTA} = concentration of EDTA (M)

V_{EDTA} = volume of EDTA (mL)

A = weight of latex (g)

B = total solids content (%TSC)

Note; The calculation of 2,431,000 can be seen in **appendix A**.

EDTA was standardized for evaluating the certain concentration prior to titration. The step of standardization can be performed by pipetting 5.00 mL of 0.02 M. $MgSO_4$ into the conical flask. Then the pH of solution was adjusted to 10.3-10.8 by adding the NH_4Cl/NH_4OH buffer pH 10.5 and 5 mL of 0.6 KCN was added as a masking agent. Amount of 0.1 g of eriochrome black T was added. The solution was titrated with EDTA until the end point was reached. The concentration of EDTA can be calculated as **equation 2.9**.

$$C_{EDTA} = \frac{C_{MgSO_4} V_{MgSO_4}}{V_{EDTA}} \times 100 \qquad \text{….}\textbf{equation 2.9}$$

Where

C_{EDTA} = concentration of EDTA (M)

V_{EDTA} = volume of EDTA (mL)

C_{MgSO_4} = concentration of $MgSO_4$ (M)

V_{MgSO_4} = volume of $MgSO_4$ (mL)

2.13 Some properties of field and concentrated latex

Some properties of field and concentrated latex sample such as total solids content (%TSC) and dry rubber content (%DRC) were determined.

2.13.1 Total Solids Content (%TSC)

The total solids content of latex is defined as the percentage by mass of the whole which is non-volatile under specified conditions of drying in an open atmosphere at an elevated temperature. Normally, TSC is demonstrated in % term that is calculated as **equation 2.10** (Blackley, 1997, volume 1, p. 418-419).

The procedure of %TSC determination can be performed as follows. First, the aluminium cup used as a container was weighed. Then, amount of 2.0 g of field or concentrated NR latex sample was added in the aluminum cup and weighed again. Next, 1 mL of DI water was added and the latex was spread out over the aluminum cup surface by swirling. Then, the field or concentrated NR latex in the aluminum cup was heated at 70±2°C in the oven for 16 hrs and then sample was left to cool in a desiccator. The aluminum cup which contained latex sample was weighed and heated again for 15 mins and after leaving to cool in desiccator, it was weighed again. This step was repeated until the constant weight obtained. Five replicates were performed for each sample (ISO 124:1997(E)).

$$\%TSC = \frac{C - A}{B - A} \times 100$$ ….**equation 2.10**

Where

A = weight of aluminum cup

B = weight of aluminum cup + latex sample before heating

C = weight of aluminum cup + latex sample after heating

2.13.2 Dry Rubber Content (%DRC)

Dry rubber content is defined as the percentage by mass of the latex which is coagulated under specified conditions of colloidal destabilization (Blackley, 1997, volume 1, p. 420-421). It can be determined by weighing amount of 10.0 g of NR latex sample into a beaker and 20±1% by weight of DI water was added into the beaker. The latex sample was coagulated by adding 35±5 mL of 2% v/v acetic acid and then the coagulated latex sample was placed in the water bath at 70°C for 15 to 30 min. The coagulated latex sample was brought up from the beaker and rinsed with water several times until the rinsed water was no longer acidic to litmus. The coagulated latex sample was pressed as a sheet (nearest 2 mm-thin) and rinsed with tap water for at least 5 min. Then, it was heated at 70±5°C until it had no white patches. The dry sheet was left to cool in desiccators for 30 min and finally, it was weighed. Five replicates were performed at each sample (ISO 126:2005(E)). % DRC was calculated by using **equation 2.12**.

$$\%\ DRC = \frac{m_1}{m_0} \times 100$$ ….**equation 2.12**

Where

m_0 = the mass of the test portion

m_1 = the mass of the dry sheet

CHAPTER 3

RESULTS AND DISCUSSION

The principle for copper determination in this work was based on the formation of a yellow complex obtained from Cu(II) and ZDEC which was extracted in DCM. Field and concentrated latex sample were digested by the developed digestion system. The conditions for Cu(II) determination in field and concentrated latex such as absorption spectra of Cu(II) complex, concentration of ZDEC and number of extraction were optimized. Moreover, the condition for NR latex digestion such as type of acid, digestion temperature and amount of NR latex were optimized. The analytical performance of proposed method was evaluated. The proposed method was applied to field and concentrated latex samples. The results were discussed as follows.

3.1 Optimization of vis-spectrophotometry

3.1.1 Choice of solvent

Type of solvent for Cu(II) extraction is one of the factor that is important for the proposed method. The distribution coefficient (K_d) of copper with solvent, the commercial availability, toxicity and type of danger material were considered for choosing type of solvent. Normally, these solvents such as chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), 1,1,1-trichloroethane (CH_3CCl_3) and dichloromethane (CH_2Cl_2) were used for the extract of the copper content. The results showed no significant differences of copper extraction by these solvents (Rathore *et al.*, 2006). In this work, dichloromethane (DCM) was applied for copper extraction due to its commercial availability whereas the other solvents are restricted chemicals. Moreover, 1,1,1-trichloroethane is hazardous chemical which is no longer produced.

3.1.2 Absorption Spectra of Cu(II) complex

The absorption spectra of copper complex (2.0 and 4.0 mg L^{-1} of standard Cu(II) solution) against reagent blank (200 mg L^{-1} ZDEC in DCM) over the wavelength range 300-800 nm were recorded. The absorption of yellow Cu-ZDEC complex showed maximum absorbance value (λ_{max}) at 435 nm as shown in **Figure 3-1**. The molar extinction coefficient of 1.37×10^4 L mol^{-1} cm^{-1} was provided, which was similar to the molar absorptivity ($\varepsilon = 1.4\times10^4$) of sodium diethyldithiocarbamate (NaDDTC) (Staden *et al.* 1999).

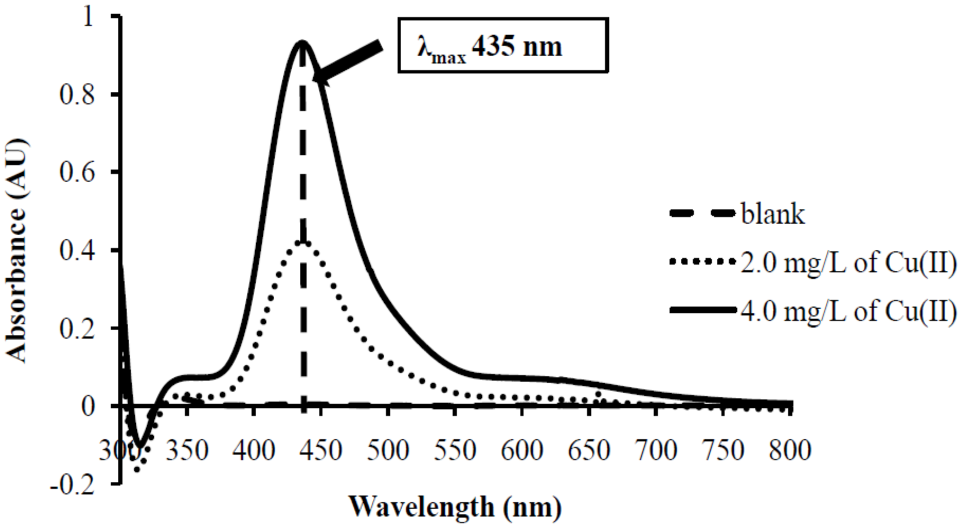


Figure 3-1 The yellow copper complex spectra against reagent blank at pH 8 (blank; 200 mg L^{-1} ZDEC in DCM; 2.0 mg L^{-1} Cu(II) + 200 mg L^{-1} ZDEC in DCM and 4.0 mg L^{-1} Cu(II) + 200 mg L^{-1} ZDEC in DCM).

3.1.3 Optimization of ZDEC concentration

Determination of 2 mg L^{-1} Cu(II) standard solution with various concentrations of ZDEC in DCM were conducted. ZDEC in DCM were prepared in the range of 50-200 mg L^{-1}, amount of 25.00 mL ZDEC at each concentration was applied for ZEDC concentration studying. The results of the optimization of ZDEC concentration are shown in **Table 3-1**.

Table 3-1 Determination of standard 2.0 mg L^{-1} Cu(II) by using various ZDEC concentrations (n=3)

Concentration of ZDEC (mg L^{-1})	Absorbance ± SD
50	0.320 ± 0.000
100	0.343 ± 0.015
150	0.383 ± 0.015
200	0.420 ± 0.000

As the results, it was found that the absorbance slightly increased with the increasing of ZDEC concentration and the absorbance of copper complex at 200 mg L^{-1} of ZDEC was slightly higher than other concentrations. However, at higher concentration of ZDEC more than 200 mg L^{-1} can't be prepared because it can't be dissolved by DCM. Consequently, 200 mg L^{-1} of ZDEC was chosen for copper determination in NR latex sample because ZDEC reacted sufficiently with copper

3.1.4 Number of extraction

To obtain most copper from the extraction, number of the extraction was optimized. Various numbers of extractions such as 1, 2 and 3 times were studied under the condition as mentioned in **section 2.4.2** and **2.4.5**. The influence of extraction number at 1, 2 and 3 times on the amount of copper was evaluated from the absorbance value of 2.0 mg L^{-1} Cu(II) determination. It was found that no significant difference in signal value between the numbers of extraction was observed. Therefore, a single extraction was enough for copper extraction in order to reduce the time of extraction and volume of complexing agent. The results of absorbance for copper complex at various numbers of extractions are shown in **Table 3-2**.

Table 3-2 Absorbance of 2.0 mg L^{-1} standard Cu(II) complex when using various extraction numbers (n=3)

Number of extraction	Absorbance ± SD
1	0.419 ± 0.002
2	0.418 ± 0.003
3	0.419 ± 0.002

3.2 Optimization of latex digestion

NR latex sample was digested by wet digestion using the developed digestion system. The digestion efficiency of NR latex depends on type of the acid, digestion temperature and amount of NR latex sample. These parameters were optimized.

3.2.1 Type of acid

The influence of acid type for NR latex digestion efficiency was studied. The acids such as conc. HNO_3, conc. H_2SO_4 and mixture of conc. H_2SO_4 and conc. HNO_3 were investigated for sample digestion.

(a) Conc. HNO_3 or conc. H_2SO_4: The result from digestion by a single acid showed that the digestion was incomplete and the digestion time was longer than 5 hrs.

(b) Acid mixture of conc. HNO_3 and conc. H_2SO_4: It was found that approximately total volume up to 16 mL of conc. HNO_3 must be added to obtain complete digestion. The sample can be digested and clear solution was obtained within 2.5 hrs.

Results showed that different types of acid had different effects on digestion of NR latex. The digestion was not completed by either individual acid conc. HNO_3 or conc. H_2SO_4. The efficient digestion was successful by using the mixture of conc. H_2SO_4 and conc. HNO_3. Therefore, this mixture of conc. H_2SO_4 and conc. HNO_3 was chosen for the digestion. The results of latex digestion from using a single acid (H_2SO_4 or HNO_3) and as acid mixture (conc. H_2SO_4 and conc. HNO_3) are demonstrated in **Figure 3-2**.

(a) (b) (c)

Figure 3-2 NR latex digestion by (a) conc. H_2SO_4, (b) conc. HNO_3 and (c) conc. H_2SO_4 and conc. HNO_3 (4:3, v/v).

3.2.2 Digestion temperature

The influence of the digestion temperature at 130, 160, 180 and 200ºC on recovery (%) of copper determination was investigated. Approximately, 1 g NR latex sample was weighed and digested with the mixture of conc. H_2SO_4 and conc. HNO_3. It was indicated that high temperature at least 160ºC was required to obtain good recovery (%) of copper. In addition, digestion at higher temperature also shortened the digestion time. However, the digestion could be impractically performed at 200ºC because the solution was hanged out under this condition. Hence, the digestion temperature of 180ºC was selected for the digestion. The result of digestion temperature for NR latex digestion is shown in **Table 3-3** and **Figure 3-3**.

Table 3-3 Recoveries (%) and digestion time of NR latex digestion at each various temperature (n=3)

Temperature (ºC)	Cu(II) concentration ($mg\ L^{-1}$)		Recovery (%)	Digestion time (hrs)
	Added	Found		
130	0.0	0.07±0.01	-	4.0
	2.0	1.70±0.00	81±0	
160	0.0	0.13±0.00	-	3.5
	2.0	2.01±0.02	94±1	
180	0.0	0.13±0.00	-	2.5
	2.0	2.01±0.02	94±1	

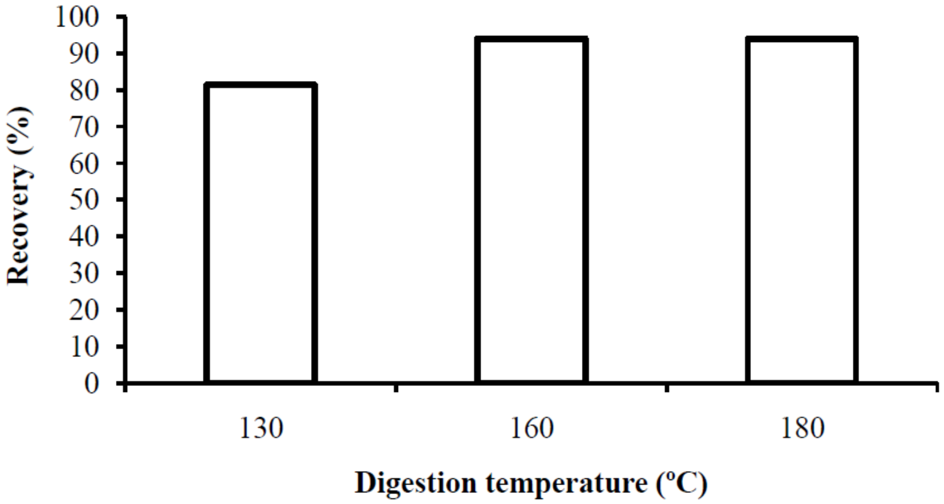


Figure 3-3 Recoveries (%) of copper determination at various digestion temperatures (130, 160 and 180ºC).

3.2.3 Amount of NR latex sample

Normally, the amount 2-10 g of NR latex is required as mentioned by ISO 8053 (1995) method. In this study, the appropriate amount of NR latex sample which can be digested efficiently with the developed digestion system was investigated. The amounts of NR latex sample in the range of 1.0-2.0 g were weighed and digested with mixture acids at 180ºC. The 2 mg L^{-1} Cu(II) was spiked into the sample to evaluate the recovery. The results are shown in **Table 3-4** and **Figure 3-4**.

Table 3-4 Determination of copper in various amounts of latex sample by using the developed method (n=3)

Weight of NR latex (g)	**Cu(II) concentration (mg L^{-1})**		**Recovery (%)**
	Added	**Found**	
1.0	0.0	0.54±0.02	-
	2.0	2.38±0.02	92±1
1.5	0.0	0.73±0.05	-
	2.0	2.39±0.02	84±1
2.0	0.0	0.90±0.03	-
	2.0	2.42±0.01	76±0

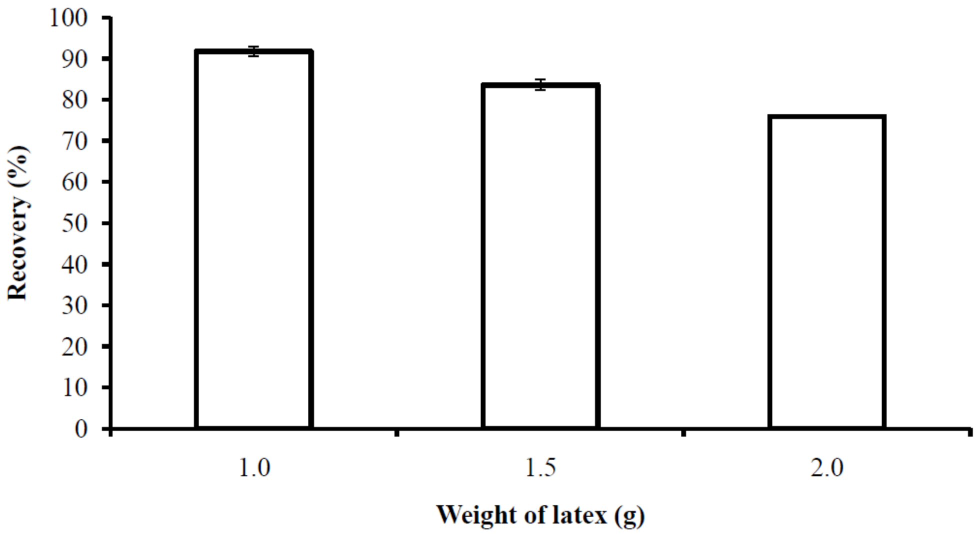


Figure 3-4 Recoveries (%) of copper determination at various weights of NR latex (1.0, 1.5 and 2.0 g).

It was indicated that sample weight affected the recovery. The sample weight of 1.0 g produced the best recovery. However, the sample weight was limited. The reaction mixture became violent when too much amount of sample (more than 1 g) was digested. The sample was hanged out, resulting in inefficient digestion with unsatisfactory recovery. Therefore, sample weight not more than 1 g was used to determine copper content by the proposed method.

According to the optimizations of latex digestion, the optimal conditions are summarized in **Table 3-5**.

Table 3-5 The optimal conditions for NR latex digestion

Parameter	**Optimum condition**
Type of acid	conc. H_2SO_4 (4 mL) and conc. HNO_3 (≈ 16 mL)
Temperature (°C)	180
NR latex amount (g)	≈ 1
Time consuming (hrs)	2.5

3.3 Analytical performances

3.3.1 Linearity range

Standard working solution of Cu(II) concentrations in the range of 0.1-6.0 mg L^{-1} were studied for evaluating the linear dynamic range. The linear range plotted between the absorbance of the signal and the concentration of Cu(II) working standard solution was established. Triplicates were performed at each concentration. The results for precision of calibration curve (n=15) are shown in **Table 3-6** and **Figure 3-5**.

Table 3-6 The precision of linearity for copper determination (n=15)

Linearity range	Slope	Intercept	R^2
0.2-4.0 mg L^{-1}	0.202±0.022	0.0145±0.0024	0.9962±0.013

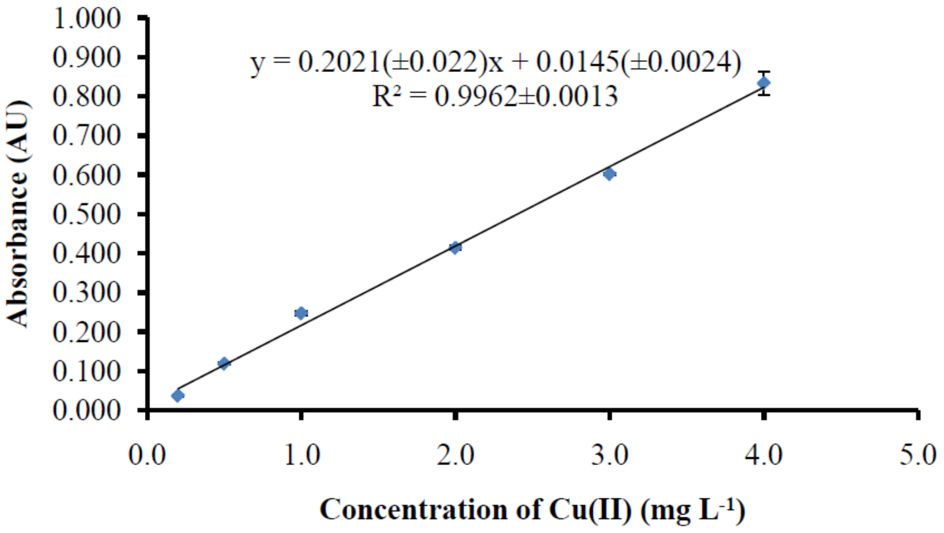


Figure 3-5 The linear range of Cu(II) determination by developed method.

Linearity based on Beer's law was in the concentrations range of 0.2-4.0 mg L^{-1} Cu(II). The mean linear regression equation for fifteen replicated calibration curves was y= (0.2021±0.0022)x + (0.0145±0.0024) with a good correlation coefficient (R^2 = 0.9962±0.0013). The results showed day-to-day minor variability in the slopes and intercepts. The good precision with relative standard deviation (%RSD) lower than 5% was provided. Analysis of variance of the slopes, intercepts and correlation coefficients of the 15 standard plots indicates no significant differences. The coefficience of variation for the slopes was 1.1% which indicates a high stability and precision of the method.

3.3.2 Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the calibration curve. The LOD and LOQ based on $3S_{y/x}/b$ and $10S_{y/x}/b$, respectively, were determined. The results of LOD and LOQ are expressed in **Table 3-7**.

Table 3-7 The limit of detection (LOD) and limit of quantification (LOQ) calculated from the calibration curve

$*X_i$	$*Y_i$	Y_{ii}	$(Y_i-Y_{ii})^2$
0.2	0.040	0.043	7.18×10^{-6}
0.5	0.100	0.103	7.84×10^{-6}
1.0	0.200	0.203	9.00×10^{-6}
2.0	0.420	0.403	2.76×10^{-4}
3.0	0.600	0.604	1.44×10^{-5}
4.0	0.800	0.804	1.76×10^{-5}
Sum of $(Y_i-Y_{ii})^2$			2.50×10^{-4}

*3 replications, %RSD ≤ 5%

Note: X_i = concentration of standard Cu(II), mg L^{-1}

Y_i = average absorbance of the measurements (n=3)

Y_{ii} = absorbance calculated from calibration curve

(Yii = 0.2025Xi + 0.0086; linear equation of calibration curve)

n = number of X_i constructed calibration curve (n=6)

b = slope of the calibration curve (b=0.2025)

Calculation:

$$S_{y/x} = \sqrt{\left[\left(\frac{\sum\left(Y_i - Y_{ii}\right)^2}{(n-2)}\right)\right]} \quad \text{(Tateo \textit{et al.}, 2010)}$$

$$= \sqrt{\frac{2.50\times10^{-4}}{4}}$$

$= 0.00003$

LOD = $3S_{y/x}/b$

$= (3\times0.00003)/0.2025$

$= 0.0004$ mg L^{-1}

LOQ = $10S_{y/x}/b$

$= (10\times0.00003)/0.2025$

$= 0.0014$ mg L^{-1}

Regarding the calculation, LOD and LOQ of 0.0004 mg L^{-1} and 0.0014 mg L^{-1}, respectively, were achieved.

3.3.3 Accuracy and precision of proposed method

In order to evaluate the accuracy of the method, recovery study was performed. The 1 mg L^{-1} and 2 mg L^{-1} Cu(II) were spiked into the field and concentrated latex sample. Triplicate portions of non-spiked and spiked samples were determined. Ten replicates determinations were performed for each portion. The results are shown in **Table 3-8** and **Table 3-9**.

As shown in **Table 3-8** and **Table 3-9**, recoveries (%) of spiked (1 mg L^{-1} and 2.0 mg L^{-1} Cu(II) standard solution) field and concentrated latex samples varied in the range of 92-101% with good precision (% RSD < 5%). Consequently, the good accurate and precise of the proposed method was achieved.

The precision study was expressed as % RSD. Results are shown in **Table 3-8** and **Table 3-9.** The intra-day and inter-day precisions of copper analysis in the field and concentrated latex sample were evaluated. The intra-day precision was carried out during the same experimental conditions by examining ten replicates of sample spiking Cu(II) at different concentrations (1 and 2 mg L^{-1}) within the same day. The inter-day precision was verified by examining sample spiking Cu(II) at different concentrations (1 and 2 mg L^{-1}) for six different days within two weeks. Ten replicate measurements were performed for each day. Results are shown in **Table 3-8** and **Table 3-9.**

Table 3-8 The intra-day accuracy and precision of copper determination by the proposed method (n=10*)

Sample	Field latex			Concentrated latex		
	Cu content (mg L^{-1})	**%RSD**	**%Recovery**	**Cu content (mg L^{-1})**	**%RSD**	**%Recovery**
Sample	0.25 ± 0.01	3.2	-	0.17 ± 0.00	2.4	-
Sample + 1 mg L^{-1}	1.17 ± 0.01	0.9	92 ± 1	1.11 ± 0.01	1.0	94 ± 1
Sample + 2 mg L^{-1}	2.26± 0.01	0.4	101 ± 0	2.18 ± 0.01	0.6	101 ± 1

n=10* means to ten portions of spiked and non-spiked samples and triplicates were determined for each sample.

Table 3-9 The inter-day accuracy and precision of copper determination by the proposed method (n=6*)

Sample	Field latex			Concentrated latex		
	Cu content (mg L^{-1})	**%RSD**	**%Recovery**	**Cu content (mg L^{-1})**	**%RSD**	**%Recovery**
Sample (n=10*)	0.25 ± 0.00	1.2	-	0.17 ± 0.00	2.4	-
Sample + 1 mg L^{-1} (n=10*)	1.17 ± 0.01	1.0	92 ± 2	1.12 ± 0.01	1.7	95 ± 1
Sample + 2 mg L^{-1} (n=10*)	2.26 ± 0.02	0.8	100 ± 1	2.20 ± 0.02	2.0	101

n=6* means to 6 days of inter-day precision and n=10* means to ten portions of spiked and non-spiked samples and triplicates were determined for each sample in each day.

From the results, the repeatability or intra-day and reproducibility or inter-day precision of the method varied from 0.4 to 3.2% and 0.8 to 2.4%, respectively. The proposed method revealed good repeatability and reproducibility with good % RSD (less than 5%). Moreover, the method demonstrated good accuracy with satisfactory recovery in the range of 92-101%. Both accuracy and precision obtained were in the acceptable range.

3.4 Interference study

The effect of some cations such Fe^{3+}, Mn^{2+} and Zn^{2+} which present in NR latex (Kang *et al.*, 2000; Scott *et al.*, 2003) on copper determination was studied. Various concentrations of interfering ion were added into 2 mg L^{-1} Cu^{2+}. The tolerance limit defined as the maximum concentration of interfering ion added causing a change in the absorbance not greater than ± 5% (%deviation $\leq$ 5%) was evaluated (Khlyntseva *et al.*, 2011). The results are given in **Table 3-10**.

Table 3-10 Tolerance limits of interfering ions for 2 mg L^{-1} Cu(II) determination by the developed method (n=3)

Interfering ion	**Concentration of interfering ion (mg L^{-1})**	**Signal (absorbance ± SD)**	**% Deviation**	**Tolerance limit (mg L^{-1})**
Fe^{3+}	1.0	0.420±0.000	0.0	9.0
	5.0	0.420±0.000	0.0	
	9.0	0.400±0.001	4.8	
	10.0	0.393±0.002	6.4	
	11.0	0.378±0.003	9.9	
Mn^{2+}	1.0	0.419±0.001	0.3	9.0
	5.0	0.410±0.000	2.4	
	9.0	0.402±0.002	4.4	
	10.0	0.392±0.002	6.8	
	11.0	0.375±0.000	10.7	
Zn^{2+}	50.0	0.420±0.000	0.0	> 500.0
	100.0	0.420±0.000	0.0	
	500.0	0.412±0.002	2.0	

Results showed that high concentration of Zn^{2+} up to 500.0 mg L^{-1} had no significant effect on 2 mg L^{-1} of Cu^{2+} determination, while the concentration of $Fe^{3+}$and Mn^{2+} higher than 9.0 mg L^{-1} interfere seriously to the analytical signal of copper determination. However, the content of those ions present in the NR latex sample were below interfering level. Amount of 3.52, 0.77 and 27.0 mg L^{-1} of Fe^{3+}, Mn^{2+} and Zn^{2+}, respectively, in field latex were reported by Scott *et al.,* 2003. In case of high content of interfering ions such as Fe(III) and Mn(II) present in NR latex, they will be eliminated by adding the ammonium citrate or tartrate as masking agent (Jankiewicz *et al.,* 1998). Therefore, this method was selective for copper determination in the NR latex sample.

3.5 Application

3.5.1 Copper determination in NR latex samples by vis-spectrophotometry

Copper determination in NR latex sample can be performed by digesting the NR latex with the optimum conditions. Then, the sample solution was analyzed by the developed method. Furthermore, copper determination in NR latex digested by the developed digestion system and kjeldahl digestion system were also compared.

3.5.2 Copper determination in NR latex samples by ICP-OES

The standard method (ICP-OES) was applied for the confirmation of the accuracy of developed method. By employing the conditions described previously in **section 2.2.1.2**, the calibration curve was constructed by plotting concentrations (X-axis) against their intensity values (Y-axis). The linearity in the range of 0.005-1.0 mg L^{-1} as shown in **Figure 3-7** was provided.

Copper determination in NR latex sample can be performed by digesting the NR latex with the optimum conditions. Then, the sample solution was analyzed by ICP-OES. Furthermore, copper determination in NR latex digested by the developed digestion system and kjeldahl digestion system were also compared. The linear regression equation was used to calculate the copper content. Results of copper determination in latex samples (triplicates) are given in **Table 3-11**.

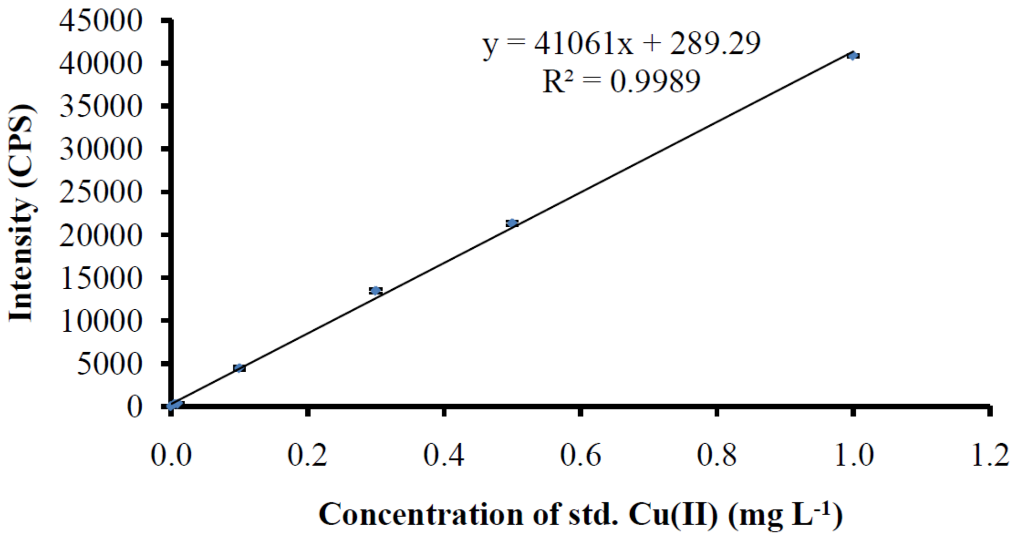


Figure 3-6 The calibration curve of Cu(II) determination (mg L^{-1}) by ICP -OES.

The developed method with optimal conditions was applied for copper determination in NR latex samples. Three replicates were performed at each sample. The results of copper content in concentrated NR latex samples by vis-spectrophotometry and ICP-OES are shown in **Table 3-11**.

Table 3-11 Copper contents in five concentrated latex samples by vis-spectrophotometry and ICP-OES (n=3)

Samples	Cu content (mg kg^{-1})			
	vis-spectrophotometry		**ICP-OES**	
	Developed digestion system	**Kjeldahl digestion system**	**Developed digestion system**	**Kjeldahl digestion system**
1	6.8 ± 0.0	7.0 ± 0.2	6.8 ± 0.1	6.7 ± 0.1
2	7.0 ± 0.2	6.9 ± 0.2	6.4 ± 0.1	6.5 ± 0.2
3	6.9 ± 0.2	6.9 ± 0.1	6.8 ± 0.1	7.0 ± 0.0
4	6.9 ± 0.2	6.9 ± 0.1	6.8 ± 0.1	6.7 ± 0.1
5	6.8 ± 0.2	6.5 ± 0.1	6.5 ± 0.1	6.5 ± 0.1

Note;1, 2, 3, 4 and 5 refers to concentrated latex prepared from field NR latex that purchased from latex traders in Na Mom, Hat Yai, Muang Songkhla, Bang Klam and Na Thawi districts, respectively.

According to the results in **Table 3-11,** the copper contents in concentrated latex samples digested and detected by the developed method in the range of 6.8-7.0 mg kg^{-1} (on %TSC) were found. The good precision (%RSD less than 5%) was achieved. Copper contents in these concentrated latex samples were lower than TIS (980-2009) guideline level (8 mg kg^{-1} on %TSC). In order to validate the method, the copper contents were compared by the standard method (ICP-OES). No significant difference was observed (at 95% confident limit; t-test and ANOVA). The results of statistical analysis are shown in **Appendix C**. Therefore, the developed method was the reliable, precise and accurate. Moreover, the sample digestion with the developed and kjeldahl digestion system were conducted and copper contents in the sample solutions obtained by both systems were compared. The results also showed no significant difference. This indicated that the developed digestion system could be an alternative system for digestion of NR latex sample.

3.5.3 Comparison of copper content in field and concentrated latex samples

The developed method was applied for copper determination in field and concentrated latex samples that were purchased from 5 traders of 5 districts in Songkhla Province. Moreover, the copper contents “before” and “after” adding diammonium hydrogen orthophosphate (DAHP) were compared.

The copper contents in field latex samples were in the range of 16.6-23.8 mg kg^{-1} on % TSC. According to the previous reports, the copper content of 0.27 mg L^{-1} reported by Kang *et al.*, (2000), 1.65 mg L^{-1} reported by Scott *et al.,* (2003) and 1.34-2.26 mg kg^{-1} (on %TSC) reported by Ng and Lai (1972) were found in field NR latex. In this research, copper contents in field and concentrated NR latex samples were also compared. The copper content in concentrated latex samples were in the range of 6.8-7.0 mg kg^{-1} (on %TSC). It was observed that copper content in field latex was higher than concentrated latex about 2-3 times. This is because DAHP added in the field latex before centrifugation to prepare concentrated latex will precipitate copper similarly to magnesium (Lunt *et al.,* 1962). The results for copper contents, %TSC and %DRC in field and concentrated latex samples are shown in **Table 3-12**.

Table 3-12 Copper content, %TSC and %DRC in field and concentrated latex samples by vis-spectrophotometry (n=3)

Samples	Field latex			Concentrated latex		
	Copper content, mg kg^{-1} (n=3)	**%TSC (n=5)**	**%DRC (n=5)**	**Copper content, mg kg^{-1} (n=3)**	**%TSC (n=5)**	**%DRC (n=5)**
1	16.6 ± 0.8	38.4±0.2	34.6±0.8	6.8 ± 0.0	62.6±0.3	60.3±0.1
2	21.7 ± 0.7	28.8±0.2	26.8±0.2	7.0 ± 0.2	62.9±0.3	60.5±0.6
3	20.7 ± 0.6	34.2±0.8	31.3±1.1	6.9 ± 0.2	63.5±0.2	61.4±0.4
4	23.5 ± 0.6	31.0±0.3	31.4±0.1	6.9 ± 0.2	62.9±0.1	61.4±0.8
5	23.8 ± 0.6	32.1±0.1	30.7±0.4	6.8 ± 0.2	63.4±0.3	63.0±0.3

3.6 Determination of magnesium content in field and concentrated NR latex samples

As mentioned before excess amount of magnesium in concentrated latex will effect to the quality of the rubber. Maximum magnesium content of 40 mg kg^{-1} on %TSC in concentrated latex was recommended by TIS 980 (2009). Hence, in concentrated latex preparation, amount of DAHP is necessary added into field latex before centrifugation to precipitate Mg^{2+}. The amount of DAHP added into field latex is depending on magnesium content. It was found that 5.43 g of DAHP is enough to precipitate 1 g of magnesium (Kajornchaiyakul, 2006). Therefore, the magnesium content (in mg kg^{-1} on %TSC as calculation in **equation 2.8)**, for calculating the amount of DAHP added in field latex before centrifugation, was determined by a titrimetric method. The results of magnesium contents are shown in **Table 3-13**. Furthermore, we estimated that the rest of DAHP can precipitate copper like magnesium. The copper precipitation will be eliminated and then, less copper content is remained in the concentrated latex. In order to proof this hypothesis, copper content “before” (in field latex) and “after” (in concentrated latex) adding DAHP was determined.

Table 3-13 Magnesium contents, %TSC and %DRC in field and concentrated NR latex samples (n=5)

Samples	Field latex			Concentrated latex		
	Magnesium content, mg kg^{-1} (n=3)	**%TSC (n=5)**	**%DRC (n=5)**	**Magnesium content, mg kg^{-1} (n=3)**	**%TSC (n=5)**	**%DRC (n=5)**
1	826±1	38.4±0.2	34.6±0.8	8.6±1.0	62.6±0.3	60.3±0.1
2	1438±1	28.8±0.2	26.8±0.2	8.9±0.0	62.9±0.3	60.5±0.6
3	1164±2	34.2±0.8	31.3±1.1	15.6±0.1	63.5±0.2	61.4±0.4
4	1364±0	31.0±0.3	31.4±0.1	1.4±0.0	62.9±0.1	61.4±0.8
5	44.8±1.0	32.1±0.1	30.7±0.4	4.1±0.0	63.4±0.3	63.0±0.3

From the results, magnesium contents in field and concentrated NR latex from 5 districts in Songkhla Province were in the range of 49-1438 and 1.4-15.6 mg kg^{-1} on %TSC, respectively. The amount of magnesium in concentrated latex was lower than in field latex due to the precipitation of DAHP with magnesium. In addition, the magnesium contents in 5 concentrated latex samples were below than TIS 980 (2009) guideline.

3.7 Properties of latex

In this work, some properties of field and concentrated NR latex such as total solids content (%TSC), dry rubber content (%DRC) were determined for calculating the copper content in field and concentrated NR latex samples.

3.7.1 Total solids content (%TSC)

Usually, copper content in mg kg^{-1} unit based on TSC is reported. Therefore, it is necessary to determined %TSC in field and concentrated latex. The results of %TSC in 5 field and concentrated NR latex samples from 5 districts in Songkhla Province are shown in **Table 3-12** and **Table 3-13**. Total solids contents in field and concentrated NR latex samples were in the range of 28.8-38.4 and 62.6-63.5%, respectively. Generally, %TSC of field and concentrated NR latex sample are in the range of 25-45 and minimum of 61%, respectively. As the results, all %TSC of all latex samples were in the acceptable value recommended by TIS 980 (2009).

3.7.2 Dry Rubber Content (%DRC)

Dry rubber content (%) is being used to indicate the amount of rubber in latex. The results of %DRC of field and concentrated latex samples are shown in **Table 3-12** and **Table 3-13**. It was found that %DRC of field and concentrated NR latex samples were in the range of 26.8-34.6 and 60.3-61.4%, respectively. Generally, %DRC of field and concentrated latex sample are in the range of 20-40% and minimum of 60%, respectively. It could be concluded that % DRC of 5 field and concentrated latex samples from 5 districts in Songkhla Province were in the acceptable value recommended by TIS 980 (2009).

CHAPTER 4

CONCLUSIONS

In this research, the developed method for copper determination in concentrated NR latex sample was successfully established. The concentrated NR latex sample was digested by the developed digestion system. The sample was digested with mixture acid of conc. H_2SO_4 and conc. HNO_3 in Pyrex digestion tube and placed in paraffin liquid oil bath connected with thermostat for temperature control. The optimal conditions are summarized in **Table 4-1**.

Table 4-1 The optimal conditions for NR latex digestion

Parameter	Optimum condition
Type of acid	conc. H_2SO_4 (4 mL) and conc. HNO_3 (≈ 16 mL)
Temperature (°C)	180
NR latex amount (g)	1

The developed digestion system offers the advantages of low cost and rapidness of sample digestion by comparing with kjeldahl digestion system utilized by ISO 8053 (1995). The Pyrex digestion tube is cheaper than kjeldahl apparatus. Moreover, the sample digestion time by the developed digestion system was shorter (2.5 hrs) than by the kjeldahl digestion system (5.0 hrs). However, amount of NR latex sample not more than 1 g digested in the developed digestion system was limited. The more amount of the sample up to 10 g can be digested in the kjeldahl digestion system.

The simple developed vis-spectrophotometry based on complex formation between Cu^{2+} and zinc diethyl dithiocarbamate (ZDEC) and extraction with dichloromethane was developed. The Cu^{2+} was formed with 200 mg L^{-1} ZDEC in dichloromethane, yielding the yellow complex. The complex was extracted in separatory funnel without re-extraction and then the absorbance of extract was measured at 435 nm. Dichloromethane was used as organic solvent instead of 1,1,1-trichloroethane (ISO 8053 (1995)). This is because of its toxicity and commercial availability. The 1,1,1-trichloroethane is highly toxic chemical and banned to import to Thailand. Moreover, 1,1,1-trichloroethane is no longer manufactured. Therefore dichloromethane, which is commercial available and less toxic, was selected.

The developed method for copper determination in concentrated NR latex provides many advantages as compared to ISO 8053 (1995), as shown in **Table 4-2**.

Table 4-2 Comparative determination of copper by developed and ISO method

Parameter	Developed method	ISO method
Digestion tube	Pyrex tube	Kjeldahl tube
Solvent	DCM	1,1,1-trichloroethane
Digestion time (hrs.)	2.5	5.0

The linear range of this method was 0.2-4.0 mg L^{-1}. The method illustrated a limit of detection of 0.0004 mg L^{-1} and a limit of quantification of 0.0014 mg L^{-1}. Furthermore, the good accuracy and precision for the method were presented in the range of 92-101% and 0.4-3.2%, respectively.

The potential interfering metal ions were investigated. The tolerance limits for Fe^{3+} and Mn^{2+} and Zn^{2+} were 9.0, 9.0 and >500 mg L^{-1}, respectively. Normally, lower contents than interfering level of these cations exist in NR latex samples. Therefore, these cations did not interfere seriously for the copper determination by the developed method.

The method was applied for copper determination in concentrated NR latex samples which were prepared from field NR latex samples by centrifugation method. Field NR latex samples were purchased from latex traders in different districts located in Songkhla Province. Diammonium hydrogen orthophosphate (DAHP) was added into field NR latex samples before centrifugation in order to precipitate magnesium. Amount of DAHP added was depending on the magnesium content. Magnesium content was determined by titrating with EDTA. The purpose for magnesium determination is to calculate the amount of DAHP that was added in field latex before centrifugation. It was observed that magnesium contents in field and concentrated NR latex were in the range of 44 -1438 and 1.4-15.6 mg kg^{-1} (on

%TSC), respectively. Lower content of magnesium in concentrated latex was provided due to the precipitation of magnesium with DAHP.

The copper contents in concentrated latex samples prepared from field NR latex was digested with the digestion system and analyzed by the developed method. Concentrations of copper in concentrated latex in the range of 6.8-7.0 mg-kg^{-1} (on %TSC) were found. Copper contents in these concentrated latex samples were lower than TIS (980-2009) guideline level (8 mg kg^{-1} on %TSC). In order to confirm the accuracy of the method, the copper contents were compared with the standard method (ICP-OES). No significant difference was observed (at 95% confident limit; t-test and ANOVA). Moreover, the sample digestion system with the developed and kjeldahl digestion system were conducted and copper contents in the sample solutions obtained by both systems were compared. The results showed no significant difference as well. This indicated that the developed digestion system could be an alternative system for sample digestion.

Copper content in field NR latex samples were determined. The copper content in field NR latex samples were found in the range of 16.6-23.8 mg kg^{-1} (on %TSC). According to the previous reports, the copper content of 0.27 mg L^{-1} reported by Kang *et al.*, (2000) and 1.65 mg L^{-1} reported by Scott *et al.*, (2003) were found in field NR latex. In this research, copper contents in field and concentrated NR latex samples were also compared in order to investigate the effect of added DAHP on copper content. It was observed that copper content in field NR latex was higher than in concentrated NR latex. By adding DAHP into the field NR latex, magnesium and other metal ions such as Fe^{2+}, Mn^{2+}, Zn^{2+} including Cu^{2+} can be precipitated with DAHP (Lunt *et al.*, 1962). The precipitation will be separated from concentrated NR latex during centrifugation process. Therefore, copper content in concentrated NR latex was lower than in field NR latex. From this observation, it is good evidence for further study. The suitable amount of DAHP added into field NR latex should be further investigated in order to reduce much more copper content.

In conclusion, the simple and effective vis-spectrophotometry for copper determination was developed. The method offers a wide linear range, good precision and accuracy. Therefore, this developed method will be further applied for copper determination in concentrated latex industries.

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APPENDIX A

Constant value calculation

Appendix A-1 Calculation of constant value for copper determination from mg L^{-1} to mg kg^{-1} by vis-spectrophotometry

From **equation 2.6**

Copper content (mg kg^{-1}) = $\frac{2500 \times A}{B \times C}$

The calculation of 2500 consists of 4 steps that can be described as follows;

1.) Converting mg $L^{-1}$to mg 25 mL^{-1};

The solution 1000 mL presents copper A mg

The solution 25 mL presents copper (A×25)/1000 mg

2.) Converting mg 25 mL^{-1} to mg kg^{-1}

Rubber B g presents copper (A×25)/1000 mg

Rubber 1000 g presents copper [(A×25)/1000 mg] × (1000/B) = 25A/B

3.) Converting %TSC to g in NR latex

Rubber 100 g presents the solid content C g

Rubber B g presents the solid content (C×B)/100 g

4.) Converting based on solid content

Solid content (C×B)/100 g present copper 25A/B

Solid content B g presents copper [(25A/B)×(B)]× (100/CB)

Then **2500** was obtained from this calculation.

Appendix A-2 Calculation of constant value for copper determination in mg L^{-1} to mg $kg^{-1}$by ICP-OES

From **equation 2.7**

Copper content (mg kg^{-1}) = $\frac{5000 \times A}{B \times C}$

The calculation of 5000 consists of 4 steps that can be described as follows

1.) Converting mg $L^{-1}$to mg 25 mL^{-1};

The solution 1000 mL presents copper A mg

The solution 50 mL presents copper (A×50)/1000 mg

2.) Converting mg 50 mL^{-1} to mg kg^{-1}

Rubber B g presents copper (A×50)/1000 mg

Rubber 1000 g presents copper [(A×50)/1000 mg] × (1000/B) = 50A/B

3.) Converting %TSC to g in NR latex

Rubber 100 g presents the solid content C g

Rubber B g presents the solid content (C×B)/100 g

4.) Converting based on solid content

Solid content (C×B)/100 g present copper 50A/B

Solid content B g presents copper [(50A/B)×(B)]× (100/CB)

Then **5000** was obtained from this calculation.

Appendix A-3 Calculation of constant value for magnesium determination in mg L^{-1} to mg $kg^{-1}$by titrimetric method

From **equation 2.8**

Magnesium content (mg kg^{-1}) = $\frac{C_{EDTA} \times V_{EDTA}}{A \times B} \times 2,431,000$

The calculation of 2,431,000 consists of 3 steps that can be described as follows

1.) Converting mg L^{-1} to mg kg^{-1}

The solution (C×V)/1000 mole presents the (g/ 24.31.) of magnesium

Therefore the mg of magnesium = [(C×V×24.31)/1000]×1000

2.) Converting mg L^{-1} to mg $kg^{-1}$of rubber weight

Rubber B g presents magnesium [(C×V×24.31)/1000]×1000 mg

Rubber 1000 g presents magnesium [(C×V×24.31)×1000]/B mg

3.) Converting %TSC to g in NR latex

Rubber 100 g presents solid content C g

Rubber B g presents solid content (C×B)/100 g

4.) Converting based on solid content

Solid content (C×B)/100 g present magnesium [(C×V×24.31)×1000]/B

Solid content B g presents magnesium

({[(C×V×24.31)×1000]/B}×B)/((C×B)/100)

Then **2,431,000** was obtained from this calculation.

APPENDIX B

Copper contents of field NR latex samples from three different plantations in different regions of Thailand (collected in dry and rainy seasons) detected by vis-spectrophotometry (n=3)

Samples	**Copper content (mg kg^{-1})**					
	Burirum		**Chacoengsao**		**Songkhla**	
	Dry	**Rainy**	**Dry**	**Rainy**	**Dry**	**Rainy**
1	22.6± 0.6	8.0± 0.0	11.0± 0.7	16.3± 0.8	14.8± 0.4	14.4± 0.6
2	21.1± 0.9	15.1± 0.3	11.4± 0.8	22.0± 0.7	11.5± 0.8	13.9± 0.6
3	15.5± 0.6	15.7± 0.8	10.7± 0.7	12.3± 0.8	9.0± 0.4	9.8± 0.8
4	21.3± 0.6	25.0± 0.8	14.1± 0.6	14.0± 0.4	10.8± 0.6	12.8± 0.1
5	15.8± 0.8	20.5± 0.7	14.4± 0.4	14.2± 0.5	14.7± 0.7	16.7± 0.8

APPENDIX C
Statistical analysis (t-test and ANOVA)

Appendix C-1: Statistical analysis for comparison of copper content in 5 field NR latex samples by developed digestion system and kjeldahl digestion system (vis-spectrophotometry), ($t_{critical}$ = 2.57 and $F_{critical}$ = 6.60 at confidence limits 95%).

Samples	Copper content (mg kg^{-1})		t-value	F-value
	developed digestion system (n=3)	kjeldahl digestion system (n=4)		
1	16.6 ± 0.8	16.9 ± 0.4	0.77	0.59
2	21.7 ± 0.7	22.8 ± 0.5	2.46	6.06
3	20.7 ± 0.6	20.1 ± 0.1	0.56	0.32
4	23.5± 0.6	24.3 ± 0.2	2.42	5.87
5	23.8± 0.6	24.0 ± 0.3	0.77	0.59

Appendix C-2: Statistical analysis for comparison of copper content in 5 concentrated NR latex samples by developed digestion system and kjeldahl digestion system (vis-spectrophotometry), ($t_{critical}$ = 2.57 and $F_{critical}$ = 6.60, at confidence limits 95%).

Samples	Copper content (mg kg^{-1})		t-value	F-value
	developed digestion system (n=3)	kjeldahl digestion system (n=4)		
1	6.8 ± 0.0	7.0 ± 0.2	1.56	2.45
2	7.0 ± 0.2	6.9 ± 0.2	0.51	0.26
3	6.9 ± 0.2	6.9 ± 0.1	0.20	6.42
4	6.9± 0.2	6.9 ± 0.1	0.00	0.71
5	6.8± 0.2	6.5 ± 0.1	2.18	4.76

Appendix C-3: Statisticalanalysis for comparison of copper content in 5 concentrated NR latex samples by developed digestion system and kjeldahl digestion system (ICP-OES), ($t_{critical}$ = 2.78 and $F_{critical}$ = 7.70, at confidence limits 95%).

Samples	Copper content (mg kg^{-1})		t-value	F-value
	developed digestion system (n=3)	kjeldahl digestion system (n=3)		
1	6.8 ± 0.1	6.7 ± 0.1	1.41	2.00
2	6.4 ± 0.1	6.5 ± 0.2	0.97	0.94
3	6.8 ± 0.1	7.0 ± 0.0	2.68	0.50
4	6.8± 0.1	6.7 ± 0.1	0.44	0.20
5	6.5± 0.1	6.5 ± 0.1	1.40	0.50

Appendix C-4: Statistical analysis for comparison of copper content in 5 concentrated NR latex samples by developed digestion system detect by vis-spectrophotometry and ICP-OES ($t_{critical}$ = 2.78 and $F_{critical}$ = 7.70, at confidence limits 95%).

Samples	Copper content (mg kg^{-1})		t-value	F-value
	vis-spectrophotometry (n=3)	ICP-OES (n=3)		
1	6.8 ± 0.0	6.8 ± 0.1	2.00	1.00
2	7.0 ± 0.2	6.4 ± 0.1	6.00	36.1
3	6.9 ± 0.2	6.8 ± 0.1	1.94	3.77
4	6.9 ± 0.2	6.8 ± 0.1	2.00	4.00
5	6.8 ± 0.2	6.5 ± 0.1	2.66	7.11

Appendix C-5: Statistical analysis for comparison of copper content in 5 concentrated NR latex samples by developed digestion system (visible-spectrophotometry) and kjeldahl digestion system (ICP-OES), ($t_{critical}$ = 2.78 and $F_{critical}$ = 7.70, at confidence limits 95%).

Samples	Copper content (mg kg^{-1})		t-value	F-value
	vis-spectrophotometry (n=3)	ICP-OES (n=3)		
1	6.8 ± 0.0	6.7 ± 0.1	2.00	4.00
2	7.0 ± 0.2	6.5 ± 0.2	3.24	10.24
3	6.9 ± 0.2	7.0 ± 0.0	1.58	6.40
4	6.9± 0.2	6.7 ± 0.1	1.58	6.40
5	6.8± 0.2	6.5 ± 0.1	1.40	0.50

Appendix C-6: Statistical analysis for comparison of copper content in 5 concentrated NR latex samples by kjeldahl digestion system (visible-spectrophotometry) and kjeldahl digestion system (ICP-OES), ($t_{critical}$ = 2.57 and $F_{critical}$ = 6.60, at confidence limits 95%).

Samples	Copper content (mg kg^{-1})		t-value	F-value
	vis-spectrophotometry (n=3)	ICP-OES (n=4)		
1	7.0 ± 0.2	6.7 ± 0.1	2.54	6.49
2	6.9 ± 0.2	6.5 ± 0.2	2.77	7.68
3	6.9 ± 0.1	7.0 ± 0.0	0.09	0.01
4	6.9 ± 0.1	6.7 ± 0.1	1.89	3.57
5	6.5 ± 0.1	6.5 ± 0.1	0.25	0.06

APPENDIX D

The reaction between magnesium and DAHP

$Mg^{++} + (NH_4)_2HPO_4 \longrightarrow Mg(NH_4)PO_4 + H^+ + NH_4^+$

Atomic mass; Mg^{++} = 24.31

Molecular weight; $(NH_4)_2PO_4$ = 131.97

$\therefore Mg^{++}$ 24.31 g ≡ DAHP 131.97 g

Mg^{++} 1 g ≡ DAHP $\frac{131.97}{24.31}$ g

$\therefore Mg^{++}$ 1 g ≡ DAHP 5.43 g

From this ratio, it can be concluded DAHP 5.43 g is added to precipitate amount 1 g of magnesium in field latex.