# Simple digestion and visible spectrophotometry for copper determination in natural rubber latex

Supunnee Duangthong<sup>a,\*</sup>, Khwannapha Rattanadaecha<sup>a</sup>, Wilairat Cheewasedtham<sup>b</sup>, Puchong Wararattananurak<sup>a</sup>, Pipat Chooto<sup>a</sup>

- <sup>a</sup> Department of Chemistry, Analytical Chemistry Division, Faculty of Science, Prince of Songkla University, Songkhla 90112 Thailand
- <sup>b</sup> Department of Science, Faculty of Science and Technology, Prince of Songkla University, Pattani 94000 Thailand

\*Corresponding author, e-mail: supunnee.d@psu.ac.th

Received 11 Aug 2017 Accepted 14 Nov 2017

**ABSTRACT**: A simple method for determination of copper contents in natural rubber (NR) latex based on the complex formation between Cu(II) and zinc diethyldithiocarbamate (Zn(II)-DDTC) and extraction with dichloromethane followed by visible spectrophotometry at 435 nm was developed. A low-cost wet digestion system for NR latex was adopted by digesting a sample in a Pyrex tube with the a mixture of concentrated  $H_2SO_4$  and concentrated  $HNO_3$  at 180 °C. Under the optimum conditions, the linear range of 0.1–4.0 mg/l ( $R^2 > 0.99$ ), the limit of detection and the limit of quantification were 0.0356 and 0.1188 mg/l, respectively, the intra-day (n = 10) and inter-day (n = 6) relative standard deviations were 0.4–3.2 and 0.8–3.4%, respectively, and recoveries of 92–101% were obtained. Fe(III), Mn(II), and Zn(II) did not interfere with the tolerance limit of 9.0, 9.0, and more than 500 mg/l, respectively, which are higher than their usual concentrations in latex. The method was applied to copper determination in a concentrated latex sample prepared from field latex samples by centrifugation. The copper content was found to be 6.8–7.0 mg/kg (on total solids content) and copper content in the field NR latex sample was 16.6–23.8 mg/kg (on total solids content). No difference was observed between the proposed method and ICP-OES as well as between the proposed digestion and Kjeldahl digestion.

KEYWORDS: zinc diethyldithiocarbamate, Hevea brasiliensis, Kjeldahl digestion

#### INTRODUCTION

Copper is recognized as one of essential nutrient elements required by rubber  $plants^1$ . It plays an important role in the growth and is present in latex by absorption from soil and fertilization<sup>2</sup>.

Copper content is one character to be considered in the specifications of latex<sup>1</sup>. Generally, copper found in natural rubber (NR) is in the forms of CuO and Cu-oleate<sup>3</sup> ( $C_{36}H_{66}CuO_4$ ) which can catalyse oxidative degradation of olefinically-unsaturated rubber<sup>4</sup>. Generally, the solubility of Cu-oleate in natural rubber latex is higher than that of CuO, therefore, catalytic action of copper mainly arises from Cu-oleate. According to the previous reports<sup>5–7</sup>, the copper content of 0.27 mg/l, 1.65 mg/l, and 1.34–2.26 mg/kg (on total solids content, TSC) were found in field NR latex.

Copper contamination can cause discoloration of rubber products during ageing<sup>4</sup>. Only 0.001% of Cu(II) and Mn(II) content was found to act as catalysts for the autoxidation of raw rubber and vulcanized rubber<sup>3</sup>. Consequently, these metal ions can be called 'rubber poisons' and the autoxidation effects on both natural and synthetic rubber. Furthermore, darken spots on rubber glove can be observed if high amount of copper contaminates in the container of concentrated latex during dipping process<sup>3</sup>. According to the Thai Industrial Standards (TIS), copper concentrated content in latex must not exceed 8 mg/kg (on TSC)<sup>8</sup>.

The way trace copper content (mg/kg) in NR latex affects on rubber degradation necessitates the use of a sensitive and accurate method for determination copper in real samples. Various techniques are employed for copper determination<sup>9</sup> in NR latex samples. The most inexpensive and simple method is visible spectrophotometry<sup>10</sup>. Alternative several techniques such as graphite furnace atomic absorption spectrophotometry<sup>11</sup>, and inductively coupled plasma optical emission spectrophotometry (ICP-

OES)<sup>6</sup> can be used for the copper determination in NR latex samples. In this study, the simple digestion, complexation and copper analysis by means of visible spectrometric method which industry can perform in their own lab is developed for copper determination in natural rubber latex. The developed digestion system was compared with Kjeldahl system for both visible spectrophotometry and ICP-OES detection.

# MATERIALS AND METHODS

#### Chemicals

All chemicals used throughout the analysis were of highest purity as commercially available. Cu(II) stock solution (100 mg/l) was prepared by dissolving 0.3930 g of  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$  (Carlo Erba, USA) in DI water. Three millilitres of concentrated  $\text{H}_2\text{SO}_4$  were added in the beaker. The resulting solution was transferred to a 1000-ml volumetric flask and the volume was adjusted to the mark with DI water. Standard working solution of Cu(II) was prepared by properly diluting 100 mg/l standard Cu(II) with DI water just before use. The complexing agent was prepared by dissolving 0.2000 g zinc diethyldithiocarbamate (Zn(II)-DDTC) in 1000 ml of dichloromethane.

#### Apparatus

Spectronic 21 (Italy, wavelength 380–800 nm) was used for the 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 and UV-Vis Spectrophotometer with Diode Array Detector (Hewlett-Packard 8453) for scanning absorption spectra in interference study.

ICP-OES model Optima 4300 DV (Perkin Elmer, USA) was used for copper content determination in concentrated NR latex sample.

# General digestion procedure

The simple wet digestion system consists of digestion tube (Pyrex;  $3 \times 25$  cm) for accurately weighed NR latex sample which was placed in paraffin liquid oil bath (Boiling point: 300-500 °C). The sample was heated for digestion on hotplate connected with thermostat for constant digestion temperature. Glass bead was added in the digestion tube during sample digestion to avoid bumping of the solution. The developed system for latex digestion is depicted in Fig. 1.



Fig. 1 Digestion system for NR latex sample.

#### Procedure of copper determination

The procedure for copper content determination was modified from ISO 8053 (1995) method<sup>12</sup>. Latex samples were digested by wet digestion before determining copper content. Five millilitres 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<sub>4</sub>OH and then adding drop by drop until the solution was just basic (pH 8) with litmus paper as an indicator. After that, the solution was left to cool and transferred into the separatory funnel. Two millilitres of 28% (w/v) ammonium hydroxide was used for rinsing the beaker. A portion of 25.00 ml of 0.2 g/l Zn(II)-DDTC in dichloromethane was added in the separatory funnel to form complex with copper in sample or 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 anhydrous Na<sub>2</sub>SO<sub>4</sub> was added to remove water. The organic layer was then filtered through the Whatman filter paper No. 42 and its absorbance was measured at 435 nm.

# Total solids content

The total solids content (TSC) of latex is defined as the fraction by mass of the whole which is nonvolatile under specified conditions of drying in an open atmosphere at an elevated temperature. Normally, TSC is calculated as (C-A)/(B-A), where *A* is weight of aluminium cup, *B* is weight of aluminium cup with latex sample before heating, *C* is weight of aluminium cup with latex sample after heating<sup>4</sup>.

For TSC determination, first, the aluminium cup as a container was weighed. Next, 2.0 g of field or concentrated NR latex sample was added and weighed again. One millilitre of DI water was then added and the latex was spread out over the aluminium cup surface by swirling. Field or concentrated NR latex in the aluminium cup was heated at  $70\pm2$  °C in the oven for 16 h and then the sample was left to cool in a desiccator. The aluminium cup with latex sample was weighed and heated again for 15 min and weighed again after cooling in desiccator. This step was repeated until constant weight was obtained. Five replicates were performed for each sample (ISO 124:1997)<sup>13</sup>.

# Dry rubber content

Dry rubber content (DRC) is defined as the fraction by mass of the latex which is coagulated under specified conditions of colloidal destabilization<sup>4</sup>. It can be determined by weighing 10.0 g of NR latex sample into a beaker and adding DI water into the beaker to obtain  $(20 \pm 1)\%$  by weight. The latex sample was coagulated by adding  $(35 \pm 5)$  ml of 2% v/v acetic acid and then the coagulated latex sample was placed in the water bath at 70 °C for 15–30 min. The coagulated latex sample was taken from the beaker and rinsed with water several times until the rinsed water was no longer acidic (indicated by litmus). The coagulated latex sample was pressed as a sheet to the nearest thickness 2 mm and rinsed with tap water for at least 5 min. Then it was heated at  $(70 \pm 5)$  °C until it had no white patches. The dry sheet was left to cool in desiccator for 30 min and finally weighed. Five replicates were performed at each sample (ISO 126:2005)<sup>14</sup>. DRC was calculated as  $m_1/m_0$ , where  $m_0$  is the mass of the test portion and  $m_1$  is the mass of dry sheet.

# **RESULTS AND DISCUSSION**

# **Optimization of extraction**

Types of solvent for Cu(II) extraction is one of important factors for the proposed method which can be selected by distribution coefficient ( $K_d$ ) of copper with solvent, commercial availability, toxicity and dangers. Efficiency of solvents, i.e., chloroform (CHCl<sub>3</sub>), carbon tetrachloride (CCl<sub>4</sub>), 1,1,1trichloroethane (CH<sub>3</sub>CCl<sub>3</sub>), and dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) in copper extraction showed no significant differences<sup>15</sup>. Dichloromethane (DCM) was chosen due to its commercial availability and the fact that the other solvents are restricted chemicals and 1,1,1-trichloroethane is a hazardous chemical which is no longer produced.

# Absorption spectra of Cu(II) complex

The absorption spectra of yellow Cu(II)-DDTC complex (2.0 and 4.0 mg/l of standard Cu(II) solu-

**Fig. 2** The yellow copper complex spectra against blank at pH 8. Blank: 200 mg/l Zn(II)-DDTC in DCM; 2.0 mg/l Cu(II) + 200 mg/l Zn(II)-DDTC in DCM; and 4.0 mg/l Cu(II) + 200 mg/l Zn(II)-DDTC in DCM.

tion) against reagent blank (200 mg/l Zn(II)-DDTC in DCM) over the wavelength range 300–800 nm showed maximum absorbance value  $\lambda_{max} = 435$  nm (Fig. 2). The molar extinction coefficient of  $1.37 \times 10^4 \, \mathrm{l \ mol^{-1} cm^{-1}}$  was similar to the molar absorptivity ( $\varepsilon = 1.4 \times 10^4 \, \mathrm{l \ mol^{-1} cm^{-1}}$ ) of copper complex when sodium diethyldithiocarbamate (NaDDTC)<sup>1</sup> was used as complexing agent.

#### Suitable Zn(II)-DDTC concentration

Cu(II) reacts with Zn(II)-DDTC to yield Cu(II)-DDTC complex. The stability constant for Cu(II)-DDTC is much greater than Zn(II)-DDTC. The logarithm of the stability constant (log  $K_{\rm ML}$ ) of Cu(II)-DDTC and Zn(II)-DDTC was 28.8 and 11.4, respectively<sup>16</sup>. To achieve the highest sensitivity, concentration of Zn(II)-DDTC in the range of 50– 200 mg/l was optimized. The results in Table 1 revealed that the absorbance slightly increased with the increasing of Zn(II)-DDTC concentration and the absorbance of copper complex at 200 mg/l of Zn(II)-DDTC was slightly higher than at other concentrations. However, at higher concentration of Zn(II)-DDTC more than 200 mg/l DCM did not

**Table 1** Determination of 2.0 mg/l Cu(II) with various concentrations of 25 ml of Zn(II)-DDTC in DCM (n = 3).

| Absorbance $\pm$ SD |
|---------------------|
| $0.320 \pm 0.000$   |
| $0.343 \pm 0.015$   |
| $0.383 \pm 0.015$   |
| $0.420 \pm 0.000$   |
|                     |

 $76 \pm 1^{c}$ 

| Temperature | Cu(I  | I) (mg/l)       | Recovery*      | Digestion |  |
|-------------|-------|-----------------|----------------|-----------|--|
| (°C)        | Added | Measured        | (%)            | (h)       |  |
| 130         | 0.0   | $0.07 \pm 0.01$ | _              | 4.0       |  |
| 130         | 2.0   | $1.70\pm0.00$   | $81\pm0^{a}$   | 4.0       |  |
| 160         | 0.0   | $0.13\pm0.00$   | _              | 3.5       |  |
| 160         | 2.0   | $2.01\pm0.02$   | $94 \pm 1^{b}$ | 3.5       |  |
| 180         | 0.0   | $0.13\pm0.00$   | _              | 2.5       |  |
| 180         | 2.0   | $2.01 \pm 0.02$ | $94 \pm 1^{b}$ | 2.5       |  |

**Table 2** Recovery and digestion time of NR latex digestion at different temperatures (n = 3).

Values followed by the same letter are not significantly different (p < 0.05) according to the one-way ANOVA.

completely dissolve. Consequently, 200 mg/l of Zn(II)-DDTC was chosen for copper determination in NR latex sample.

# Number of extraction

To obtain copper content in extracts as much as possible, the number of the extraction was experimented under optimum conditions. The influence of extraction number at 1, 2, and 3 times on the amount of copper was evaluated by the absorbance of 2.0 mg/l Cu(II). No significant difference in signal value between the numbers of extraction was found (data not shown). Hence single extraction was found enough for copper extraction to save both extraction time and complexing agent volume.

# Optimization of latex digestion

The investigation of the effect of acid type on NR latex digestion efficiency revealed that a single acid, either concentrated  $HNO_3$  or concentrated  $H_2SO_4$ , showed incomplete digestion and longer than 5 h digestion time. For a mixture of concentrated  $HNO_3$  and concentrated  $H_2SO_4$ , 4 ml of  $H_2SO_4$  and approximately total volume up to 16 ml of concentrated  $HNO_3$  must be added to obtain complete digestion and clear solution within 2.5 h, and this condition was chosen for further experiments.

# **Digestion temperature**

The influence of the digestion temperature at 130, 160, and 180 °C on recovery (%) of copper determination was investigated. Approximately 1 g NR latex sample was weighed and digested with the mixture of concentrated  $H_2SO_4$  and concentrated  $HNO_3$ . It was found that high temperature of at least 160 °C was required to obtain good recovery (%) of copper as shown in Table 2 as well as short digestion time. However, to ensure the completeness of

Weight of NR latex Cu(II) (mg/l) Recovery\* (%) (g) Added Measured 1.0 0.0  $0.54 \pm 0.02$ 1.0 2.0  $2.38 \pm 0.02$  $92 \pm 1^{a}$ 1.5 0.0  $0.73 \pm 0.05$ 1.5 2.0  $2.39 \pm 0.02$  $84 \pm 1^{b}$ 

**Table 3** Determination of copper with different weights of latex sample by the developed method (n = 3).

<sup>\*</sup> Values followed by the different letter are significantly different (p < 0.05) according to the one-way ANOVA.

 $0.90 \pm 0.03$ 

 $2.42 \pm 0.01$ 

digestion, temperature of 180 °C was selected.

0.0

2.0

# Amount of NR latex sample

2.0

2.0

The amount 2–10 g of NR latex is normally required by ISO 8053 (1995) method <sup>12</sup>. In this study, the appropriate amount of field NR latex sample which can be digested efficiently with the developed digestion system was investigated in the range of 1.0-2.0 g. The 2.0 mg/l Cu(II) was spiked into the sample to evaluate the recovery. As shown in Table 3, the best recovery was obtained with the sample weight of 1.0 g at 92%. Greater quantity resulted in violent reaction and suspended sample and cause inefficient digestion and unsatisfactory recovery at less than 76%. Hence sample weight of 1 g was used to determine copper content by the proposed method.

#### **Analytical Performance**

Linearity was found to be in the concentrations range of 0.1–4.0 mg/l Cu(II) (Fig. 3). The mean linear regression equation for 15 replicated calibration curves was  $y = (0.2021\pm0.0022)x + (0.0145\pm0.0024)$  with a good correlation coefficient ( $R^2 =$ 



Fig. 3 The linear range of Cu(II) determination by developed method.

| Sample            | ample Field latex |         | Concentrated latex |                 |         |              |
|-------------------|-------------------|---------|--------------------|-----------------|---------|--------------|
|                   | Cu (mg/l)         | RSD (%) | Recovery (%)       | Cu (mg/l)       | RSD (%) | Recovery (%) |
| Sample            | $0.25 \pm 0.01$   | 3.2     | _                  | $0.17 \pm 0.00$ | 2.4     | _            |
| Sample + 1.0 mg/l | $1.17 \pm 0.01$   | 0.9     | $92 \pm 1$         | $1.11 \pm 0.01$ | 1.0     | $94 \pm 1$   |
| Sample + 2.0 mg/l | $2.26 \pm 0.01$   | 0.4     | $101\pm0$          | $2.18 \pm 0.01$ | 0.6     | $101 \pm 1$  |

**Table 4** The intra-day accuracy and precision of copper determination by the proposed method (n = 10).

**Table 5** The inter-day accuracy and precision of copper determination by the proposed method (n = 10).

| Sample Field latex |                 | Concentrated latex |              |                 |         |              |
|--------------------|-----------------|--------------------|--------------|-----------------|---------|--------------|
|                    | Cu (mg/l)       | RSD (%)            | Recovery (%) | Cu (mg/l)       | RSD (%) | Recovery (%) |
| Sample             | $0.25 \pm 0.01$ | 1.2                | _            | $0.17 \pm 0.00$ | 2.4     | _            |
| Sample + 1.0 mg/l  | $1.17\pm0.01$   | 1.0                | $92 \pm 1$   | $1.12\pm0.01$   | 1.7     | $95 \pm 1$   |
| Sample + 2.0 mg/l  | $2.26\pm0.02$   | 0.8                | $100 \pm 1$  | $2.20 \pm 0.02$ | 2.0     | $101\pm0$    |

 $0.9962 \pm 0.0013$ ) and relative standard deviation (RSD) lower than 5%. ANOVA of the slopes, intercepts and correlation coefficients of the 15 standard plots indicated no significant differences and the coefficient of variation for the slopes was 1.1%.

# Limit of detection and limit of quantification

The limit of detection and limit of quantification calculated from the calibration curve based on  $3 S_{y/x}/b$ and  $10 S_{y/x}/b$ , where  $S_{y/x}$  is the standard deviation on the regression and *b* is the slope, was found to be 0.0356 and 0.1188 mg/l, respectively.

# Accuracy and precision

Recovery study was performed to evaluate the accuracy of the method by spiking 1.0 and 2.0 mg/lCu(II) into the field and concentrated latex samples. Ten portions of spiked and non-spiked samples were analysed. Recoveries of spiked (1.0 and 2.0 mg/l Cu(II) standard solution) field and concentrated latex samples were found to be in the range of 92-101% with good precision (RSD < 5%) as shown in Tables 4 and 5. The intra-day precision was carried out during the same experimental conditions by examining ten replicates of sample spiked with Cu(II) at different concentrations (1.0 and 2.0 mg/l) within the same day. The inter-day precision was verified by examining the sample for 6 different days within 2 weeks with 10 replicates measurements each day. The repeatability or intra-day and reproducibility or inter-day precision of the method were found to be 0.4-3.2% and 0.8-2.4%, respectively, revealing good repeatability and reproducibility with RSD < 5%. Furthermore, the method demonstrated satisfactory accuracy with recovery in the range of 92–101%.

# Interferences

The effect of other transition cations including Fe(III), Mn(II), and Zn(II) normally present in NR latex<sup>6,17</sup> on copper determination was studied by adding various concentrations of interfering ions into 2.0 mg/l Cu(II) standard solution. The tolerance limit defined as the maximum concentration of interfering ion added to cause a change in the absorbance not greater than  $\pm 5\%$  (deviation  $\leq 5\%$ )<sup>18</sup> was then evaluated. It was found that high concentration of Zn(II) up to 500 mg/l had no significant effect on 2.0 mg/l of Cu(II) while the concentration of Fe(III) and Mn(II) at only  $\ge$  9.0 mg/l interfere seriously. However, the concentrations of those ions in the NR latex sample were found to be below interfering level of 3.52, 0.77, and 27.0 mg/l for Fe(III), Mn(II), and Zn(II), respectively<sup>6</sup>. In case of high content of interfering ions such as Fe(III) and Mn(II) in NR latex, ammonium citrate or tartrate can be added as a masking agent<sup>2</sup>. The method was therefore selective for copper determination in the NR latex sample.

# Applications

Copper in NR latex sample can be determined by digesting the NR latex with the optimum conditions and then analysing by the developed method. The standard method (ICP-OES)<sup>19</sup> was applied to confirm the accuracy of the developed method. With the conditions previously described, the calibration curve was constructed to obtain the linearity in the range of 0.005–0.500 mg/l with the linear regression equation to calculate the copper content in latex samples. The digestion system was also compared with the Kjeldahl method and the results are shown in Table 6.

**Table 6** Copper contents (mg/kg) in 5 concentrated latex samples by vis-spectrophotometry and ICP-OES (n = 3).

| Sample <sup>†</sup> | Vis-spectrop  | ohotometry    | ICP-C         | DES           |
|---------------------|---------------|---------------|---------------|---------------|
|                     | Developed     | Kjeldahl      | Developed     | Kjeldahl      |
| 1                   | $6.8 \pm 0.0$ | $7.0 \pm 0.2$ | $6.8 \pm 0.1$ | $6.7 \pm 0.1$ |
| 2                   | $7.0 \pm 0.2$ | $6.9 \pm 0.2$ | $6.4 \pm 0.1$ | $6.5\pm0.2$   |
| 3                   | $6.9 \pm 0.2$ | $6.9 \pm 0.1$ | $6.8 \pm 0.1$ | $7.0\pm0.0$   |
| 4                   | $6.9 \pm 0.2$ | $6.9 \pm 0.1$ | $6.8 \pm 0.1$ | $6.7 \pm 0.1$ |
| 5                   | $6.8 \pm 0.2$ | $6.5\pm0.1$   | $6.5\pm0.1$   | $6.5\pm0.1$   |

<sup>†</sup> The concentrated latex prepared from field NR latex purchased from latex traders in Na Mom, Hatyai, Muang Songkhla, Bang Klam, and Na Thawi District, respectively.

# **Properties of latex**

Certain properties of field and concentrated NR latex including total solids content (TSC) and dry rubber content (DRC) were determined as follows to supplement copper content report in field and concentrated NR latex.

Normally, copper content is reported in mg/kg unit based on TSC. It was found that TSC in 5 field and concentrated NR latex samples from 5 districts in Songkhla Province as shown in Table 7 were in the range of 28.8–38.4% and 62.6–63.5%, respectively, corresponding with general TSC of field and concentrated NR latex samples in the range of 25–45% and minimum of 61%, respectively. All TSC of all latex samples were therefore in the acceptable value recommended by TIS 980<sup>8</sup>.

Dry rubber content (%) is used to indicate the quantity in gram of rubber in 100 g of latex as shown in Table 7 and was found in the range of 26.8–34.6 and 60.3–61.4%, respectively, in good agreement with normal DRC of field and concentrated latex sample 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<sup>8</sup>.

The copper content in concentrated latex sample prepared from the same batch of field NR latex was digested by means of the developed digestion system and analysed method. Copper concentrations in concentrated latex in the range of 6.8– 7.0 mg/kg (on TSC) were found. Copper contents in these concentrated latex samples were lower than TIS (980–2009) guideline level (8 mg/kg on TSC). To confirm the accuracy of the method, the copper contents were compared with the standard method (ICP-OES)<sup>6</sup>. No significant difference was observed

(at 95% confident limit, ANOVA). The sensitivity of the developed spectrophotometric method was also found in the same range with that of ICP-OES. Furthermore, the sample digestion system with the developed digested method and Kjeldahl digestion system were conducted and copper content in the sample solutions obtained by both systems were compared. The results showed no significant difference. 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 (on TSC) or 6.1–7.4 mg/l which were higher than those obtained from previous reports<sup>5,6</sup> of 0.27 mg/l and 1.65 mg/l.

In this study, copper contents in field and concentrated NR latex sample were also compared in order to investigate the effect of added diammonium hydrogen phosphate (DAHP) and centrifugation effect on the remaining copper content. It was observed that copper content in concentrated NR latex was found in the range 6.8–7.0 mg/kg (on TSC) which was around 60–70% lower than in field NR latex. It can be assumed that adding of DAHP into the field NR latex before centrifugation process, other divalent transition metals such as Fe<sup>2+</sup>, Mn<sup>2+</sup>, Zn<sup>2+</sup> can be precipitated.

# Effect of rubber growing regions and tapping seasons on copper content of field latex

The copper contents in different growing regions and tapping seasons were investigated. The rubber (Hevea brasiliensis) plantations in Buriram, Chachoengsao, and Songkhla provinces were selected to be the study areas representing Northeastern, Eastern, and Southern Thailand, respectively. Field NR latex samples were collected in both dry and rainy seasons. In sample from three different parts of Thailand, the concentrations of copper in field latex samples were found in the range of 12.2-19.3 mg/kg and 13.5–16.9 mg/kg in dry and rainy season, respectively (as shown in Table 8). There was no significant difference of copper concentration in field latex between dry and rainy season from each region of Thailand, except in sample from Buriram for which latex in dry season contained 57% higher significantly than that of rainy season (p < 0.05). In rainy season, there was no significant difference of copper concentration in field latex among rubber tree from three different regions of Thailand.

In conclusion, the simple and effective di-

# ScienceAsia 43 (2017)

| Sample |                | Field latex    |                |               | Concentrated latex |                |  |
|--------|----------------|----------------|----------------|---------------|--------------------|----------------|--|
|        | Cu (mg/kg)     | TSC (%)        | DRC (%)        | Cu (mg/kg)    | TSC (%)            | DRC (%)        |  |
| 1      | $16.6 \pm 1.0$ | $38.4 \pm 0.2$ | $34.6 \pm 0.8$ | $6.8 \pm 0.0$ | $62.6 \pm 0.3$     | $60.3 \pm 0.1$ |  |
| 2      | $21.7 \pm 1.0$ | $28.8 \pm 0.2$ | $26.8 \pm 0.2$ | $7.0 \pm 0.2$ | $62.9 \pm 0.3$     | $60.5 \pm 0.6$ |  |
| 3      | $20.7 \pm 2.0$ | $34.2 \pm 0.8$ | $31.3 \pm 1.1$ | $6.9 \pm 0.2$ | $63.5 \pm 0.2$     | $61.4 \pm 0.4$ |  |
| 4      | $23.5 \pm 0.0$ | $31.0 \pm 0.3$ | $31.4 \pm 0.1$ | $6.9 \pm 0.2$ | $62.9 \pm 0.1$     | $61.4 \pm 0.8$ |  |
| 5      | $23.8 \pm 1.0$ | $32.1 \pm 0.1$ | $30.7 \pm 0.4$ | $6.8 \pm 0.2$ | $63.4 \pm 0.3$     | $63.0 \pm 0.3$ |  |

**Table 7** Copper contents, TSC, and DRC in field and concentrated NR latex samples by vis-spectrophotometry (n = 3).

 Table 8 Copper contents (mg/kg) in field latex samples by vis-spectrophotometry.

|              | Duradian     | 0                   | (              |  |
|--------------|--------------|---------------------|----------------|--|
| Region       | Province     | Cu contents (mg/kg) |                |  |
|              |              | Dry season          | Rainy season   |  |
| Northeastern | Buriram      | $19.3 \pm 3.4$      | $16.9 \pm 6.4$ |  |
| Eastern      | Chachoengsao | $12.3 \pm 1.8$      | $15.8 \pm 3.8$ |  |
| Southern     | Songkhla     | $12.2\pm2.5$        | $13.5\pm2.5$   |  |

gestion system for copper determination by visspectrophotometry was developed with the advantages of low cost and speed of sample digestion in compared with Kjeldahl digestion system utilized by ISO 8053<sup>12</sup>. The Pyrex digestion tube is also cheaper than Kjeldahl apparatus. Furthermore, the sample digestion time by the developed digestion system was shorter (2.5 h) than that by the Kjeldahl digestion system (5.0 h). The method offers a linear working range of 40-fold, good precision and accuracy. Hence this developed method can be an alternative method for copper determination in natural rubber latex industries.

Acknowledgements: The authors are grateful to the Centre of Excellence for Innovation in Chemistry; Postgraduate Education and Research Programme in Chemistry (PERCH-CIC), Office of the Higher Education Commission, Ministry of Education; the Graduate School as well as the Department of Chemistry, Faculty of Science, of Prince of Songkla University for the scholarship and research support.

# REFERENCES

- 1. van Staden JF, Botha A (1999) Spectrophotometric determination of Cu(II) with sequential injection analysis. *Talanta* **49**, 1099–108.
- Jankiewicz B, Ptaszynski B, Turek A (1999) Spectrophotometric determination of copper(II) in samples of soil from selected allotment gardens in Lodz. *Pol J Environ Stud* 8, 35–8.
- 3. Kajornchaiyakul V (2009) Natural Rubber: Produc-

tion and Applications, The Thailand Research Fund (TRF), Bangkok, pp 81–5, [in Thai].

- Blackley DC (1997) Polymer Latices: Science and Technology, Vol 2: Types of Latices, 2nd edn, Chapman and Hall, London, United Kingdom.
- Kang H, Kang MY, Han KH (2000) Identification of natural rubber and characterization of rubber biosynthetic activity in fig tree. *Plant Physiol* 123, 1133–42.
- Scott DJ, da Costa BM, Espy SC, Keasling JD, Cornish K (2003) Activation and inhibition of rubber transferases by metal cofactors and pyrophosphate substrates. *Phytochemistry* 64, 123–34.
- Ng SK, Lai PT (1972) Simultaneous determination of copper, manganese, iron, and calcium in natural rubber by emission spectrography. *Appl Spectros* 26, 369–72.
- 8. Thai Industrial Standards (2009) Natural Rubber Latex Concentrate (TIS 980-2009).
- Yang S, Fang X, Duan L, Yang S, Lei Z, Wen X (2015) Comparison of ultrasound-assisted cloud point extraction and ultrasound-assisted dispersive liquid liquid microextraction for copper coupled with spectrophotometric determination. *Spectrochim Acta A* 148, 72–7.
- Shishehborea MR, Nasirizadeha NH, Shabanib AM, Tabatabaeea M (2005) Spectrophotometric determination of copper with 1,5-diphenylcarbazone (DPC) on microcrystalline naphthalene of various samples. *Can J Anal Sci Spectros* 50, 130–4.
- 11. ASTM International (2006) Standard Test Methods for Rubber—Determination of Metal Content by Flame Atomic Absorption (AAS) Analysis (ASTM D4004-06), ASTM International, West Conshohocken, PA.
- ISO International Organization for Standardization (1995) Rubber and Latex—Determination of Copper Content—Photometric Method (ISO 8053).
- ISO International Organization for Standardization (2008) Latex, Rubber—Determination of Total Solids Content (ISO 124).
- 14. ISO International Organization for Standardization (2005) Natural Rubber Latex Concentrate—Determination of Dry Rubber Content (ISO 126).
- 15. Rathore HS, Kumar M, Ishratullah K (2006) Metal ion chromatography on sodium diethyldithiocarbamate. *Indian J Chem Tech* **13**, 84–7.

ScienceAsia 43 (2017)

- 16. Yang Y, Zuo B, Li J, Chen G (1996) Studies on the stability of four-membered ring chelates. Part V. The stability of dialkyl dithiocarbamate chelates. *Spectrochim Acta A* **52**, 1915–9.
- 17. Kang H, Kim SY, Chung GC (2000) Characterization of natural rubber biosynthesis in *Ficus benghalensis*. *Plant Physiol Biochem* **38**, 979–87.
- 18. Khlyntseva SV, Vishnikin AB, Al-Shwaiyat MKEA, Sklenarova H, Solich P, Bazel YR, Andruch V (2011) Sequential injection determination of orthophosphate as ion associate of 12-molybdophosphate with Astra Phloxine. *Talanta* **84**, 1355–60.
- 19. ISO International Organization for Standardization (2015) Rubber, Raw, Vulcanised—Determination of Metal Content by ICP-OES (ISO/DIS 19050).