CHAPTER III

RESEARCH METHODOLOGY

This chapter describes the detail of methodology on the digestion of natural (NR) latex by using a thermoreactor and UV digestion unit before the determination of total phosphorus residue. This chapter covers the experimental details in the order of following;

1. Application of thermoreactor to NR latex
   1.1 Study the types of oxidizing agent
   1.2 Study the effect of oxidizing agent on phosphorus molybdenum blue complex spectra
   1.3 Study the effect of concentration of oxidizing agent
   1.4 Study the effect of digestion temperature
   1.5 Study the effect of digestion time
   1.6 Study the effect of reaction time for color development
2. Application of UV digestion to NR latex
   2.1 Fabrication of UV digestion unit
   2.2 Study the effect of digestion tube position
   2.3 Study the effect of oxidizing agent on phosphorus molybdenum blue complex spectra
   2.4 Study the effect of concentration of oxidizing agent
   2.5 Study the effect of digestion time
   2.6 Study the effect of reaction time for color development
3. Analytical performance for the determination of total phosphorus in NR latex using thermoreactor and UV digestion unit
   3.1 Study the linearity range
   3.2 Study the recoveries for the determination of total phosphorus in NR latex sample
   3.3 Study the effect of interfering ions on recoveries of total phosphorus
   3.4 Study the analytical accuracy and precision
4. Determination of total phosphorus in NR latex samples

Instrument and apparatus

1. Double beam UV-Vis spectrophotometer, Lambda 20 Perkin Elmer, U.S.A.
2. Digital image-based colorimeter, Laboratory-made, (In the process of for petty patent submission).
3. CMOS webcam camera, Microsoft HD-5000, China.
4. Quartz cuvette, 10 mm Hellma, U.S.A.
5. AA dry cell rechargeable batteries, Panasonic HHR-3LVT, China.
6. 10 kΩ variable resistors, Thailand.
7. White high intensity light-emitting diode (LEDs), Thailand.
8. Laptop, Lenovo G640, Lenovo, China.
9. Digital lux meter, LX-802, Nicety, China.
10. Thermoreactor, VELP – ECO 16 COD Thermoreactor, Italy.
11. Hotplate stirrer, Hamony LMS-1003, Japan.
12. Analytical balance (4 digits), Sartorius, Germany.
13. UV lamp 300W, Osram, Slovakia.
14. Digital thermometer, Newlite, China.
15. Deionized water equipment, Elga, England.
16. Borosilicate glass test tube (14 mm ID), Macherey-Nagel, Germany.

Chemicals

1. Potassium dihydrogen orthophosphate, KH_2PO_4 (136.09 g mol^{-1}), AR grade, Rankem, India.
2. Ammonium peroxodisulphate, $(NH_4)_2S_2O_8$ (228.19 g mol^{-1}), AR grade, Loba Chemie, India.
3. Potassium peroxodisulphate, $K_2S_2O_8$ (270.32 g mol^{-1}), AR grade, Ajax Finechem, Australia.
4. Potassium antimonyl tartrate, $K(SbO)C_4H_4O_6 \cdot 1/2H_2O$ (667.87 g mol^{-1}), AR grade, Riedel-de Haen, Germany.
5. Ammonium molybdate, $(NH_4)_6Mo_7O_{24}$ (1235.86 g mol^{-1}), AR grade, Ajax Finechem, Australia.
6. Ascorbic acid, $C_6H_8O_6$ (176.13 g mol^{-1}), AR grade, Rankem, India.

7. Potassium chromate, K_2CrO_4 (194.20 g mol^{-1}), AR grade, Merck, Germany.

8. Sodium sulphide, $Na_2S{\cdot}9H_2O$ (240.18 g mol^{-1}), Carlo ERBA, Italy.

9. Potassium nitrite, KNO_2 (85.10 g mol^{-1}), Ajax Finechem, Australia.

10. Sodium arsenate hydrated, $Na_2HAsO_4{\cdot}7H_2O$ (312.01 g mol^{-1}), BDH Chemicals Ltd., England.

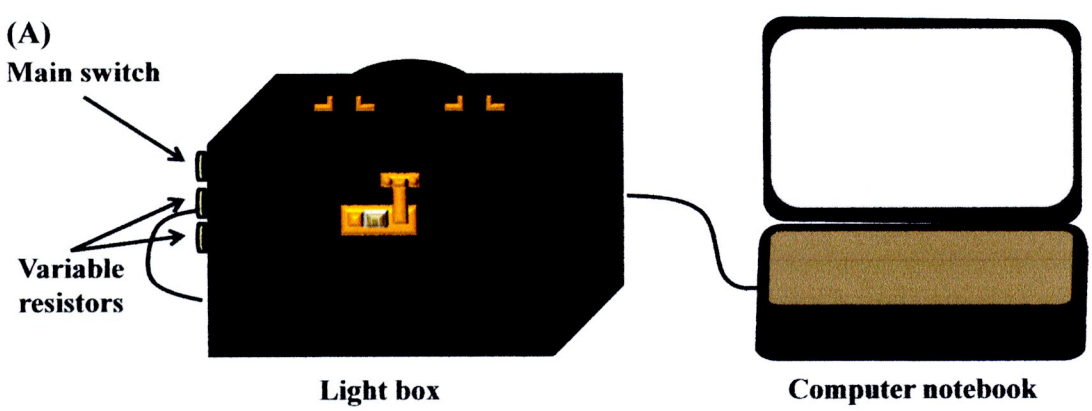
11. Sodium silicate, Na_2SiO_3 (122.07 g mol^{-1}, 1,350 g mL^{-1}), Panreac, Spain.

12. 96 % Sulfuric acid, H_2SO_4 (98.08 g mol^{-1}), AR grade, Carlo ERBA, Italy.

13. 37% Hydrochloric acid, HCl (36.46 g mol^{-1}), AR grade, RCl Labscan Limited, Thailand.

Digital image-based colorimeter-artificial neuron network (DIC-ANN) [70]

A schematic diagram of the system assembled for digital images acquisition is illustrated in Figure 19. A light box was made of a 30 × 20 × 20 cm (width × height × depth) black cardboard to protect the system from outside light. The CMOS webcam camera connected with computer notebook was secured on the center floor of the box and in front of the sample cell which was located in a sample cell holder made by a white plastic. Two white high intensity LEDs were placed at the camera backside to give constant light intensity throughout the experiment. The LEDs driver were powered by a 4.5 V DC from 3 AA dry cell rechargeable batteries and the illumination was adjusted by 10 kΩ variable resistors.



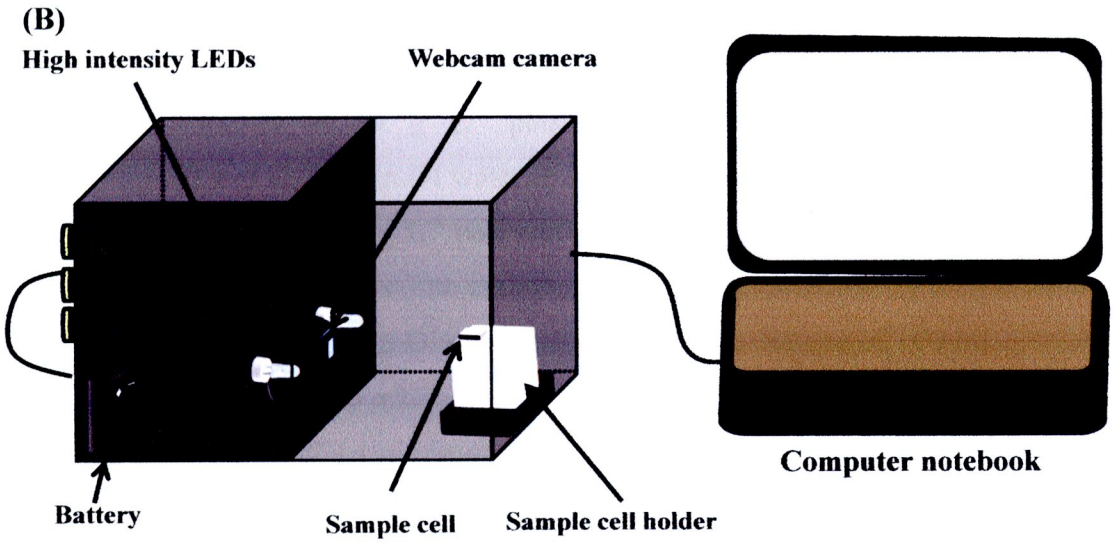


Figure 19 Schematic diagram of a DIC system (A) outside view and (B) inside view [70]

The phosphorus standard solution and sample solution were photographed and stored as "JPEG" compressed files and 1280 pixels x 720 pixels of dimension by software written in Visual Basic version 6.0 [70]. All images were processed with the software by random learning for 3,000 times in each image to obtain the mean of red, green and blue (RGB) value. Then, the RGB value (each color representing different intervals from 0 to 255) was inputted into database. Finally, the software will predict the total phosphorus concentration in NR latex by comparing the RGB value of the sample image with as close as possible of the RGB value of phosphorus standard solution image in the database.

Preparation of standard solutions and reagents

1. **Standard solution of phosphorus**

A stock of 1,000 mg L^{-1} standard phosphorus solution was prepared by dissolving 0.4390 g of KH_2PO_4 in 50 mL of de-ionized water (DI water). Then, final volume of 100 mL was adjusted with DI water at room temperature. Store the standard phosphorus solution at 4 °C. This standard solution is stable for 1 month.

2. **Solution of 30 g L^{-1} ammonium peroxodisulphate**

Solution of 30 g L^{-1} ammonium peroxodisulphate was prepared by dissolving 3.0 g of $(NH_4)_2S_2O_8$ in DI water and adjusted to a volume of 100 mL.

3. Solution of 50 g L^{-1} potassium peroxodisulphate

Solution of 50 g L^{-1} potassium peroxodisulphate was prepared by dissolving 5.0 g of $K_2S_2O_8$ in DI water and adjusted to a volume of 100 mL.

4. Solution of 2.7 g L^{-1} potassium antimonyl tartrate

Solution of potassium antimonyl tartrate was prepared by dissolving 0.27 g of $K(SbO)C_4H_4O_6 \cdot 1/2H_2O$ in DI water and adjusted to a volume of 100 mL.

5. Solution of 40 g L^{-1} ammonium molybdate

Solution of ammonium molybdate was prepared by dissolving 4.0 g of $(NH_4)6Mo_7O_{24} \cdot 4H_2O$ in DI water and adjusted to a volume of 100 mL.

6. Solution of 17.6 g L^{-1} ascorbic acid

Solution of ascorbic acid was prepared by dissolving 1.76 g of $C_6H_8O_6$ in DI water and adjusted to a volume of 100 mL. The ascorbic acid solution is stable for 1 week at 4°C.

7. Solution of 5 N sulfuric acid

Solution of 5 N sulfuric acid was prepared by diluting 35 mL of concentrated H_2SO_4 to a volume of 250 mL with DI water.

8. Solution of molybdenum blue [6]

Molybdenum blue solution was prepared by mixing 5 N sulfuric acid solution, potassium antimonyl tartrate solution, ammonium molybdate solution and ascorbic acid solution in the ratio of 10:1:3:6.

Preparation of interfering solutions

1. Stock solution of arsenate, AsO_4^{3-}

A stock of 1,000 mg L^{-1} arsenate solution was prepared by dissolving 0.1123 g of $Na_2HAsO_4 \cdot 7H_2O$ in 50 mL of DI water. Then, final volume of 100 mL was adjusted with DI water at room temperature. The pentavalent arsenic solution was stored at 4 °C. The solution is stable for 1 month.

2. Stock solution of sulphide, S^{2-}

A stock of 1,000 mg L^{-1} sulphide solution was prepared by dissolving 0.7490 g of $Na_2S \cdot 9H_2O$ in 50 mL of DI water. Then, final volume was adjusted to 100 mL with DI water at room temperature. The sulphide solution was stored at 4 °C. The solution is stable for 1 month.

3. Stock solution of nitrite, NO_2^-

A stock of 1,000 mg L^{-1} nitrite solution was prepared by dissolving 0.1850 g of KNO_2 in 50 mL of DI water. Then, final volume was adjusted to 100 mL with DI water at room temperature. The nitrite solution was stored at 4 °C. The solution is stable for 1 month.

4. Stock solution of chromium, Cr^{6+}

A stock of 1,000 mg L^{-1} hexavalent chromium solution was prepared by dissolving 0.3734 g of K_2CrO_4 in 50 mL of DI water. Then, final volume was adjusted to 100 mL with DI water at room temperature. The hexavalent chromium solution was stored at 4 °C. The solution is stable for 1 month.

5. Stock solution of silicate, SiO_3^{2-}

A stock of 1,000 mg L^{-1} silicate solution was prepared by diluting 0.12 mL of concentrated Na_2SiO_3 to a volume of 100 mL with DI water. The silicate solution was stored at 4 °C. The solution is stable for 1 month.

Preparation of NR latex

A 5.0 g of the NR latex sample was stirred to evaporate ammonia for 20 minutes. The sample was stored in polyethylene tube and was freshly prepared for daily use.

Application of thermoreactor to NR latex

Study the type of oxidizing agent

Ammonium peroxodisulphate and potassium peroxodisulphate were used in this study in order to compare the efficiency of both oxidizing agents for the decomposition of organic matrices in NR latex samples. A 0.03 g of NR latex was placed in five borosilicate glass test tubes. Each concentration of phosphorus standard solution (0, 1.0, 2.0, 3.0 and 4.0 mg L^{-1}) and 5 mL of 50 g L^{-1} oxidizing agent were added into the test tubes, respectively. The mixture solutions were digested at 100 °C for 60 minutes by using a thermoreactor. After that, the resultant solutions were cooled down to room temperature and diluted to 10 mL with DI water. A 1.6 mL of molybdenum blue solution was added. The resultant solutions were stood for 10 minutes and then determined by using UV-Vis spectrophotometry at 880 nm.

Study the effect of oxidizing agent on phosphorus molybdenum blue complex spectra

The common spectroscopic methods of identifying (or detecting) compound are the UV-Vis spectroscopy. This technique was used for spectra characterization of phosphorus in NR latex sample with molybdenum blue solution. After the digestion procedure, the absorbance of phosphorus standard solution in the range of 0.1 - 5.0 mg L^{-1} and NR latex sample were measured by UV-Vis spectrophotometer at the wavelength range from 400 - 900 nm.

Study the effect of concentration of oxidizing agent

The concentrations of ammonium peroxodisulphate as oxidizing agent were studied. A 0.03 g of NR latex was placed into eight borosilicate glass test tubes. Then a 5 mL of eight concentrations of oxidizing agent ranging from 10 to 80 g L^{-1} were added into the test tubes. The mixture solutions were digested in thermoreactor at 100 °C for 60 minutes. After that the resultant solutions (without filtration) were cooled down to room temperature and followed by adjusting to 10 mL with DI water. A 1.6 mL of molybdenum blue solution was added and then left it standing for 10 minutes. Finally, the absorbances of the resultant solutions were measured at the wavelength 880 of nm by UV-Vis spectrophotometer.

Study the effect of digestion temperature

The digestion temperature by thermoreactor was varied in the range of 60 to 150 °C. A 0.03 g of NR latex and 5 mL of 30 g L^{-1} ammonium peroxodisulphate solution were taken into ten borosilicate glass test tubes. The mixture solutions were digested by using a thermoreactor at different temperatures from 60 to 150 °C for 60 minutes. Then, the resultant solutions were cooled down to room temperature and adjusted to 10 mL with DI water. A 1.6 mL of molybdenum blue solution was added and left the reaction taken place for 10 minutes. Finally, the absorbances of the resultant solutions were measured by using UV-Vis spectrophotometer at the wavelength of 880 nm.

Study the effect of digestion time

The effect of digestion time was investigated in the range of 10 - 120 minutes. A 0.03 g of NR latex and 5 mL of 30 g L^{-1} ammonium peroxodisulphate solution were added into the twelve borosilicate glass test tubes. The mixture solutions

were digested at different times varied from 10 minutes to 120 minutes at 100 °C by using a thermoreactor. After that, the resultant solutions were cooled down to room temperature and diluted to 10 mL with DI water. A 1.6 mL of molybdenum blue solution was added and then left it standing for 10 minutes. Finally, the absorbances of the resultant solutions were measured at the wavelength of 880 nm by UV-Vis spectrophotometer.

Study the effect of reaction time for color development

The reaction time of phosphorus with molybdenum blue solution was varied between 5 to 60 minutes. The complexes of molybdenum blue solution with a 0.3 mg L^{-1} of phosphorus standard solution and NR latex sample after the digestion procedure (without and with 0.02 mg g^{-1} added phosphorus standard) were left it standing from 5 to 60 minutes. Then, the solutions were measured by UV-Vis spectrophotometer at the wavelength of 880 nm.

Application of UV digestion to NR latex

Fabrication of UV digestion unit

A schematic diagram of the system assembled for UV digestion unit acquisition is illustrated in Figure 20. A digestion unit was made of a 35 × 24 × 21 cm (width × height × depth) black cardboard in order to keep a temperature and UV light in the closed system. The UV lamp (300W, Osram, Slovakia) was secured on the wall of the box in front of the ten borosilicate glass test tubes (16 mm) which was located in the stainless steel rack. Digital thermometer was placed at the top of the box in order to monitor the digestion temperature throughout the experiment.

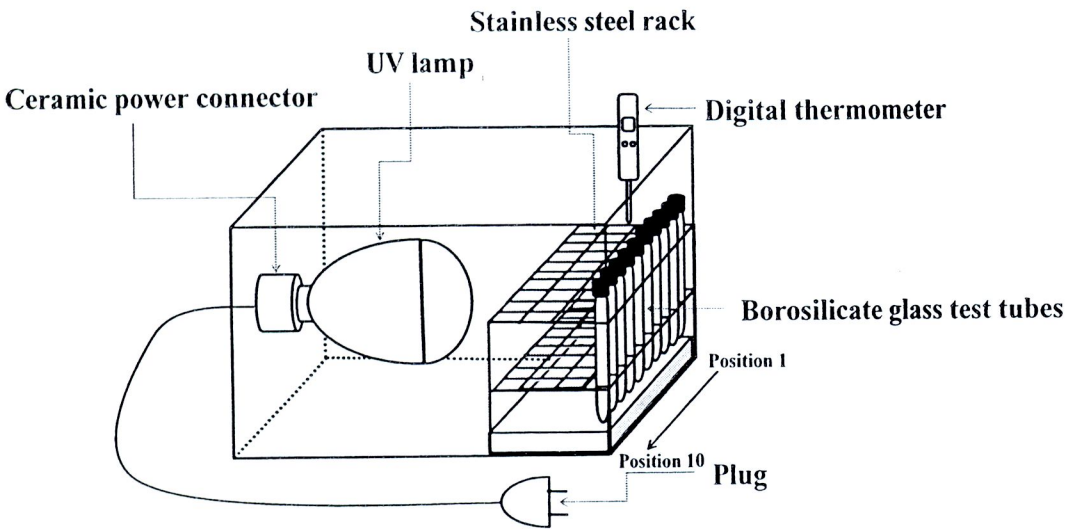


Figure 20 Schematic diagram of UV digestion unit

Study the effect of digestion tube position

The digestion tubes were experimented in 10 positions as shown in Figure 20. Under the same conditions, NR latex sample solutions and oxidizing agent in ten digestion tubes were set to the digestion unit. Then, the solutions were digested by UV radiation for 50 minutes. All resultant solutions were diluted to 10 mL with DI water and then mixed with 1.6 mL of molybdenum blue solution. The resultant solutions were left for 10 minutes for complete color development and then measured by using UV-Vis spectrophotometry at the wavelength of 880 nm.

Study the effect of oxidizing agent on phosphorus molybdenum blue complex spectra

UV-Vis spectroscopy was used for spectra characterization of phosphorus in NR latex sample with molybdenum blue solution. After NR latex digestion by UV radiation, the phosphorus standard solution at the concentration range of 0.1 - 5.0 mg L^{-1} and NR latex sample were measured by a UV-Vis spectrophotometer at the wavelength range from 400 - 900 nm.

Study the effect of concentration of oxidizing agent

The concentrations of ammonium peroxodisulphate as oxidizing agent were studied in the range of 10 - 100 g L^{-1}. A 0.03 g of NR latex sample was weighed in ten borosilicate glass test tubes, followed by adding 5 mL of each concentrations of the oxidizing agent. The solution mixtures were digested by using UV digestion unit for

50 minutes. Then, the resultant solutions (without filtration) were cooled down to room temperature and adjusted to 10 mL with DI water. A 1.6 mL of molybdenum blue solution was added and left for 10 minutes followed by the absorbance measurement using UV-Vis spectrophotometer at the wavelength of 880 nm.

Study the effect of digestion time

The digestion time was optimized in the range of 10 - 60 minutes. A 0.03 g of NR latex and 5 mL of 30 g L^{-1} ammonium peroxodisulphate mixture of in six borosilicate glass test tubes were digested in UV digestion unit at different times ranging from 10 to 60 minutes. After cooling to room temperature, the resultant solutions were adjusted to 10 mL with DI water followed by adding 1.6 mL of molybdenum blue solution and left for 10 minutes. Finally, the absorbances of the solution were determined by UV-Vis spectrophotometer at the wavelength of 880 nm.

Study the effect of reaction time for color development

The reaction time of phosphorus with molybdenum blue solution was investigated in the range of 5 to 60 minutes. The complexes of molybdenum blue solution with the 0.3 mg L^{-1} of phosphorus standard solution and NR latex sample after the digestion procedure by UV radiation (without and with added 0.02 mg g^{-1} phosphorus standard) were left from 5 to 60 minutes for completed reaction. Then, the absorbances of the resultant solutions were measured at the wavelength of 880 nm every 5 minutes.

Analytical performance for the determination of total phosphorus in NR latex using thermoreactor and UV digestion unit

Study the linearity range

The phosphorus standards at the concentration range of 0.1 - 1.0 mg L^{-1} were used for studying the linearity range for the determination of total phosphorus with and without digestion. A 10 mL of all phosphorus standard solutions were mixed with 1.6 mL of molybdenum blue solution and left for 10 minutes for completed reaction. The absorbances of the resultant solutions were measured at 880 nm by UV-Vis spectrophotometer.

Study the recoveries for the determination of total phosphorus in NR latex sample

The recoveries of total phosphorus were studied at appropriate level of phosphorus ion. In these experiments, solutions of phosphorus standard at different levels (0.01 - 0.05 mg g^{-1}) were added to 5.0 g of NR latex in polyethylene beaker followed by stirring for 20 minutes to evaporate ammonia. A 0.03 g of the spiked NR latex and 5 mL of 30 g L^{-1} ammonium peroxodisulphate solution were added into the borosilicate glass test tubes. The solutions were mixed and digested by thermoreactor and UV digestion unit under the optimum conditions. After that, the resultant solutions were diluted to 10 mL with DI water. A 1.6 mL of molybdenum blue solution was added and then left for 10 minutes. Finally, the absorbances of the resultant solutions were determined at the wavelength of 880 nm by UV-Vis spectrophotometer.

Study the effect of interfering ions on recoveries of total phosphorus

The effect of interfering ions on recoveries of total phosphorus was studied. In these experiments, solutions of 0.02 mg g^{-1} of phosphorus standard solution containing each interfering ions namely, arsenate (AsO_4^{3-}), sulfide (S^{2-}), nitrite (NO_2^-), hexavalent chromium (Cr^{6+}) and silicate (SiO_3^{2-}) at different levels were added to 5.0 g of NR latex in polyethylene beaker followed by stirring for 20 minutes to evaporate ammonia. A 0.03 g of the spiked NR latex and 5 mL of 30 g L^{-1} ammonium peroxodisulphate solution were added into the borosilicate glass test tubes. The solutions were digested by thermoreactor and UV digestion unit under the same conditions as the procedure of studying recoveries of total phosphorus. The tolerance level of interfering ions defined as the largest amount making the recoveries of total phosphorus less than 80%.

NR latex containing 0.02 mg g^{-1} of phosphorus standard solution along with varying amounts of interfering ions as described in Tables 6 were prepared and tested.

Table 6 The concentration of interfering ions on the recoveries of total phosphorus.

Interfering ions	Concentration (mg kg^{-1})
AsO_4^{3-}	0.1
	0.01
	0.001
S^{2-}	10.0
	1.0
	0.1
NO_2^-	100.0
	10.0
	1.0
Cr^{6+}	10.0
	1.0
	0.1
SiO_3^{2-}	100.0
	10.0
	1.0

Study the analytical accuracy and precision

The accuracy and precision of thermoreactor and UV digestion unit were evaluated by studying the recoveries of total phosphorus within day and between day. In this study, solution of 0.02 mg g^{-1} phosphorus standard was added to 5.0 g of NR latex in polyethylene beaker. Then, the NR latex was stirred for 20 minutes to evaporate ammonia. Then, the solutions were digested by thermoreactor and UV digestion unit and measured under the optimum conditions. Besides, the within day and between day precision were determined in order to evaluate the precision of the proposed methods as the relative standard deviation (%RSD) of phosphorus standard solution (at the concentration of 0.02 mg g^{-1}). The within day precision was carried out for seven replicate measurement in one day. The between day precision was studied in seven day for seven replications of determination.

The limit of detection and limit of quantitation of the UV-Vis spectrophotometer were studied by using the blank solution. Under the optimum conditions, 10 mL of blank solution was mixed with 1.6 mL of molybdenum blue

solution and left for 10 minutes. Then, the resultant solutions were measured at the wavelength of 880 nm by UV-Vis spectrophotometer.

Determination of total phosphorus in NR latex samples

The NR latex sample (high ammonia latex, 60% of dry rubber content (DRC)) was purchased from Thai Rubber Latex Corporation (Thailand) Public Company Limited. A 0.03 g of the NR latex sample and 5 mL of 30 g L^{-1} ammonium peroxodisulphate solution were added into the borosilicate glass test tube followed by digestion under the optimized conditions in a thermoreactor and UV digestion unit. Then, the resultant solution was cooled down to room temperature and diluted to 10 mL with DI water. A 1.6 mL of the molybdenum blue solution was added and left for 10 minutes for completed reaction. The absorbance of the resultant solution was measured by using UV-Vis spectrophotometer at the wavelength of 880 nm and compared with the measurement by digital image-based colorimeter-artificial neural network (DIC-ANNs). For the DIC-ANNs, phosphorus standard solutions and sample solution complexes with molybdenum blue reagent were transferred into quartz cell and set in the DIC light box (Figure 19). The solutions were pictured then the images were processed by the ANNs written program [70] to determine the RGB value in the database. Finally, the amount of total phosphorus containing in NR latex was predicted by comparing the RGB value of the sample image with the nearest RGB value of the phosphorus standard solutions in the database.