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Full Length Research Paper

Development of UV digestion unit for natural rubber latex preparation before the determination of phosphorus residue with artificial neural network-digital image-based colorimetry

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Digital image–based colorimetry (DIC) coupled with artificial neural network (ANN) was studied for the determination of phosphorus in natural rubber (NR) latex. The method is based on the RGB (Red, Green, and Blue) value of an image of phosphorus standard solution reacted with molybdenum blue solution. The picture of the complex solution was captured by using a complementary metal oxide semiconductor (CMOS) camera. Because of a colloidal property of NR latex, phosphorus residue could not be directly determined by this technique. Therefore, a UV digestion unit was designed and fabricated for the digestion of NR latex before color developing and processing with DIC-ANN. The digestion time needed for complete digestion was 50 min. The digestion capability of the UV digester to process 10 samples simultaneously led to an acceptable samples analysis frequency of 12 h^{-1}. Quantitative measurement of phosphorus was made in the range 0.1 to 1.0 mg L^{-1}. The proposed method was successfully applied to the determination of total phosphorus in NR latex and it was found to be simple, rapid, accurate, precise and low cost method.

Key words: Digital image-based colorimetry, artificial neural network, UV digestion, natural rubber latex, phosphorus.

INTRODUCTION

Natural rubber (NR) latexis a major agricultural product of Thailand due to the hot climate being conducive to the growth of the rubber tree. Thai rubber latex industries such as rubber manufacturing plants, gloves, condoms, tires, etc. require a high quality of NR latex. One parameter in the pricing of NR latex is the residual amount of magnesium. Magnesium is a chemical that affects the stability of NR latex. Therefore, it is necessary to precipitate magnesium before manufacturing process. Diammonium hydrogen phosphate (DAHP) or diammonium phosphate (DAP) is used for the precipitating process. Then magnesium sediment (magnesium ammonium phosphate) is removed in the form of sludge by centrifugation. The excessive use of DAHP or DAP causes phosphate residues in NR latex. The phosphate residues will react with some chemicals in production process effecting the formation of the products (Karunanayake and Perera, 2006; Patthanakul et al.,

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2009). Therefore, the detection of phosphate residues is very important. However, phosphate in NR latex consists of many types such as phospholipids, free orthophosphate, sugar phosphate and phosphate from precipitation of magnesium (Loadman, 1998). Thus, the total phosphate is determined in the form of total phosphorus in this research. Recently, a new technique called digital image–based colorimetry coupled with artificial neural network (DIC-ANN) was developed in our laboratory for protein assay in NR latex and medical latex gloves which could be used instead of spectrophotometry (Bang-iam et al., 2013). Unfortunately, phosphorus residues in NR latex cannot be directly determined by this technique because the NR latex has a colloidal characteristic which obstructs the penetration of light beams. In addition, the procedure for color developing by the molybdenum blue method involves certain acidic chemicals (American Public Health Association, 1992). When the NR latex is exposed to acid, it becomes agglomerated. Thus, digestion of NR latex before the determination of phosphorus in NR latex is a crucial step. The digestion process is a procedure for destroying the rubber constituents until achieving a clear solution which is ready for the color developing process. It normally uses energy such as heat (UV light, microwave, thermoreactor) or chemical reagents such as acids or a combination of the two methods (Matusiewicz, 2003; VelpScientifica, n.d.). In previous studies, the phosphorus content in environmental and pharmaceutical samples was determined using ammonium persulphate or potassium persulphate as the oxidizing agentor used in conjunction with acid such as perchloric acid or sulfuric acid and with UV light providing the energy (Benson et al., 1996; Tzanavaras and Themelis, 2003; Tue-Ngeun et al., 2005a, b). For NR latex sample, a previous study used the Kjeldahl technique to obtain the clear and appropriate solution (Loadman, 1998). Concentrated sulfuric acid and nitric acid were used in the digestion procedure together with high pressure and temperature. There are no findings on the sample preparation of NR latex using UV-assisted digestion before the detection of phosphorus with molybdenum blue method. Consequently, the purpose of this research was to develop a simple UV-assisted digestion unit for the preparation of NR latex before the determination of total phosphorus residues by molybdenum blue spectrophotometric method and digital image–based colorimetry coupled with artificial neural network (DIC-ANN). In the paper, a description of the fabrication is given and the optimal conditions are explored. Initial results are presented based on real NR latex samples.

EXPERIMENTAL

Chemicals and reagents

All chemicals were of analytical grade and all solutions were prepared by using deionized (DI) water (Prima Reverse Osmosis, Maxima water purification system, Elga Ltd., England). A stock solution (1,000 mg L^{-1}) of phosphorus was prepared by dissolving 0.4390 g of KH_2PO_4 (Rankem, India) in DI water and diluting to 100 ml in a volumetric flask. The standard phosphorus solution was stored at 4°C. This solution is stable for 1 month. Solution of 30 g L^{-1} ammonium peroxodisulphate ($(NH_4)_2S_2O_8$, LobaChemie, India) was prepared by dissolving 3.0 g in DI water and adjusting to a volume of 100.0 ml. Solution of potassium antimonyl tartrate ($K(SbO)C_4H_4O_6.1/2H_2O$, Riedel-de Haen, Germany) was prepared by dissolving 0.27 g in DI water and adjusting to a volume of 100.0 ml. Solution of ammonium molybdate ($(NH_4)6Mo_7O_{24}.4H_2O$, Ajax Finechem, Australia) was prepared by dissolving 4.0 g in DI water and adjusting to a volume of 100.0 ml. Solution of ascorbic acid ($C_6H_8O_6$, Rankem, India) was prepared by dissolving 1.76 g in DI water and adjusting to a volume of 100.0 ml. The ascorbic acid solution is stable for 1 week at 4°C. Solution of 5 N sulfuric acid (H_2SO_4, Carlo ERBA, Italy) was prepared by diluting 35.0 ml to a volume of 250.0 ml with DI water. 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, respectively (American Public Health Association, 1992). Stock solution (1,000 mg L^{-1}) each interfering ion was prepared by dissolving an appropriate amount of the suitable salt.

5.0 g each of the NR latex samples (high ammonia latex, 60% of dry rubber content, Thai Rubber Latex Corporation, Public Company Limited, Thailand) were stirred to evaporate ammonia for 20 min. The sampleswere stored in polyethylene tube and were freshly prepared for daily use.

Apparatus

A doublebeam UV-Vis Spectrophotometer (Lambda 20 Perkin Elmer, USA) was used for the optimization of the proposed digestion method and also results comparison with the DIC-ANN measurement. A digital image-based colorimeter (under Petty Patent since 2012, Thailand) coupled with an ANN-program (under copyright since 2011, Thailand) utilized in this work was fabricated and gave satisfactory results for protein assay in NR latex and medical latex gloves (Bang-iam et al., 2013). The data for calibration set and real samples sets were obtained by taking images in the DIC light box. The ANN program which was trained with the back-propagation of errors learning algorithm, was used to extract the red (R), green (G), and blue (B) values from the images. The ANN has three input nodes (one for each of the R, G, B channels), two hidden layers with 11 nodes (because the output is the concentration of standard phosphorus from 0-1.0 mg L^{-1}), and one output node (phosphorus concentration). In contrast to UV-Vis spectrophotometry, the construction of calibration curve is not required when using DIC-ANN. After ANN training (random learning for 3,000 iterations) of the standard phosphorus images, the program could directly predict the concentration of the phosphorus residue in the unknown NR latex samples.

Laboratory-made UV digestion unit

A schematic diagram of the system assembled for UV digestion unit (under Petty Patent since 2013, Thailand) acquisition is illustrated in Figure 1. A digestion unit was made from a 35 × 24 × 21 cm (width × height × depth) black cardboard adapted from a first aid box (ESM Medical, Thailand) to maintain the temperature and UV light in the closed system. The UV lamp (300 W, Osram, Slovakia) was secured on the wall of the box and in front of the ten screw cap test tubes (16 mm) which was located in the stainless steel rack. The temperature can go up to 160± 5°C after warming up for 10 min. The distance between the UV lamp and the screw cap test

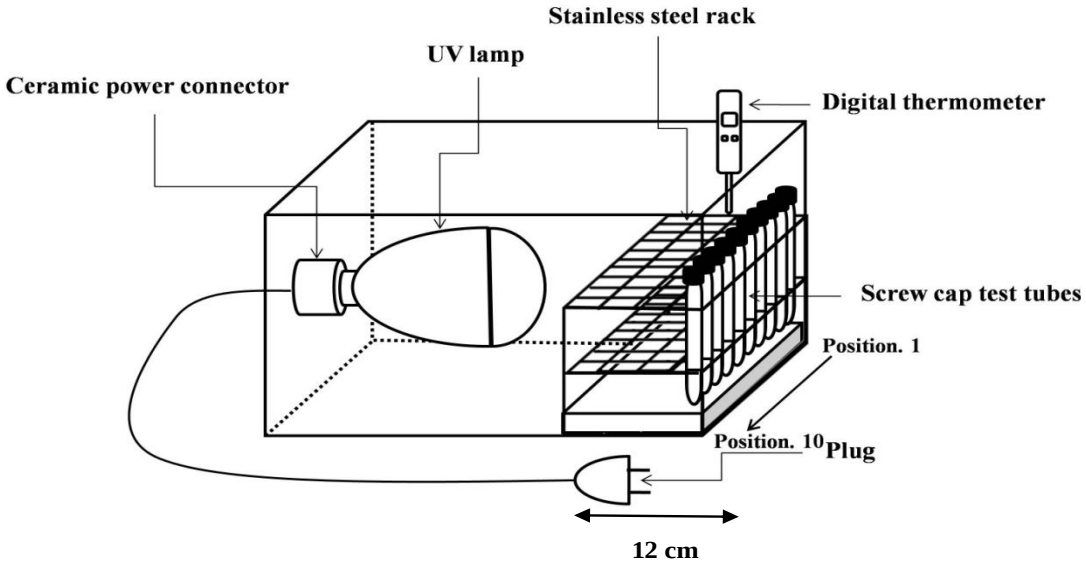

Figure 1. Schematic diagram of a laboratory-made UV digestion unit.

tubes at the back row of the rack is 12 cm. A thermometer was placed at the top of the box in order to monitor the digestion temperature throughout the experiment.

The digestion tube position is likely to have great effect on the reproducibility of the results. The fabricated UV digestion unit used a stainless steel test tube rack as the sample holder. The distance between the UV lamp and the first row of the rack is 3 cm. Ten digestion tubes were placed in the end row of the rack (12 cm from the UV lamp) because the first four rows at the front are too near the UV lamp causing vigorous boiling of solution. Thus, in this experiment, digestion tube positioning was studied in ten positions at back row of the rack. The results indicated that all positions provide no difference in normalized absorbance. It was confirmed by the standard deviation (SD) and the relative standard deviation (RSD) which was 0.23 absorbance g^{-1} and 2.14%, respectively. Therefore, the digestion tube positioning at all positions could be used for NR latex digestion.

RESULTS AND DISCUSSION

In this work, the UV digestion unit was constructed and applied to NR latex digestion coupled with the oxidizing agent. Five parameters including type of oxidizing agent, effect of oxidizing agent on phosphorus molybdenum blue complex spectra, concentration of oxidizing agent, digestion time and reaction time for color development were studied and optimized. Normalized absorbance was used for the study of these effects because of the viscosity of NR latex. It is difficult to accurately pipette the NR latex thus the sample weighing was carried out and normalized absorbance which means absorbance divided by weight of NR latex was utilized instead of absorbance.

The effect of types of oxidizing agent

Types of oxidizing agents were studied in order to select the optimum oxidizing agent for the decomposition of biological matrices in NR latex. The use of oxidizing agents such as hydrogen peroxide, nitric acid, sulfuric acid, perchloric acid, ammonium peroxodisulphate and potassium peroxodisulphate were reported for various samples in the literature reviews (Benson et al., 1996; Golimowski and Golimowska, 1996; Zhang et al., 1996; Lyengar et al., 1998; Tzanavaras and Themelis, 2003; Tue-Ngeun et al., 2005a, b). Non oxidizing acid were chosen in this study because the NR latex is coagulated during treatment with acid. Thus, the strong oxidizing agents such as ammonium peroxodisulphate and potassium peroxodisulphate were considered instead. The phosphorus standard solutions in the concentration range of 0 to 4 mg L^{-1} were spiked with NR latex samples followed by adding oxidizing agent before digestion. The slope when using ammonium peroxodisulphate and potassium peroxodisulphateas oxidizing agent for the NR latex digestion shows similar results. Thus, it can be concluded that the sensitivity is unaffected by the choice of these oxidizing agents. However, the solubility of ammonium peroxodisulphate is higher than potassium peroxodisulphate. Moreover, ammonium peroxodisulphate is cheaper than potassium peroxodisulphate. Therefore, ammonium peroxodisulphate was chosen as oxidizing agent for the next study.

The effect of oxidizing agent on phosphorus molybdenum blue complex spectra

Under the optimum conditions, the effect of the ammonium peroxodisulphate used as oxidizing agent on the absorbance of phosphorus molybdenum blue

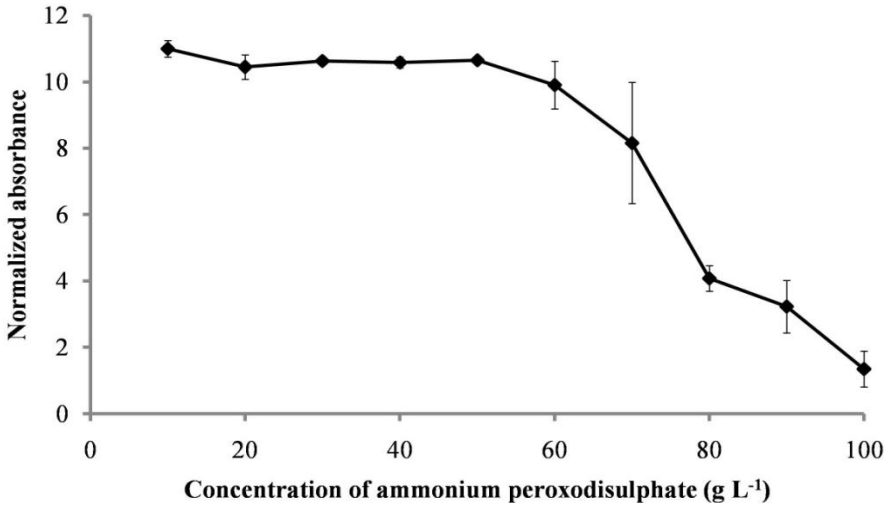

Figure 2. Effect of concentration of ammonium peroxodisulphate used as oxidizing agent coupled with UV-assisted digestion on normalized absorbance.

complex in digested NR latex sample was studied by scanning the wavelength in the range of 400 to 900 nm. The spectrum of the complex compound indicated the maximum wavelength at 710 and 880 nm that is similar to the spectra of phosphorus standard solutions. It can be concluded that the oxidizing agent has no effect on the complex compound of phosphorus in NR latex with molybdenum blue solution. Thus, the maximum wavelength at 880 nm was chosen for the next experiment.

The effect of concentration of oxidizing agent

The effect of concentration of the oxidizing agent for NR latex digestion by UV digestion unit was studied in the range of 10 to 100 g L^{-1}. From Figure 2, the signal is constant up to 50 g L^{-1} of ammonium peroxodisulphate solution. Then the signal decreased with increasing the concentration of the oxidizing agent from 60 to 100 g L^{-1}. However, the solutions obtained using 10 to 20 g L^{-1} of the oxidizing agent are not clear and they have a lot of pieces of the coagulated rubber particles owing to incomplete decomposition reaction. Therefore, a filtration step was carried out in the sample preparation. Moreover, the phosphorus complex compound might be adsorbed on the filter paper. The best result was obtained when using 30 g L^{-1} of the oxidizing agent providing clear solution and low reagent consumption. Therefore, concentration of the oxidizing agent at 30 g L^{-1} was selected for the next study.

The effect of digestion time

The UV digestion unit (after a 10 min warm-up) operates at a constant temperature of 160 ± 5°C. However, the digestion time has the effect on the rate of decomposition reaction. The digestion time was studied in the range of 10 to 60 min. Figure 3 shows that the normalized absorbance increased with increasing digestion time, nevertheless a plateau was reached after 20 min. Moreover, the resultant solutions at the range of 20 to 30 min are not clear and the standard deviation at 40 min is higher than that at 50 min. Therefore, the digestion of NR latex for 50 min was selected in the next experiment.

The effect of reaction time for color development

After the NR latex digestion, the reaction time of the colorimetric reaction between phosphorus and molybdenum blue reagent was studied in the range of 5 to 60 min. The results of 0.3 mg L^{-1} of phosphorus standard solution, NR latex sample with and without added phosphorus standard (0.02 mg g^{-1}) were obtained as illustrated in Figure 4. It was found that, all colorimetric reactions are stable after 10 min. Therefore, the standing time for 10 min before phosphorus determination was chosen for the next experiment.

Analytical performance for the determination of total phosphorus in NR latex

The linear range

The linear range of phosphorus standard solution was studied in the range of 0.1 to 1.0 mg L^{-1} for the determination of total phosphorus in NR latex sample. Under the optimum conditions, the results of the calibration solutions set without digestion procedure and

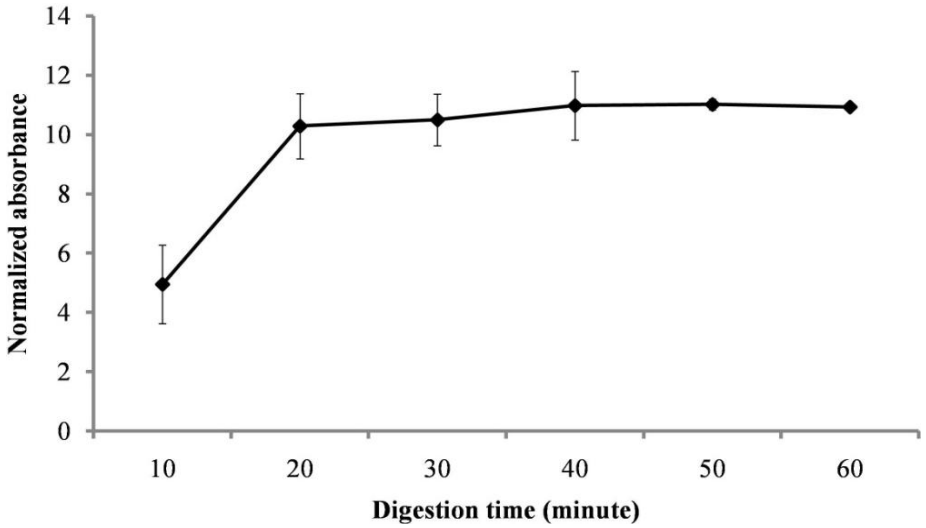

Figure 3. Effect of digestion time of NR latex sample on normalized absorbance by using UV-assisted digestion couple with 30 g L^{-1} ammonium peroxodisulphate.

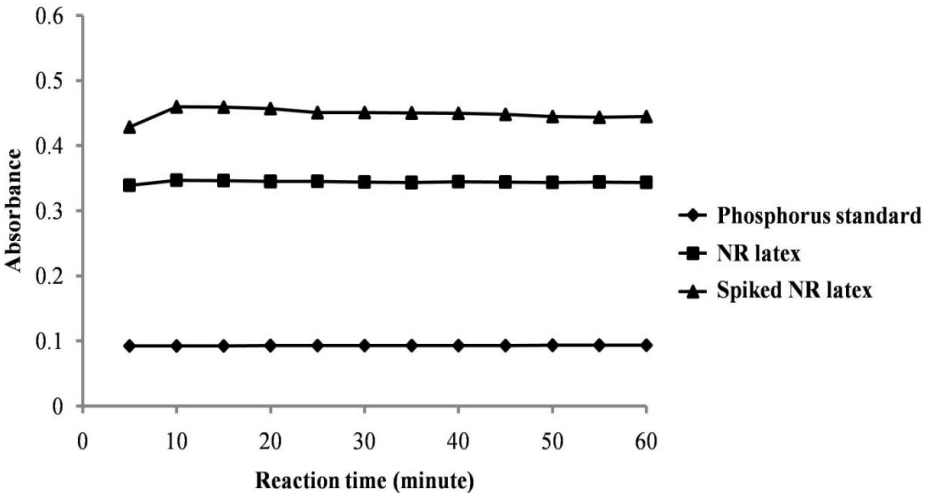

Figure 4. Effect of reaction time for color developing of phosphorus standard solution (0.3 mg L^{-1}), phosphorus in NR latex sample after digestion using UV digestion unit and spiked (0.02 mg g^{-1} of phosphorus standard) NR latex sample after digestion using UV digestion unit with molybdenum blue solution.

with digestion procedure in which 5 ml of 30 g L^{-1} oxidizing agent was added before coupled with UV digestion unit. It was found that the digestion step has little effect on calibration curves and the slope values of these curves are not significantly different. It can be concluded that the calibration method with digestion and without digestion are equally sensitive. Nevertheless, the construction of calibration solutions without the digestion step reduces preparation time, energy and reagent. Therefore, the calibration curve construction without the digestion step was selected for total phosphorus estimation in NR latex sample.

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

NR latex samples were digested using UV digestion unit and analyzed for recoveries of the total phosphorus by UV-Vis spectrophotometry. NR latex containing added phosphorus standard solutions were analyzed and represented in Table 1. The recoveries of total phosphorus using the UV digestion unit are in the range of 84.7 to 103%. These recoveries indicated that the proposed digestion techniques can be used for the determination of total phosphorus in NR latex.

Table 1. Recoveries of UV digestion unit for the analysis of total phosphorus in NR latex samples.

Samples	Added (mg g^{-1})	Found (mg g^{-1}, n=3)	% Recovery[a]
1	0	0.176	-
	0.01	0.186 ± 0.0006	103 ± 5.77
2	0	0.171	-
	0.02	0.187 ± 0.0005	84.7 ± 2.70
3	0	0.164	
	0.03	0.190 ± 0.0010	86.7 ± 3.33
4	0	0.168	-
	0.04	0.206 ± 0.0010	95.0 ± 2.50
5	0	0.163	-
	0.05	0.207 ± 0.0015	87.3 ± 3.00

[a]Mean value ± standard deviation (n = 3).

Table 2. Effect of interfering ions on recovery of total phosphorus.

Interfering ions	Tolerance limit concentration (mg kg^{-1})	%Recovery [a]
AsO_4^{3-}	0.001	94.1 ± 7.29
S^{2-}	1.0	97.7 ± 6.42
NO_2^-	10.0	93.8 ± 1.61
Cr^{6+}	1.0	90.3 ± 4.58
SiO_3^{2-}	1.0	85.8 ± 1.73

[a] Mean value ± standard deviation (n =3)

The effect of interfering ions on recoveries of total phosphorus determination

The effect of potential interferences upon the molybdenum blue reaction was studied at 0.02 mg g^{-1} phosphorus ions in NR latex samples. Quantitative recoveries of the analyte were shown in Table 2. The presence of arsenate (AsO_4^{3-}), sulfide (S^{2-}), nitrite (NO_2^-), hexavalent chromium (Cr^{6+}) and silicate (SiO_3^{2-}) can interfere with the process of determining the phosphorus content (American Public Health Association, 1992) when interfering ions concentration are higher than the concentration as presented in Table 2.

Validation of the method

Under the chosen conditions described above, by using UV-Vis spectrophotometry, the calibration graphs were linear over the range 0.1 to 1.0 mg L^{-1}.The value of LOD ($3SD_{blank}$ / slope, n=15) was 3.14×10^{-4} mg L^{-1} and LOQ ($10SD_{blank}$ / slope, n=15) was 1.1×10^{-3} mg L^{-1}. The relative standard deviations (%RSD) of intraday and interday analyses for recoveries of phosphorus using the laboratory-made UV digestion unit were 2.48 and 5.98%, respectively (n=7).

The validation and estimation performance of an ANN prediction model were normally determined by mean square error (MSE) or root mean square error (RMSE) or mean absolute deviation (MAD) or mean absolute percentage error (MAPE) (Elmolla et al., 2010; Roush et al., 2006; Salle et al., 2003; Twomey and Smith, 1996; Yetilmezsoy and Demirel, 2008). Therefore, the accuracy and precision of the ANN written program used in this work were validated by MSE and the relative standard error (%RSE).It was found that the average MSE from phosphorus standard solutions testing at 0.1, 0.3, 0.5, 0.7 and 0.9 mg L^{-1} was 0.0020. The low MSE indicated that the accuracy of the DIC-ANN is relatively high. Thus, the DIC-ANN could be used for the determination of total phosphorus in NR latex. The %RSE is the standard error expressed as a fraction of the estimate value and is usually displayed as a percentage. The high %RSE is subject to high sampling error. The %RSE was 0.05 when reading the RGB values for 3,000 times. This indicated that the proposed method gave high precision. Therefore, the numbers of iteration for 3,000 times were selected for the RGB values reading in the ANN written

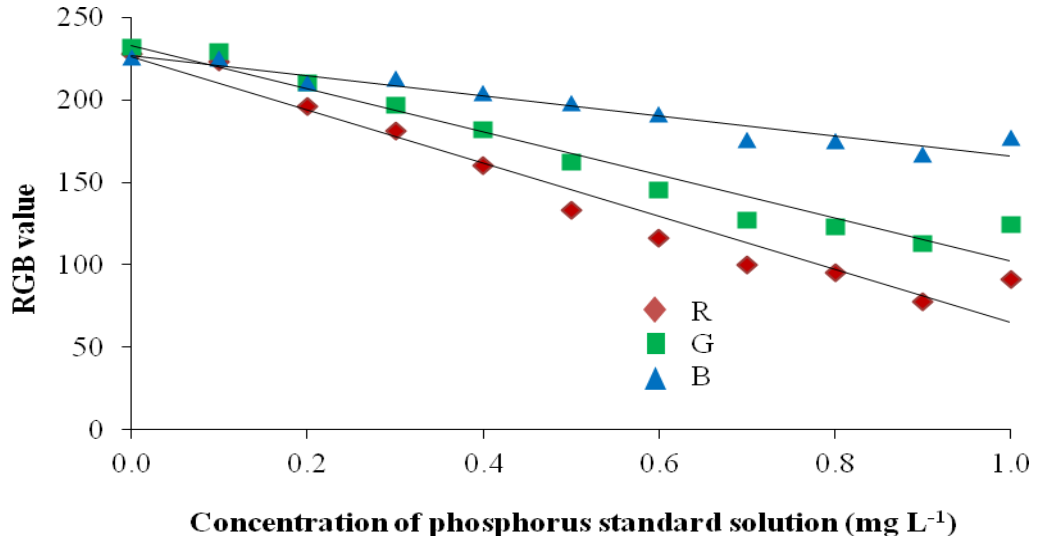

Figure 5. Plots of relationships between RGB values and concentration of phosphorus standard solution.

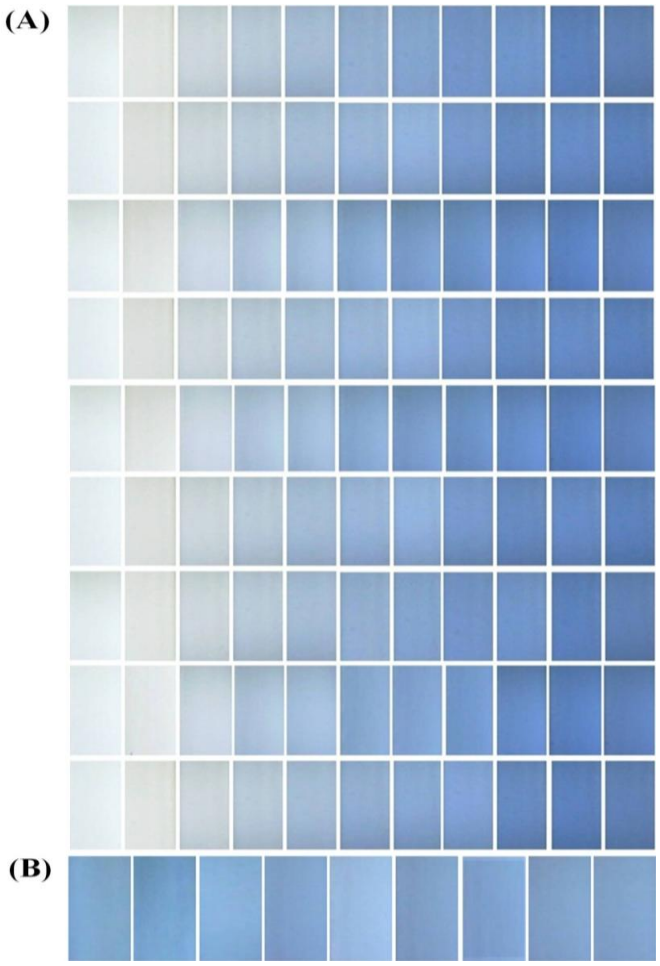

Figure 6. (A) Digital images of phosphorus standard solutions (0-1.0 mg L^{-1}) with molybdenum blue reagent (n=9) and (B) digital images of NR latex sample after digestion using UV digestion unit with molybdenum blue reagent (n=9).

program (only 5 min for data processing). The reproducibility of the phosphorus determination in NR latex was verified by the %RSD carried out between days for 11 replications. The %RSD of phosphorus standard solutions at 0.1, 0.3 and 0.5 mg L^{-1} were 3.72, 2.59 and 1.36%, respectively. This method showed good reproducibility for phosphorus determination at the concentration more than 0.1 mg L^{-1}. Moreover, the LOD could not be calculated because there was no calibration curve construction by the DIC-ANN method. The LOQ by the DIC-ANN defined as the concentration that could be photographed and processed by an ANN program was 0.1 mg L^{-1}.

Analysis of real samples

In this study, UV digestion unit was chosen for NR latex preparation before determination of total phosphorus by DIC-ANN compared with UV-Vis spectrophotometer. As illustrated in Figure 5, RGB value decreased with increasing phosphorus standard concentration whereas the intensity of color of the solution increased (Figure 6 A).The images of the complexes between phosphorus in NR latex with molybdenum blue solution after digestion were shown in Figure 6 B. The results from the determination of total phosphorus in NR latex samples after UV digestion were 0.170 ± 0.0181 mg g^{-1} (n=5) and 0.173 ± 0.0022mg g^{-1} (n=5) by DIC-ANN and UV-Vis spectrophotometry, respectively, which show no statistical difference at 95% confidence level by applying the paired t-test. These results do not exceed the level as compared to the DAP addition in NR latex recommended by Pollution Control Department (0.180 - 0.987 mg g^{-1} of phosphorus ion) (Pollution Control Department, 2005).

However, Karunanayake found that the phosphorus at aconcentration of more than 0.0098 mg g^{-1} would affect the stability of NR latex and the physical properties of products (Karunanayake and Perera, 2006).

Conclusions

The laboratory-made UV digestion unit used in combination with 30 g L^{-1} ammonium peroxodisulphate as oxidizing agent at 50 min digestion time was proved to be a successful NR latex preparation technique. It provided recovery ≥ 85% for phosphorus residue determination in NR latex samples using DIC-ANN and UV-Vis spectrophotometry. Therefore, it could be emphasized that the developed digestion procedure is simple, rapid, safety, accurate, precise and low cost (only USD 140) which are the main advantages. The UV digestion unit could be improved on increasing the number of sample per batch (more than 10 samples). Although, it could not be used continuously for the digestion in order to prevent the overheat in the UV digestion unit, it is still more interesting than the original technique such as Kjeldahl because using UV radiation assisted with thermal energy provides higher radical generating from oxidizing agent for decomposition reaction than using only thermal energy. Consequently, UV digestion unit is suitable for laboratory and small industrial factory in NR latex digestion before total phosphorus determination.

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