CHAPTER IV

RESULTS AND DISCUSSION

This chapter presents the results of this research including application of thermoreactor and UV digestion unit for natural rubber (NR) latex preparation before the determination of total phosphorus by the UV-Vis spectrophotometry compared with the determination by digital image-based colorimetry-artificial neural networks (DIC-ANNs). The detail of each topic is described below.

Application of thermoreactor to NR latex

Normally, thermoreactor was used for the decomposition of matrices in water and waste water [24]. In this work, thermoreactor was applied to NR latex digestion coupled with the use of oxidizing agent. Six parameters including type of oxidizing agents, effect of oxidizing agent on phosphorus molybdenum blue complex spectra, concentration of oxidizing agent, digestion temperature, digestion time and reaction time for color development were optimized. The results and discussion are presented as followed;

Type of oxidizing agents

Type 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 [9, 10, 12, 13, 34, 39]. 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 - 4 mg L^{-1} were spiked in NR latex sample followed by adding oxidizing agent before digestion. The results were shown in Figure 21 and 22. The slope when using ammonium peroxodisulphate and potassium peroxodisulphate as oxidizing agent for the NR latex digestion shows similar result.

Thus, it can be concluded that the sensitivity when using both oxidizing agents are not difference. 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.

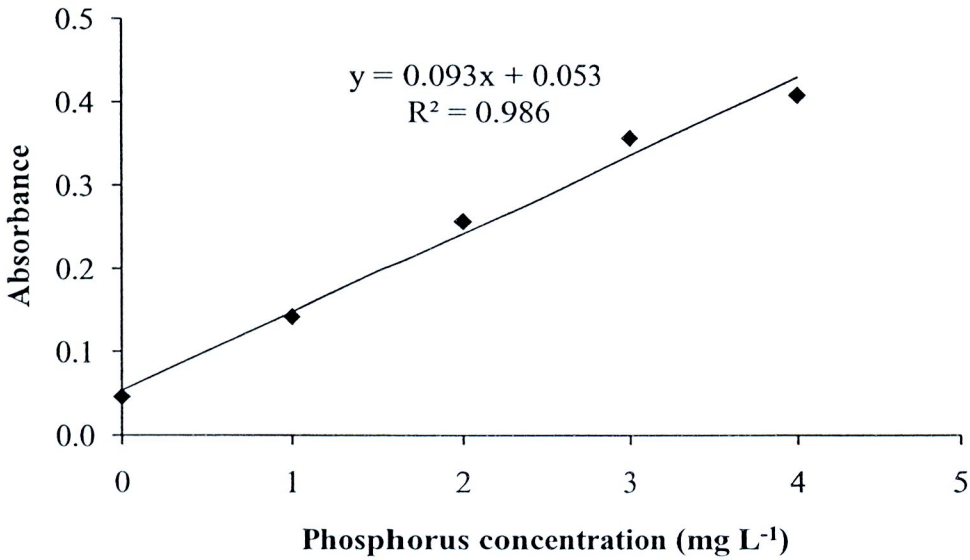

Figure 21 Standard addition curve using 50 g L^{-1} of ammonium peroxodisulphate as oxidizing agent for NR latex digestion

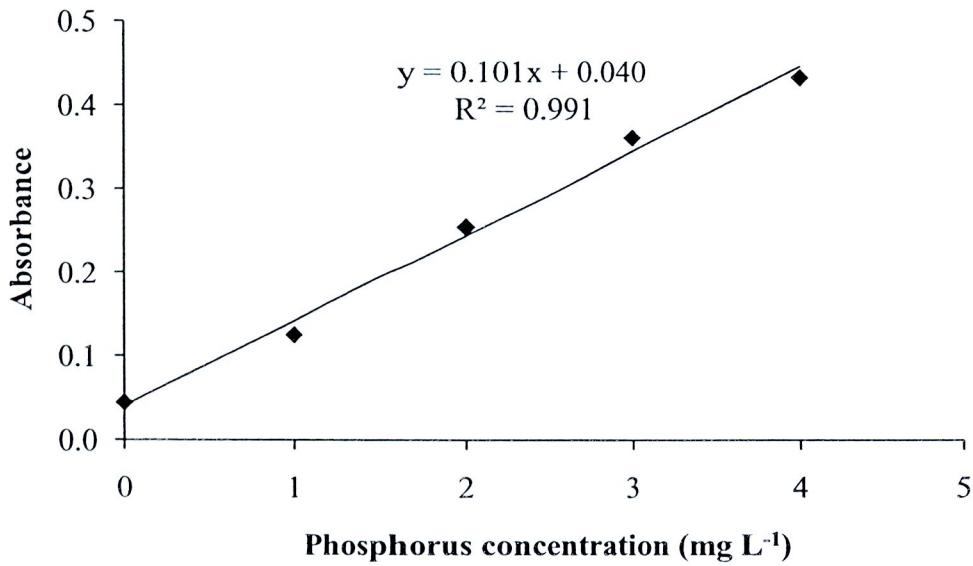

Figure 22 Standard addition curve using 50 g L^{-1} of potassium peroxodisulphate as oxidizing agent for NR latex digestion

Effect of oxidizing agent on phosphorus molybdenum blue complex spectra

The oxidizing agent might affect the spectrum of phosphorus reacted with molybdenum blue solution if it remains after digestion. Thus, after color development, phosphorus standard solutions (0.1, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg L^{-1}) and NR latex sample were scanned for absorption the spectrum in the range of 400 - 900 nm by UV-Vis spectrophotometer. The results obtained were illustrated in Figure 23 and 24. The spectra of phosphorus standard and the sample show the same maximum wavelength at 710 and 880 nm. It can be concluded that the oxidizing agent is not effect the complexation of phosphorus with molybdenum blue solution in NR latex sample. The maximum wavelength at 880 nm was chosen for the next experiment because the sensitivity obtained with this wavelength is higher than the other wavelengths.

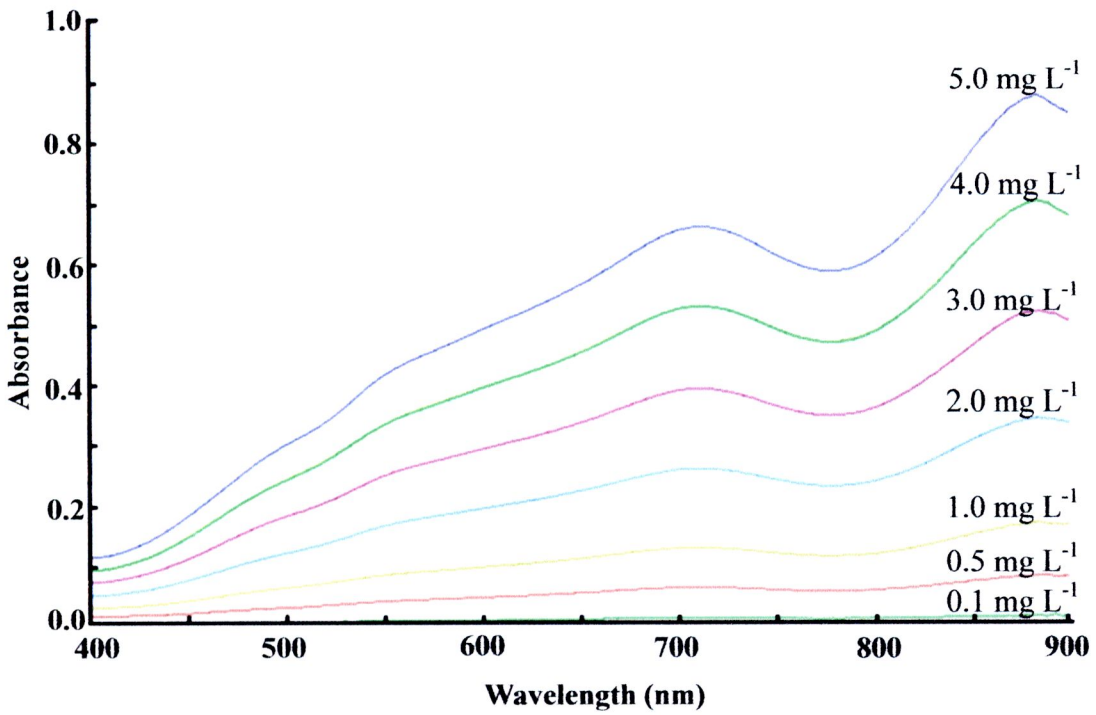

Figure 23 UV spectra of phosphorus standard solutions

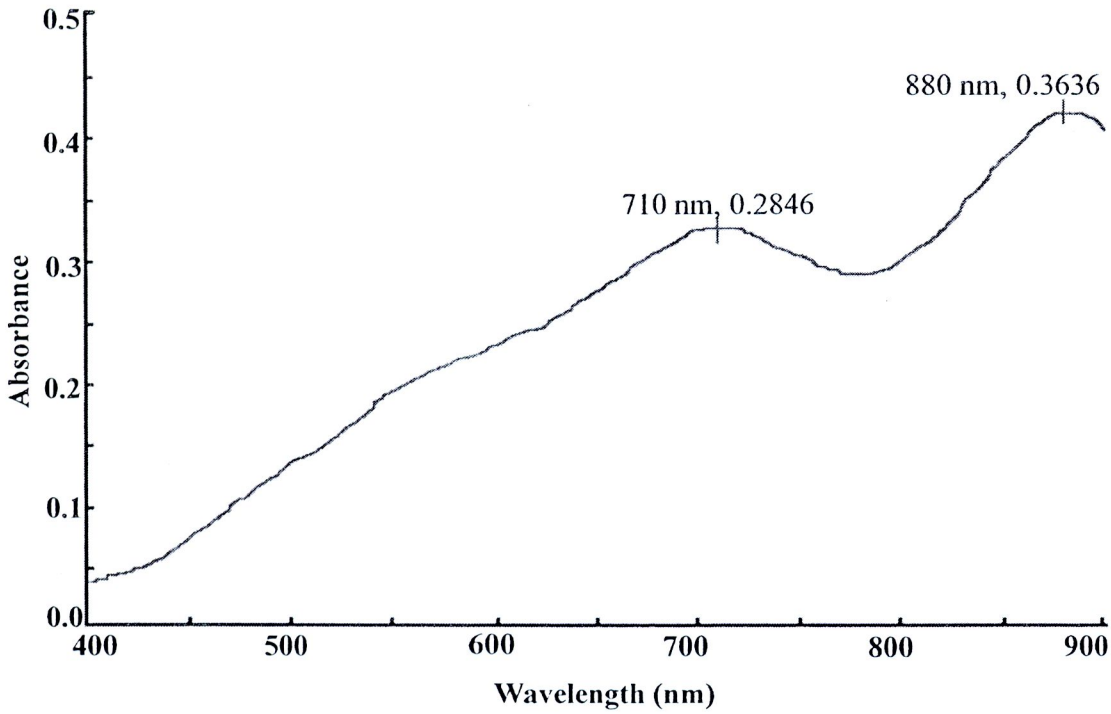

Figure 24 UV spectrum of phosphorus in NR latex sample after digestion using thermoreactor

Effect of concentration of oxidizing agent

Normalized absorbance was used for the study of this effect because of the viscosity of NR latex. It is difficult to accurately pipette the NR latex thus weighing sample was carried out and normalized absorbance which means absorbance divided by weight of NR latex was utilized instead of absorbance in the study of concentration of oxidizing agent, digestion temperature and time. The concentration of ammonium peroxodisulphate is important parameter for the equilibrium of the decomposition reaction of the matrices in NR latex. It was studied in the range of 10 to 80 g L^{-1}. The result obtained was demonstrated in Figure 25. The high signals were obtained when adding 10 and 20 g L^{-1} of ammonium peroxodisulphate. However, the solutions obtained by using these concentrations are not clear and there are a lot of pieces of the coagulated rubber particles. This could be due to the amount of oxidizing agent is not sufficient for decomposition reaction. Therefore, the filtration step was required before color developing that is complicated, time consuming and the complexes may be

absorbed on the filter paper. The signal decreased with increasing the concentration of the oxidizing agent from 50 to 80 g L^{-1} which could be attributed to the remaining oxidizing agent after digestion procedure. The remaining oxidizing agent will react with the reducing agent (ascorbic acid) lead to decreasing of the color of the molybdenum blue solution and the absorbance. Good results were obtained when using 30 and 40 g L^{-1} of the oxidizing agent. Therefore, concentration of the oxidizing agent at 30 g L^{-1} was selected for the next study as the reason of reducing cost.

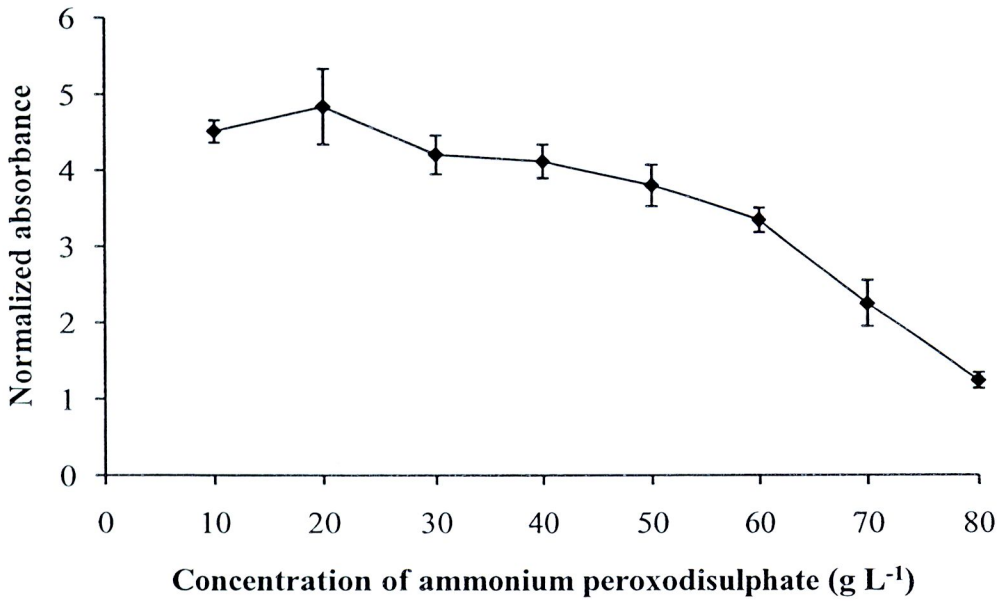

Figure 25 Effect of concentration of ammonium peroxodisulphate used as oxidizing agent coupled with thermoreactor at 100 °C for 60 minutes on normalized absorbance

Effect of digestion temperature

The digestion temperature has the effect on the rate of the decomposition reaction. The reaction is slow when using low temperature and the NR latex is burnt providing the dark yellow solution when using high temperature. This parameter was studied in the range of 60 - 150 °C for 60 minutes using 30 g L^{-1} of ammonium peroxodisulphate as oxidizing agent. From Figure 26, it was found that the normalized absorbance was observed to increase with increasing the digestion temperature up to 100 °C after which the normalized absorbance become constant. Obviously, low

temperature is not enough for catalytic activity of the decomposition reaction but when increasing the digestion temperature until suitable or more for the reaction, constant signal was obtained.

Consequently, the temperature at 100 °C was chosen for the NR latex digestion in the next experiments.

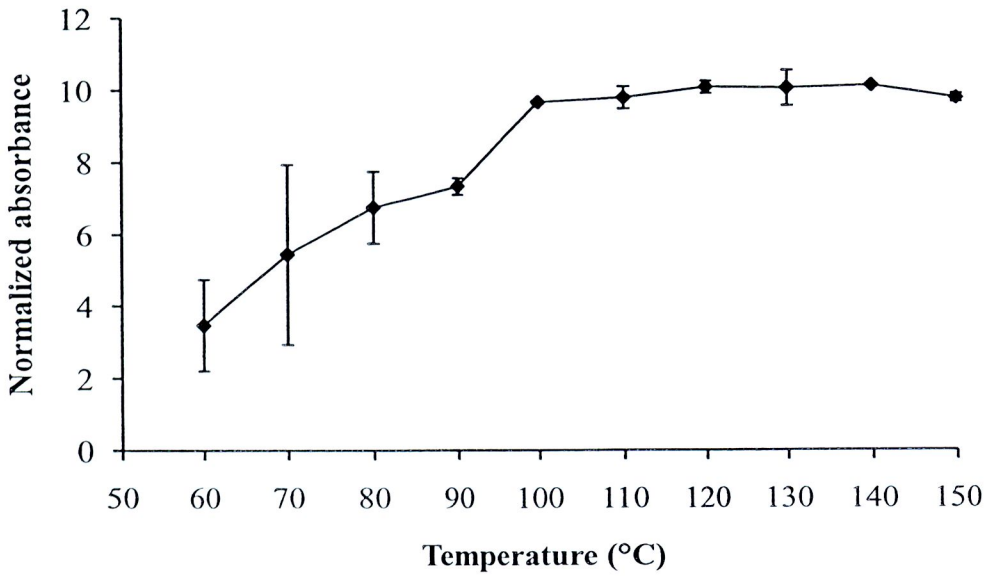

Figure 26 Effect of digestion temperature on normalized absorbance using thermoreactor for 60 minutes and 30 g L^{-1} of ammonium peroxodisulphate

Effect of digestion time

The digestion time has the effect on the completeness of the NR latex digestion. The digestion time was studied in the range of 10 to 120 minutes by controlling the digestion temperature at 100 °C. Figure 27 shows that normalized absorbance increased with increasing the digestion time. However, a plateau was reached after 70 minutes indicated that most of matrices in NR latex were completely decomposed and all forms of phosphorus were oxidized to orthophosphate form. Therefore, the digestion time for NR latex was selected at 70 minutes for the next studies.

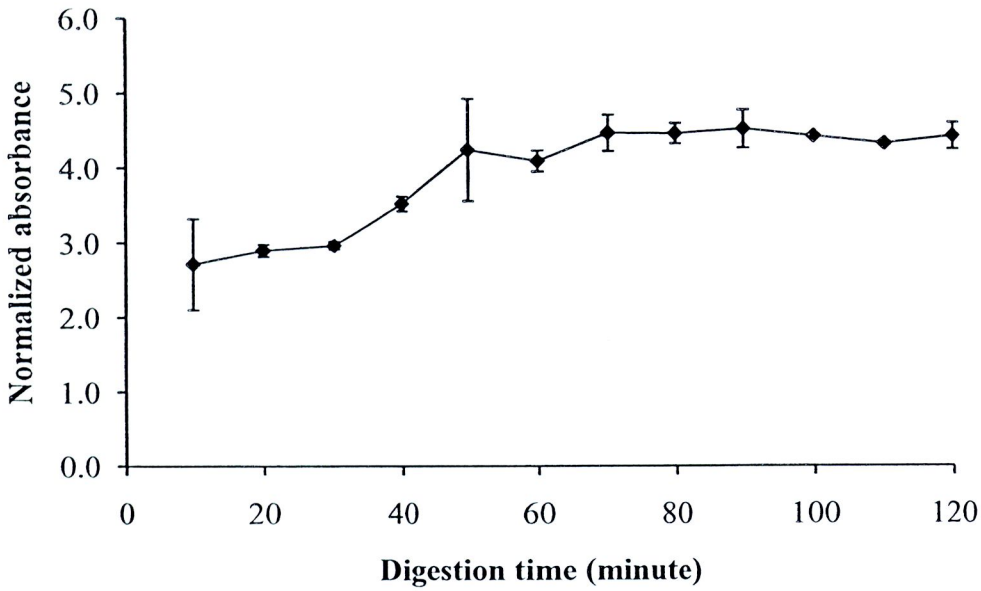

Figure 27 Effect of digestion time of NR latex sample on normalized absorbance using thermoreactor at 100 °C and 30 g L^{-1} of ammonium peroxodisulphate

Effect of reaction time for color development

The molybdenum blue method [6] was chosen for color developing of phosphorus in standard solutions and phosphorus in NR latex sample after digestion. The standing time is important for the coordination between phosphorus (in orthophosphate form) with molybdenum blue solution. The effect of reaction time for the determination of 0.3 mg L^{-1} of phosphorus standard solution, NR latex sample without and with added phosphorus standard (0.02 mg g^{-1}) were illustrated in Figure 28. It was found that the absorbance of all studies show no difference between the range of 10 to 60 minutes. Therefore, the reaction time for color development for 10 minutes was chosen for the next experiment.

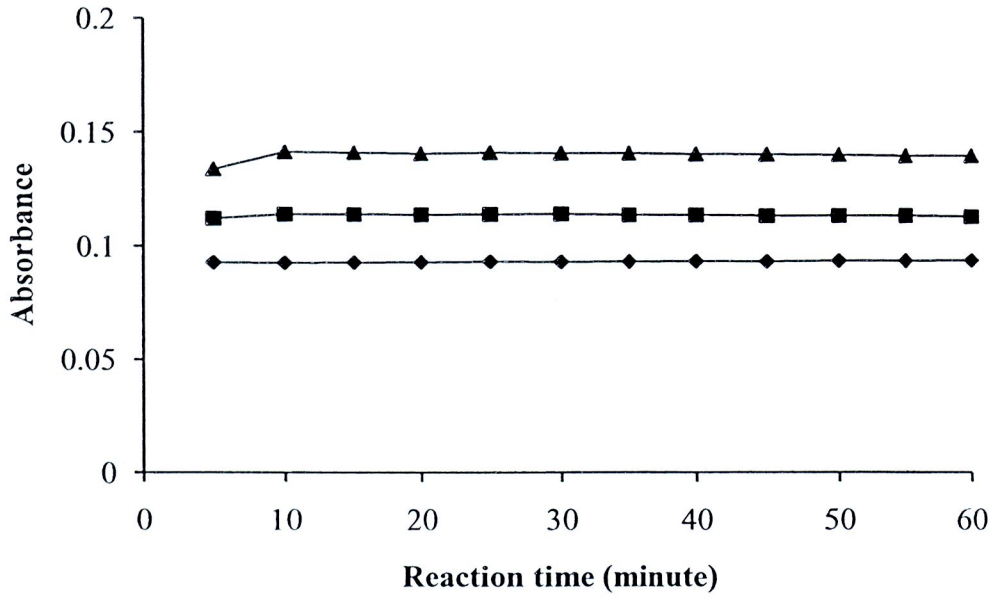

Figure 28 Effect of reaction time for color development of 0.3 mg L^{-1} phosphorus standard solution (◆), NR latex sample (■) and NR latex sample spiked with 0.02 mg g^{-1} of phosphorus standard (▲) with molybdenum blue method after digestion with thermoreactor

Application of UV-assisted digestion technique to NR latex

In this work, UV digestion unit was constructed and applied to NR latex digestion coupled with the oxidizing agent. Five parameters including digestion tube positioning, 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. Thus, the results and discussion are presented below.

Effect of digestion tube position

The digestion tube position is likely to have great effect on the reproducibility of the results. The fabricated UV digestion unit [Figure 20] used stainless steel test tube rack as sample holder. Ten digestion tubes were placed in back row of the rack because the first four rows in the front are too near the UV lamp causing the vigorous boiling of solution. Thus, in this experiment, digestion tube positioning was studied in ten positions at back row of the rack. The results in Figure 29 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 were 0.23 and 2.14, respectively. Therefore, the digestion tube positioning at all positions could be used for NR latex digestion.

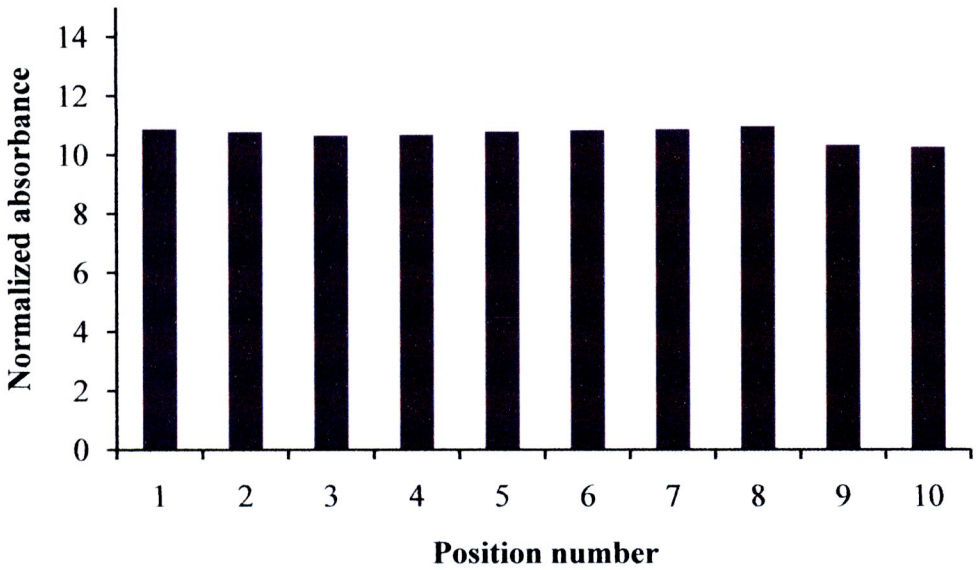

Figure 29 Effect of digestion tube position

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 complex in digested NR latex sample was studied by scanning the wavelength in the range of 400 - 900 nm. The result obtained was illustrated in Figure 30. 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 presented in Figure 23. 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.

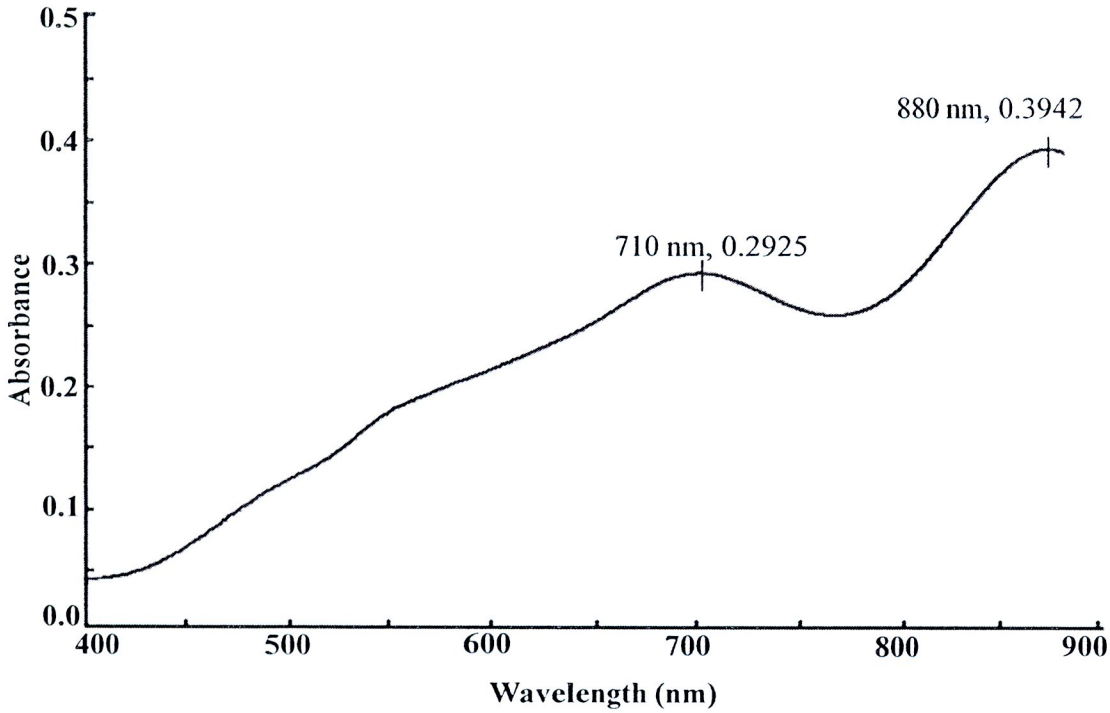

Figure 30 UV spectrum of phosphorus molybdenum blue complex in NR latex sample after UV digestion

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 31, 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 - 100 g L^{-1}. However, the solutions obtained using 10 - 20 g L^{-1} of the oxidizing agent are not clear and has a lot of pieces of the coagulated rubber particles owing to incomplete decomposition reaction. Therefore, the filtration step was carried out in this sample preparation. Moreover, the phosphorus complex compound might absorbed 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.

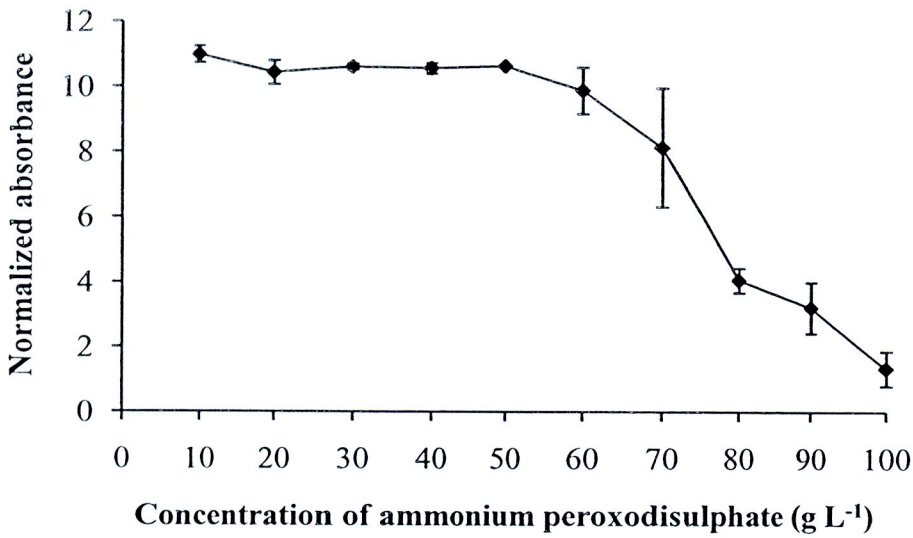

Figure 31 Effect of concentration of ammonium peroxodisulphate used as oxidizing agent coupled with UV-assisted digestion for 50 minutes on normalized absorbance

Effect of digestion time

The temperature of the UV digestion unit is a gradient temperature started from 60 ± 5 °C to 170 ± 5 °C in approximate that is uncontrolling. Therefore, the digestion temperature could not be studied. 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 minutes. Figure 32 shows that the normalized absorbance increased with increasing digestion time, however a plateau was reached after 20 minutes. Moreover, the resultant solutions at 20 - 30 minutes are not clear and the standard deviation (SD) at 40 minutes is higher than that at 50 minutes. Therefore, the digestion of NR latex for 50 minutes was selected in the next experiment.

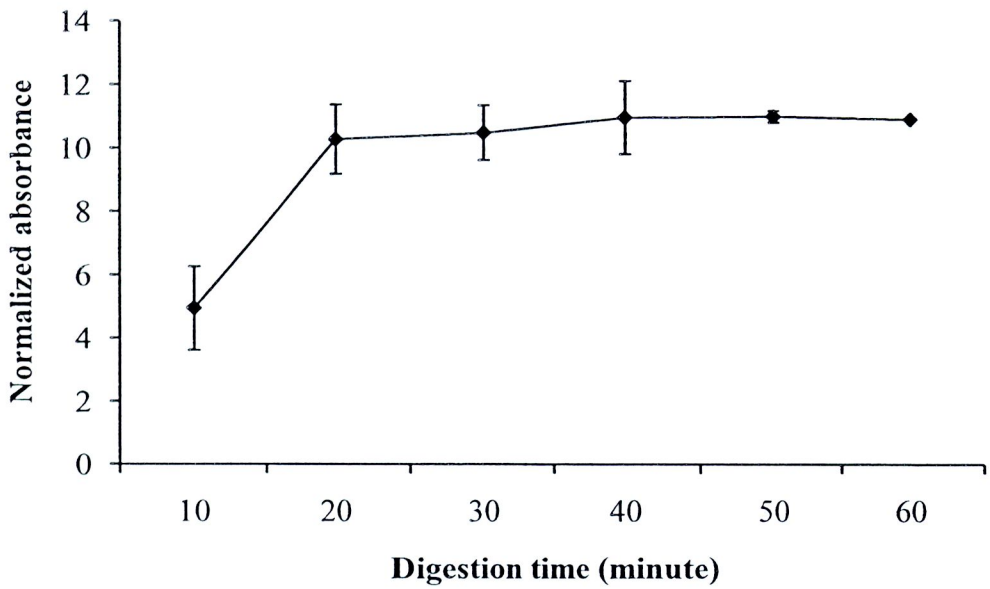

Figure 32 Effect of digestion time of NR latex sample on normalized absorbance by UV-assisted digestion couple with 30 g L^{-1} of ammonium peroxo-disulphate

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 - 60 minutes. The results of 0.3 mg L^{-1} of phosphorus standard solution, NR latex sample without and with added phosphorus standard (0.02 mg g^{-1}) were obtained as illustrated in Figure 33. It was found that, all colorimetric reactions are stable after 10 minutes. Therefore, the standing time for 10 minutes before phosphorus determination was chosen for the next experiment.

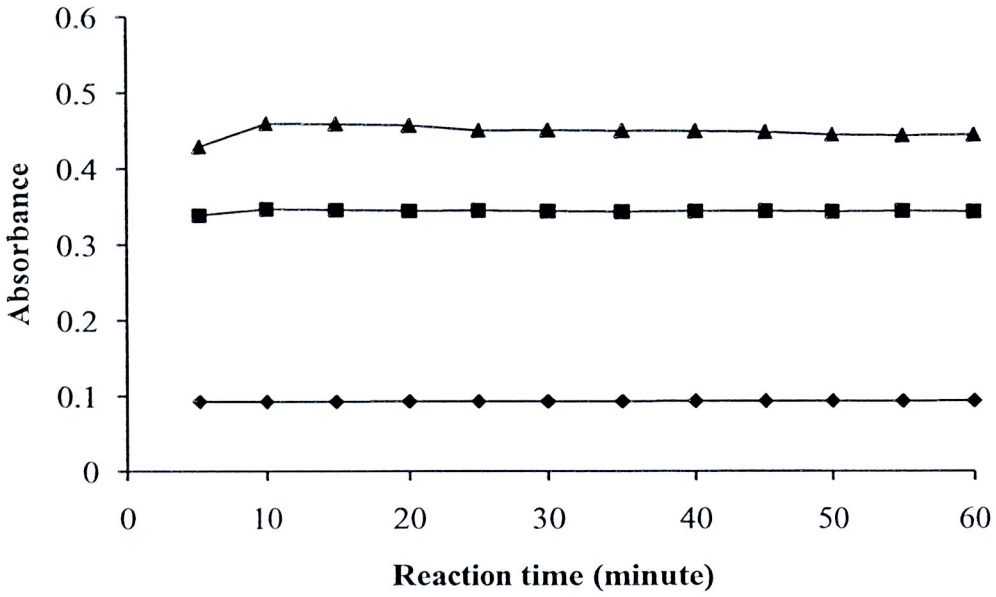

Figure 33 Effect of reaction time for color development of 0.3 mg L^{-1} phosphorus standard solution (◆), NR latex sample (■) and NR latex sample spiked with 0.02 mg g^{-1} of phosphorus standard (▲) with molybdenum blue method after digestion with UV digestion unit

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

Study of the linearity range

The linearity range of phosphorus standard solution was studied in the range of 0.1 - 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 with digestion procedure in which 5 mL of 30 g L^{-1} oxidizing agent was added before coupled with thermoreactor and UV digestion unit. The results were shown in Figure 34. It was found that the digestion step has little effect on calibration curves and the slope values of all calibration curves are not significant difference. It can be concluded that the sensitivities of the calibration method without digestion and with digestion are similar. 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.

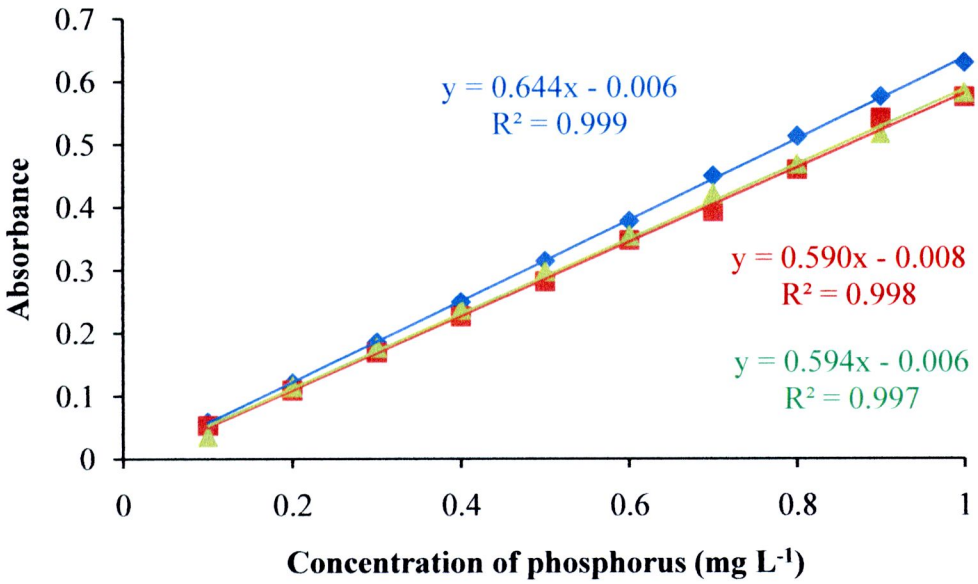

Figure 34 Phosphorus standard curves with digestion step; Thermoreactor (■) and UV digestion unit (▲) and without digestion step (◆)

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

NR latex sample was digested by the both proposed digestion methods and analyzed for the total phosphorus by UV-Vis spectrophotometry. NR latex containing added phosphorus standard solutions were analyzed and represented in Table 7. The recoveries of total phosphorus using the thermoreactor and UV digestion unit are 82.28 - 101.4% and 84.67 - 103.3%, respectively. These recoveries indicated that both digestion techniques can be used for the determination of total phosphorus in NR latex.

Table 7 Analysis of total phosphorus in NR latex

Trial	Thermoreactor			UV digestion unit		
	Added ($mg\ g^{-1}$)	Found ($mg\ g^{-1}$, n=3)	% Recovery[a]	Added ($mg\ g^{-1}$)	Found ($mg\ g^{-1}$, n=3)	% Recovery[a]
1	0	0.167	-	0	0.176	
	0.01	0.177 ± 0.0012	101.4 ± 8.19	0.01	0.186 ± 0.0006	103.3 ± 5.77
2	0	0.163	-	0	0.171	-
	0.02	0.183 ± 0.0006	99.30 ± 2.86	0.02	0.187 ± 0.0005	84.67 ± 2.70
3	0	0.174	-	0	0.164	
	0.03	0.203 ± 0.0003	96.57 ± 0.86	0.03	0.190 ± 0.0010	86.67 ± 3.33
4	0	0.169	-	0	0.168	
	0.04	0.205 ± 0.0021	88.75 ± 5.20	0.04	0.206 ± 0.0010	95.00 ± 2.50
5	0	0.161	-	0	0.163	
	0.05	0.202 ± 0.0015	82.28 ± 3.64	0.05	0.207 ± 0.0015	87.33 ± 3.00

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

Study the effect of interfering ions on recoveries of total phosphorus

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 obtained in Table 8. 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 phosphorus determination [6] when interfering ion concentration are higher than the concentration as presented in Table 8.

Table 8 Effect of interfering ions on recovery of total phosphorus

Interfering ions	Thermoreactor		UV digestion unit	
	Tolerance limit concentration (mg kg^{-1})	%Recovery [a]	Tolerance limit concentration (mg kg^{-1})	%Recovery [a]
AsO_4^{3-}	0.001	97.75 ± 5.29	0.001	94.09 ± 7.29
S^{2-}	1.0	92.77 ± 7.84	1.0	97.66 ± 6.42
NO_2^-	10.0	99.49 ± 1.07	10.0	93.75 ± 1.61
Cr^{6+}	1.0	88.84 ± 5.75	1.0	90.28 ± 4.58
SiO_3^{2-}	1.0	101.5 ± 3.41	1.0	85.80 ± 1.73

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

Study the analytical accuracy and precision

The precision of both proposed methods for NR latex digestion before total phosphorus determination was verified by using the relative standard deviation (%RSD) of within day and between day as shown in Table 9.

Table 9 Analytical characteristics for the determination of total phosphorus in NR latex samples

Parameters	Thermoreactor	UV digestion unit
Linear range (mg L^{-1})	0.1 – 1.0	
Regression equation	$y = 644x - 0.007$	
Correlation coefficient (R^2)	1.000	
LOD (mg L^{-1}), n = 15	3.4×10^{-4}	
LOQ (mg L^{-1}), n = 15	1.1×10^{-3}	
Within day precision[a] (0.02 mg g^{-1}), n = 7	5.97	2.48
Between day precision[a] (0.02 mg g^{-1}), n = 7	3.72	5.98

[a] Relative standard deviation (%RSD) of recoveries of phosphorus standard solution (at 0.02 mg g^{-1}).

Determination of total phosphorus in NR latex samples

In this study, thermoreactor and UV digestion unit were chosen for NR latex preparation before determination of total phosphorus by UV-Vis spectrophotometer compared with Digital image-based colorimeter-artificial neural networks (DIC-ANNs). As illustrated in Figures 35 and 36, RGB value decreased with increasing phosphorus standard concentration whereas the intensity of color of the solution increased [70]. The estimation performance of the DIC-ANNs was performed using the means squared error (MSE) as shown in Table 10. It was found that the average MSE by phosphorus standard testing is 0.0020. The low MSE indicated that the accuracy of the DIC-ANNs is relatively high. Thus, the DIC-ANNs can also be used for the determination of total phosphorus in NR latex.

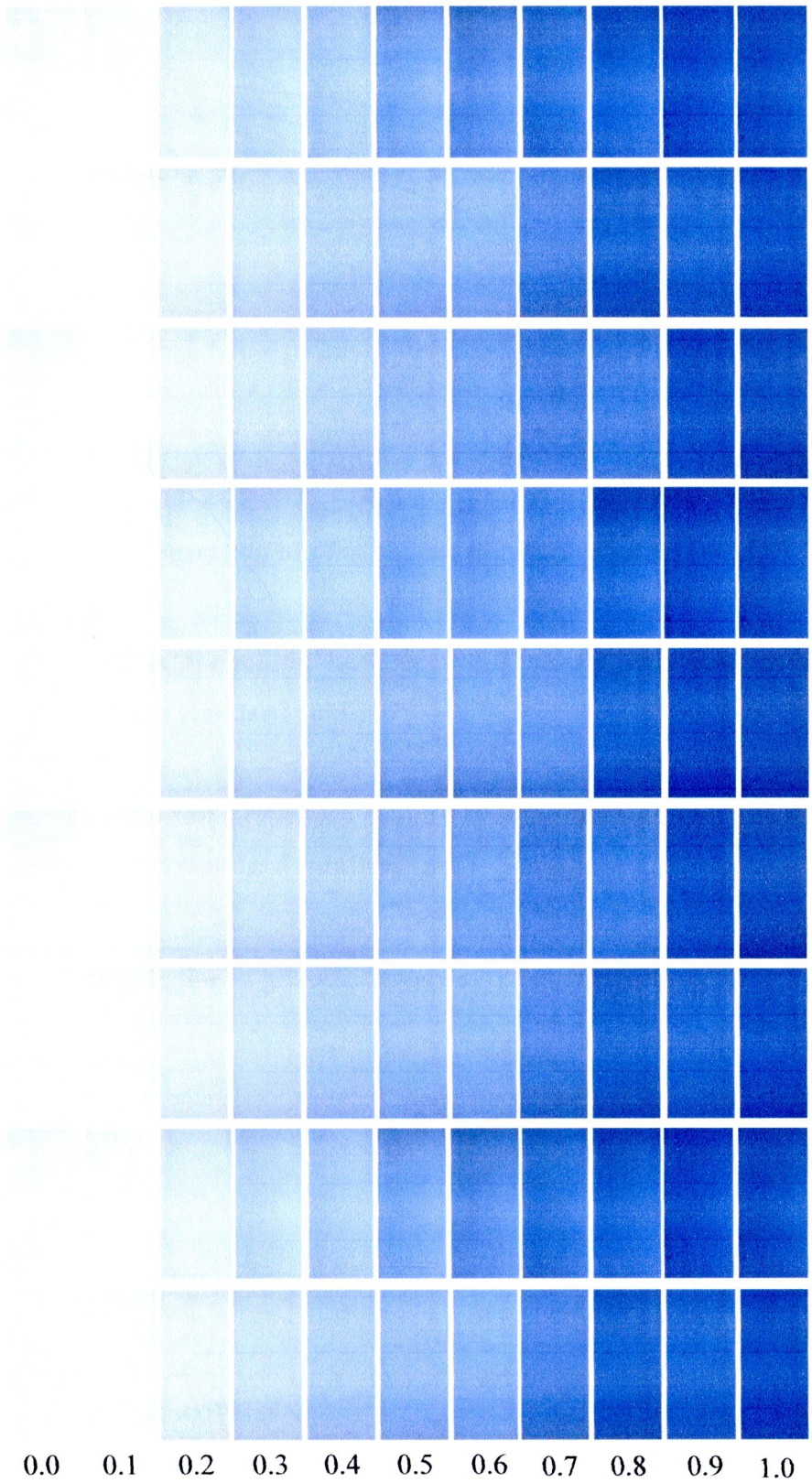

Figure 35 Digital images of phosphorus standard solutions (0-1.0 mg L^{-1}) with molybdenum blue reagent (n=9)

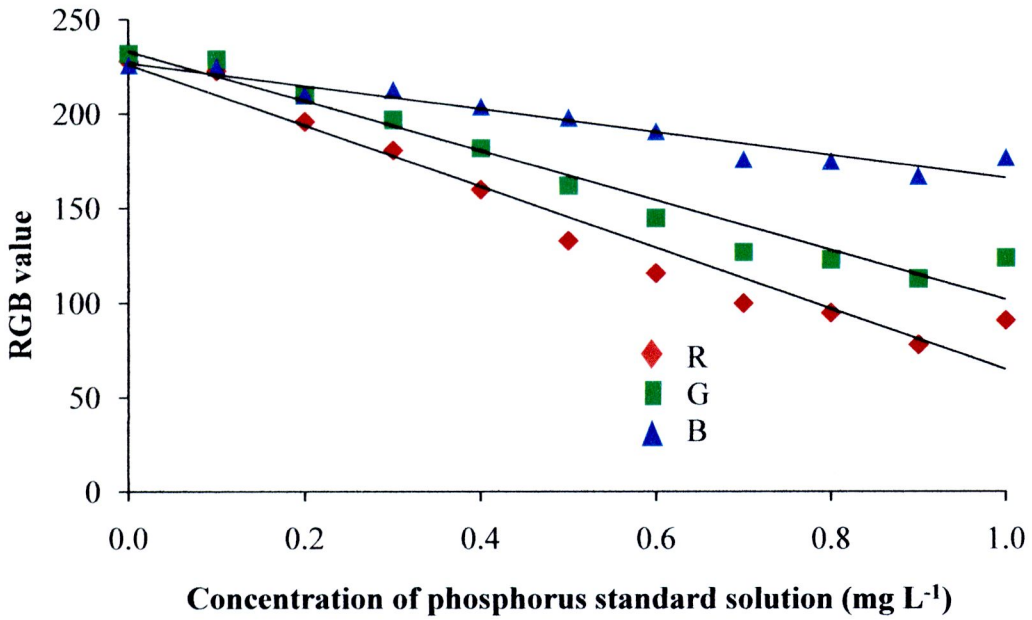

Figure 36 Plots of relationships between RGB values and concentration of phosphorus standard solution

Table 10 Composition of prediction set and means squared error (MSE) of the phosphorus standard solution, (n=9)

Concentration of phosphorus (mg L^{-1})		MSE
True concentration	Found concentration	
0.10	0.11	0.0010
0.30	0.32	0.0017
0.50	0.49	0.0010
0.70	0.68	0.0040
0.90	0.90	0.0022
Average of MSE		0.0020

The images of the complexes between phosphorus in NR latex after digestion and molybdenum blue solution were shown in Figure 37. The results from the determination of total phosphorus in NR latex sample after digestion by the proposed methods were demonstrated in Table 11. The results obtained by using thermoreactor and UV digestion unit and then measuring by the UV-Vis spectrophotometry and the DIC-ANNs show no statistical difference at 95% confidence level by applying the paired t-test.

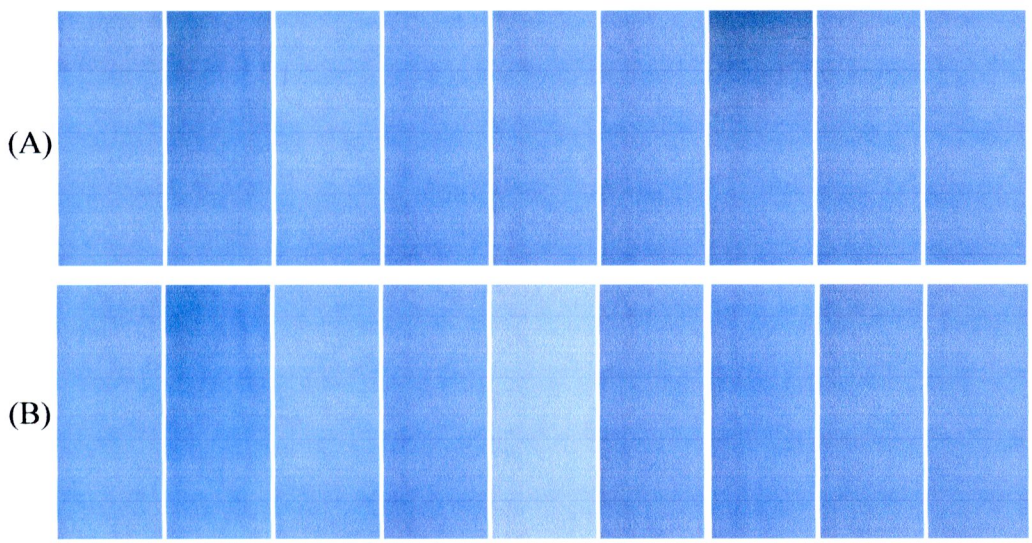

Figure 37 Digital images of NR latex samples after digestion using thermo-reactor (A) and UV digestion unit (B) with molybdenum blue solution (n=9)

Table 11 The results of total phosphorus in NR latex sample, (n=11)

Method	Amounts of total phosphorus in NR latex (mg g^{-1})	
	Thermoreactor	UV digestion unit
Spectrophotometry	0.173 ± 0.0030	0.173 ± 0.0022
DIC-ANNs	0.176 ± 0.0127	0.170 ± 0.0181

The total phosphorus concentrations as presented in Table 11 was found in the range of 0.170 - 0.176 mg g^{-1}. These were not exceed the level as compared to the diammonium phosphate (DAP) addition in NR latex recommended by Department of Industrial Works (0.180 - 0.987 mg g^{-1} of phosphorus ion) [71]. However, Karunanayake found that the phosphorus at the concentration more than 0.0098 mg g^{-1} would effect the stability of NR latex and the physical properties of products [2].