Method Development for Determination of Phosphate in Natural Rubber Concentrated Latex

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ชื่อวิทยานิพนธ์ : การพัฒนาวิธีสำหรับการวิเคราะห์ปริมาณฟอสเฟตในน้ำยางข้น

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บทคัดย่อ

ได้ทำการพัฒนาวิธีอย่างง่ายสำหรับวิเคราะห์หาปริมาณฟอสเฟตที่ละลายได้และปริมาณฟอสเฟตทั้งหมดในน้ำยางข้นด้วยวิธีกรดแอสคอบิกโดยตรวจวัดด้วยวิธีสเปกโทรโฟโต-เมตรีที่ความยาวคลื่น 890 นาโนเมตร สภาวะของน้ำยารวมที่เหมาะสมสำหรับวิธีกรดแอสคอบิกได้แก่ กรดซัลฟิวริกเข้มข้น 2.50 โมลาร์ โพแทสเซียม แอนติโมนิล ตาร์เตรทเข้มข้น 0.01 โมลาร์ แอมโมเนียม โมลิบเดตเข้มข้นร้อยละ 4.0 โดยน้ำหนัก และกรดแอสคอบิกเข้มข้น 0.10 โมลาร์ที่ปริมาตร 50.00, 5.00, 15.00 และ 30.00 มิลลิลิตร ตามลำดับ สารประกอบเชิงซ้อนเกิดสีได้ภายใน 1-2 นาทีและเสถียรนาน 130 นาที ไอออนบวก ไอออนลบ หรือสารประกอบต่างๆโดยรวมที่มีอยู่ในน้ำยางไม่มีผลต่อการวิเคราะห์ปริมาณฟอสเฟตในน้ำยางข้น

วิธีที่เหมาะสมในการเตรียมตัวอย่างน้ำยางข้นสำหรับการวิเคราะห์ปริมาณฟอสเฟตที่ละลายได้เตรียมโดยจับตัวเนื้อยางด้วยเอทานอลและอะซิโตน จากนั้นกรองซีรัมที่ได้ด้วยกระดาษกรองเบอร์ 42 ส่วนการวิเคราะห์ปริมาณฟอสเฟตทั้งหมดเตรียมตัวอย่างโดยย่อยเนื้อยางด้วยกรดไนตริกเข้มข้นร่วมกับไฮโดรเจนเปอร์ออกไซด์

ผลการศึกษาพบกราฟมาตรฐานฟอสเฟตมีความเป็นเส้นตรงอยู่ในช่วง 0.05 ถึง 1.00 มิลลิกรัมฟอสเฟตต่อลิตร มีสมการของความเป็นเส้นตรง y เท่ากับ 0.594x+0.014 ค่าโมลาร์แอบซอร์พติวิตี้เท่ากับ 8.93×10^{4} ลิตรต่อโมลต่อเซนติเมตร ขีดการตรวจวัดต่ำสุดและปริมาณต่ำสุดที่ตรวจวัดเท่ากับ 0.03 และ 0.05 มิลลิกรัมต่อลิตร ตามลำดับ ค่าการได้กลับคืนเฉลี่ยของการวิเคราะห์หาปริมาณฟอสเฟตที่ละลายได้และปริมาณฟอสเฟตทั้งหมดในตัวอย่างน้ำยางสดอยู่ในช่วงร้อยละ 82.0 ถึง 105.2 และ 81.5 ถึง 105.9 ตามลำดับ โดยมีค่าเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่าร้อยละ 5 ค่าการได้กลับคืนเฉลี่ยของการวิเคราะห์หาปริมาณฟอสเฟตที่ละลายได้

และปริมาณฟอสเฟตทั้งหมดในตัวอย่างน้ำยางข้นอยู่ในช่วงร้อยละ 82.6 ถึง 107.7 และ 82.9 ถึง 103.9 ตามลำดับ โดยมีค่าเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่าร้อยละ 5

เมื่อวิเคราะห์หาปริมาณฟอสเฟตในตัวอย่างน้ำยางข้น จำนวน 5 ตัวอย่าง พบปริมาณฟอสเฟตที่ละลายได้อยู่ในช่วง 21.6 ถึง 28.3 มิลลิกรัมต่อกิโลกรัมเทียบฐานปริมาณของแข็งทั้งหมด มีค่าเบี่ยงเบนมาตรฐานน้อยกว่า 1 เมื่อเปรียบเทียบการตรวจวัดปริมาณฟอสเฟตที่ละลายได้ระหว่างเทคนิคสปกโทรโฟโตเมตรีกับเทคนิคไอออนโครมาโทกราฟีในตัวอย่างน้ำยางข้น พบค่าที่ได้ไม่แตกต่างอย่างมีนัยสำคัญ ส่วนปริมาณฟอสเฟตทั้งหมดในตัวอย่างเดียวกันอยู่ในช่วง 257.2 ถึง 318.8 มิลลิกรัมต่อกิโลกรัมเทียบฐานปริมาณของแข็งทั้งหมด มีค่าเบี่ยงเบนมาตรฐานน้อยกว่า 5

นอกจากนี้ได้วิเคราะห์หาปริมาณฟอสเฟตในตัวอย่างน้ำยางสด จำนวน 5 ตัวอย่าง และน้ำยางข้นที่ตกตะกอนแมกนีเซียมและผ่านการปั่นเหวี่ยง พบปริมาณฟอสเฟตที่ละลายได้ในน้ำยางสดและน้ำยางข้นอยู่ในช่วง 17.8 ถึง 22.2 และ 25.1 ถึง 35.3 มิลลิกรัมต่อกิโลกรัมเทียบฐานปริมาณของแข็งทั้งหมดมีค่าเบี่ยงเบนมาตรฐานน้อยกว่า 2 และ 1 ตามลำดับ ปริมาณฟอสเฟตทั้งหมดในน้ำยางสดและน้ำยางข้นอยู่ในช่วง 133.5 ถึง 201.9 และ 251.9 ถึง 385.7 มิลลิกรัมต่อกิโลกรัมเทียบฐานปริมาณของแข็งทั้งหมด มีค่าเบี่ยงเบนมาตรฐานน้อยกว่า 5 และ 4 ตามลำดับ

Thesis Title	:	Method Development for Determination of Phosphate in Natural Rubber Concentrated Latex
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Abstract

A simple method for the determination of soluble and total phosphate in concentrated latex was developed by using ascorbic acid method with spectrophotometric detection at 890 nm. An optimum combined reagent for the ascorbic acid method were 2.50 M sulfuric acid, 0.01 M potassium antimonyl tartrate, 4.0% ammonium molybdate and 0.10 M ascorbic acid at 50.00, 5.00, 15.00 and 30.00 mL, respectively. The color development of phosphate complex occurred within 1-2 minutes and was stable up to 130 minutes. Cations, anions or total matrices in latex did not interfere the quantification of phosphate in concentrated latex.

The sample preparation of concentrated latex for soluble phosphate included latex coagulation by ethanol and acetone, followed by a serum filtration by using a Whatman no. 42 filter paper, while the sample preparation for total phosphate used nitric acid and hydrogen peroxide for latex digestion.

Under an optimum condition, a calibration was linear over the concentration ranged from 0.05-1.00 mg PO_4^{3-} L^{-1}, with a linear equation of y = 0.594x+0.014. The molar absorptivity, limit of detection and limit of quantification were found to be 8.93×10^4 L mol^{-1} cm^{-1}, 0.03 mg PO_4^{3-} L^{-1} and 0.05 mg PO_4^{3-} L^{-1}, respectively. The mean percentage recoveries of soluble and total phosphates for field latex were in the good range of 82.0-105.2% and 81.5-105.9% respectively, with the relative standard deviations (RSD) less than 5%. For concentrated latex, the mean percentage recoveries of soluble and total phosphates were in the good range of 82.6-107.7% and 82.9-103.9% respectively, with the RSD less than 5%.

The concentrations of phosphates detected in concentrated latex samples collected from five different factories. The concentrations of soluble phosphates detected in concentrated latex samples were in the range of 21.6-28.3 mg kg^{-1} based on %TSC, with the standard deviation less than 1. Moreover, soluble phosphate in these latex samples were also compared by two detection techniques, spectrophotometry and ion chromatography. It was found that there was no significant difference. The concentrations of total phosphates detected in those latex samples were in the range of 257.2-318.8 mg kg^{-1} based on %TSC, with the standard deviation less than 5.

The developed method was also carried out by determining phosphates contents in field latex samples which collected at five different districts located in the Songkhla province of Thailand and concentrated latex samples after precipitating magnesium as magnesium ammonium phosphate followed by latex centrifugation. The concentrations of soluble phosphates detected in field and concentrated latex samples were in the range of 17.8-22.2 and 25.1-35.3 mg kg^{-1} based on %TSC, with the standard deviation less than 2 and 1, respectively. The concentrations of total phosphates detected in field and concentrated latex samples were in the range of 133.5-201.9 and 251.9-385.7 mg kg^{-1} based on %TSC, with the standard deviation less than 5 and 4, respectively.

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CHAPTER 1
INTRODUCTION

1.1 Background and Rationale

The concentrated natural rubber, *Hevea brasiliensis* latex could be prepared by different methods such as centrifugation, creaming and evaporation in order to obtain at least 60% dry rubber contents. The concentrated latex has been widely used in many rubber industries involving dipping process, latex foam process and coating (Blackley, 1997, Vol. 3, p. 155, 229). To obtain a good quality control of concentrated latex, it is necessary to determine the quantity of magnesium which is found to be a major cause of flocculation and destabilization of latex (Karunanayake and Perera, 2006). After magnesium content has been known, it is principally removed by adding phosphate in the form of either diammonium hydrogen phosphate or diammonium phosphate into field latex before latex centrifugation, resulting in precipitation as magnesium ammonium phosphate. The amount of phosphate added can be calculated to reduce magnesium in latex to zero (Karunanayake and Perera, 2006), however, after treatment, concentration of magnesium varies up to 100 mg L^{-1}. Therefore, some manufacturers of dipped products add excess phosphate in order to obtain low concentration of magnesium at least 30 mg L^{-1} in latex (Karunanayake and Perera, 2006). According to this fact, effects of phosphate have been further studied by several groups. Pendle and Gorton (1978) reported that in contrast to latexes containing fillers, stability of uncompounded latexes was reduced by the presence of complex phosphates as trisodium polyphosphate and tetrasodium pyrophosphate since the remaining of phosphate in the bulk aqueous phase of latex contributes to increased ionic strength. Karunanayake and Perera (2006) concluded that the concentration of 30 mg L^{-1} phosphate was found to produce the highest latex stability during storage and better film properties of dipped products. Furthermore, they reported stability of latex was changed during storage and process by the addition of excess magnesium and phosphate into latex (Karunanayake and Perera, 2006). It has been reported that phosphate causes the destabilization of concentrated latex and compounded latex (Pendle and Gorton, 1978).

The latex industry in Suratthani and Trang provinces of Thailand were interviewed for the effect of phosphate ion in concentrated latex. It was found that too high concentration of phosphate ion residues causes the destabilization of latex. The higher phosphate concentration causes the decrease in mechanical stability time (MST) and the increase in potassium hydroxide number (KOH No.), resulting in the destabilization of compounded latex for dipped product. The phosphate contents are likely to be from the addition of diammonium hydrogen phosphate (DAHP) in field latex to remove magnesium in latex.

The manufacturers of dipped products in Songkhla and Suratthani provinces of Thailand were interviewed for the effect of phosphate ion in concentrated latex for dipping process. The compound latex provides low adhesive on the mold in dipped process when containing too high phosphate ion. The manufacturers point out that it is difficult to control the phosphate content in the concentrated latex. However, the manufacturers of the rubber industry such as glove, toys and condoms conclude that the concentration of phosphate less than 100 mg PO_4^{3-} L^{-1} is found to be suitable for film properties of dipped products. From those points of news, therefore, it is important to develop method for determination of phosphate contents in concentrated latex.

Analytical methods and instrumental methods have been applied for the determination of phosphate in a wide range of samples such as water, detergents, soils and fertilizers. Classical analysis involves gravimetric methods related to the precipitation of phosphate as, for example, magnesium pyrophosphate, magnesium ammonium phosphate hexahydrate or ammonium phosphomolybdate, while volumetric methods relates to the titration of ammonium phosphomolybdate with sodium hydroxide (Takhulee *et al.,* 2005). Due to a lack of sensitivity of classical methods, most samples have been analyzed by instrumental techniques including atomic absorption spectrometry, flow injection analysis with photometric detection (Jing-fu and Gui-bin, 2000; Estela and Cerdà, 2005), high performance liquid chromaptography with spectrophotometric detection (Jing-fu and Gui-bin, 2000), spectrophotometry (He and Honeycutt, 2005; Mahadevaiah *et al.,* 2007 and Shyla *et al.,* 2011), colorimetry (Liberatore, 2010) and ion chromatography (Karmarkar, 1999; AOAC 993.3, 2000; Sekiguchi *et al.,* 2000 and Glenn *et al.,* 2007). Compared to other

techniques, the spectrophotometric detection involving ammonium molybdate method (Pendle and Gorton, 1978; Jing-fu and Gui-bin, 2000; Haberer and Brandes, 2003; Jastrzębska, 2009 and Shyla *et al.,* 2011) and phosphovanadomolybdate method (ASTM Standard D515-78) appears to be the most practical method for phosphate. Ammonium molybdate method is frequently used and more sensitive than phosphovanadomolybdate method (ASTM Standard D515-78 and Mendham *et al.,* 2000). It relates to the reaction between phosphate and molybdate in an acidic solution, in which molybdophosphoric acid is formed and further reduced by various reducing agents such as ascorbic acid (Jing-fu *et al.,* 2000; Haberer and Brandes, 2003; Takhulee *et al.,* 2005; Jastrzębska, 2009 and Shyla *et al.,* 2011), thiourea (Shyla *et al.,* 2011), hydrazine sulfate (Mendham *et al.,* 2000; Jastrzębska, 2009 and Liberatore, 2010), the mixture of hydrazine sulfate and hydroquinone (1:1) (Jastrzębska, 2009), stannous chloride (Van der Bie, 1947; Muñoz, 1997 and Spivakov *et al.,* 1999) and ferrous sulfate (Tunnicliffe, 1956). It is apparent that ascorbic acid is most promising agent with the presence of antimony serving as a catalyst to increase the reduction rate. While a few numbers of procedures for the determination of phosphate in latex have been reported (Tunnicliffe, 1956), those procedure require cationic column for removal of interferences and time consuming of sample preparation. Moreover, no report has been made to explain which form of phosphate affecting a quality of latex. Hence, we attempt to develop simple method for sample preparation of concentrated latex to determine both soluble and total phosphate with spectrophotometric detection based on ammonium molybdate method with ascorbic acid served as a reducing agent.

1.2 Natural rubber latex

1.2.1 Characteristic and composition of *Hevea brasiliensis* latex

Natural rubber (NR) latex from *Hevea brasiliensis* has been recognized as the most important source of natural rubber among over 2,000 species of higher plants producing polyisoprene which structure is shown in Figure 1.1. The characteristic of latex is whitish milk liquid containing colloidal suspension of rubber particles (Cacioli, 1997 and Hussin *et al.,* 1998). The density of serum fraction is 1.021 g dm^{-3} (Kajornchaiyakul, 2006). Serum commonly contains water-soluble

substance such as soluble protein, amino acid, carbohydrates, inositol, organic and inorganic compound. A rubber particle as shown in Figure 1.2 has 200-20,000 angstrom and its density is 0.975-0.980 g mL^{-1}, with pH approximately 6.5-7.0 (Cacioli, 1997). The NR latex consists of approximately 25-40% dry rubber content (DRC) and 5-10% non-rubbers including proteins, carbohydrates, lipids and inorganic compositions. The composition of non-rubber components of solids NR is given in Table 1.1.

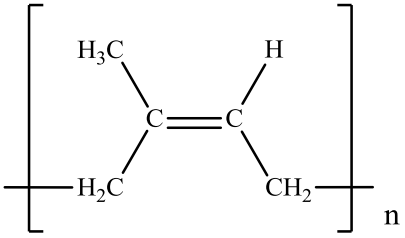


Figure 1.1 *Cis*-1, 4-polyisoprene.

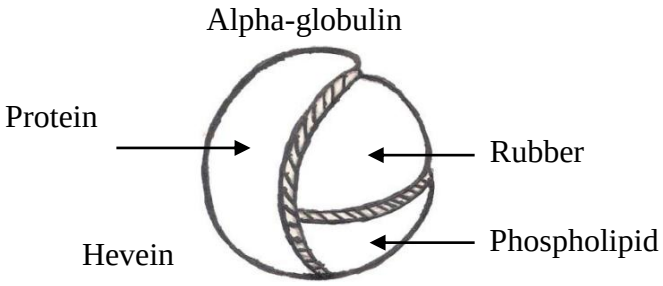


Figure 1.2 Characteristics of the rubber particles.

Table 1.1 Composition of field latex (Cacioli, 1997 and Galli *et al*., 2002)

Components	**Weight by dry rubber (%)**
Total Solids Content (TSC)	36
Dry Rubber Content (DRC)	33
Protein	1-1.5
Resin	1-2.5
Ash	1
Sugar	1
Water	27

Trace metals are one of the mineral components presented naturally in NR latex. Some research groups reported that mostly inorganic elements found in latex were potassium, sodium, calcium, magnesium, manganese, copper, sulfur and phosphorus (Ng, 1972 and Scott *et al.,* 2003). Furthermore, sodium, calcium and copper are associated within the rubber particles, while iron and potassium are found in the serum. Table 1.2 shows the average percentages of metal contents detected in *Hevea brasiliensis* latex. This latex was filtered through a membrane with 0.45-μm porosity and analyzed by inductively-coupled plasma spectrophotometry (Kang *et al.*, 2000). However, the contents of metals are variable depending on the clone of rubber tree and the age of *Hevea brasiliensis* latex. Some trace elements such as copper, manganese and iron serve as oxidative catalysts (Lee e*t al.*, 1998 and Sělih *et al.*, 2007).

Table 1.2 Metal ion content (mg L^{-1}) in *Hevea brasiliensis* latex (Kang *et al.,* 2000)

Metal	**Mean concentration (mg L^{-1}) (n=3)**
Calcium	8.9
Magnesium	587
Potassium	816
Iron	0.27
Copper	0.27
Manganese	7.45

1.2.2 Types of latex

1.2.2.1 Field latex

Natural rubber latex or field latex in Thailand are mostly tapped from *Hevea brasiliensis* without any preservatives added (Abraham *et al.,* 2009). Field latex contains about 30% DRC and is concentrated by centrifugation to about 60% DRC for commercial latex productions (Rippel *et al.,* 2003). Field latex can be self-coagulated when left at room temperature. This is because of proteins and lipids in NR latex. The soluble proteins are precipitated by denaturing the stability of natural

proteins with sodium dodecyl sulfate (SDS), followed by centrifugation process (He and Honeycutt, 2005).

1.2.2.2 Concentrated latex

The field latex is not commonly used in its original form due to high water content and susceptibility to bacterial attack. Therefore, it is necessary to preserve and concentrate it, resulting in at least 60% DRC. The process for concentrated latex involves evaporation, creaming (Allen, 1972), centrifugation (Perrela *et al.*, 2002) and electrostatic process (Blackley, 1997, Vol. 2, p. 42-69). The concentrated latex types obtained from centrifugation process and preservation, as shown in Table 1.3.

Table 1.3 Concentrated latex types obtained from centrifugation process and preservation (Kajornchaiyakul, 2006)

Concentrated latex type	**Preservation**
Concentrated latex from centrifugation process	
(a) High ammonia (HA)	0.7% ammonia
(b) Low ammonia-santobrite (LA-SPP)	0.2% ammonia + 0.2% sodiumpenta chlorophenate
(c) Low ammonia-boric acid (LA-BA)	0.2% ammonia + 0.24% boric acid + 0.05% lauric acid
(d) Low ammonia-zinc diethyl dithiocarbamate (LA-ZDC)	0.02% ammonia + 0.10% ZDC + 0.05% lauric acid
(e) Low ammonia-tetramethylthiuram disulphide (TMTD)/zinc oxide (ZnO)	0.02% ammonia + 0.013% TMTD + 0.013% ZnO + 0.05% lauric acid

1.2.3 Latex coagulation

Colloidal suspensions of latex or latex coagulation are stable because of negative charge particles which repel one another. These charges result from anions that are bound to the surface of the particles. The model of latex coagulation with acid is shown in Figure 1.3. The acids used for latex coagulation are, for

example, formic acid and acetic acid. These acids will give hydrogen ion (H^+) and react with carboxylic group ($R\text{-}COO^-$) of latex, resulting in the fatty acid being around the rubber particle (equation 1.1) (Blackley, 1997, Vol. 1, p. 242). This fatty acid does not dissolve in water, therefore, while this reaction occurs, the field latex will be coagulated quickly to obtain rubber and serum of the latex (Cacioli *et al.*, 1997). Apart from acids, alcohols such as methanol and ethanol have been used as latex coagulation substances (Braga, 2000 and Galembeck *et al.*, 2000). The addition of water-soluble salts of divalent cation such as calcium chloride and calcium nitrate can also be used to coagulate the field latex (Blackley, 1997, Vol. 1, p. 259; Karunanayake and Perera, 2006 and Santipanusopon and Riyajan, 2009).

$$H^+ + R\text{-}COO^- \longrightarrow R\text{-}COOH \text{ (Fatty acid)} \qquad (1.1)$$

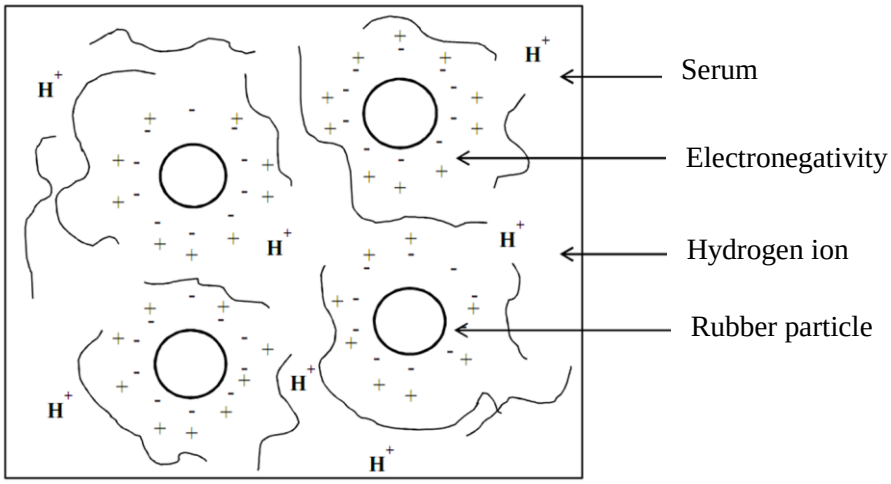


Figure 1.3 Model of rubber latex coagulation with acid (Blackley, 1997, Vol. 1, p. 242)

1.2.4 Effect of magnesium and other divalent metals on latex properties

The magnesium ions in the concentrated latex cause destability of concentrated latex. In general, the magnesium can be reduced by the addition of phosphate compounds. However, the residual phosphate can adversely affect the stability of the concentrated latex, rubber compound, dipped products and rubber products (Karunanayake and Perera, 2006). It has been reported that viscosity of the

rubber compound decreased with increasing amounts of phosphate ions (Takhulee *et al.*, 2010).

Ng *et al.* (1970) reported that the high concentration of calcium and magnesium ion caused a destabilization of *Hevea brasiliensis* latex. This was attributed to the reduction of charges on the surface rubber particles. Calcium and magnesium presented in natural rubber latex could also form ionic cross-link between amino acids or carboxylic acid, thus leading to higher gel content. The morphology of rubber particles obtained after the centrifugation process has been investigated in relation with adsorbed species. Rippel *et al.* (2005) reported that calcium salt crystallites formed around the particles affected a film formation. These crystallites are compatible with the hydrocarbon matrix of the rubber particles and associated with their membrane materials.

1.3 Phosphate compound and phosphate ions in natural rubber latex

Phosphorous are the eleventh most abundant element and its known terrestrial minerals are orthophosphates (Mahadevaiah *et al.*, 2007). The phosphate ion, PO_4^{3-}, is an inorganic polyatomic ion, and is found in lakes and streams because of agricultural operations from either animal or fertilizers (Hasma, 1992). Phosphate is used in detergents and its acid, H_3PO_4 is used as a tart flavor in cola beverages (Hasma *et al.*, 1986 and Hasma, 1992). Excessive use of fertilizers and detergents leads to over growth of aquatic plants with reducing the dissolved oxygen level of water and further is detrimental to aquatic life (Hasma, 1992). Therefore, the analytical chemistry of phosphorus is very important in many fields, for example, medical and clinical science, agriculture and environmental science (Sakdapipanich *et al.*, 2007 and Mahadevaiah *et al.*, 2007).

1.3.1 Type of phosphate compound

1.3.1.1 Orthophosphates

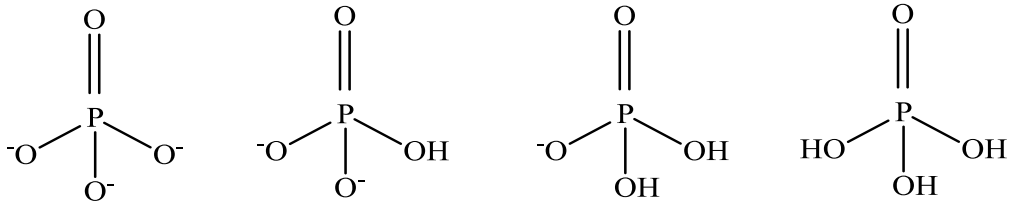
Orthophosphates are, for example, trisodium phosphate (Na_3PO_4), disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4) and diammonium hydrogen phosphate ($(NH_4)_2HPO_4$). These forms will give PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^{-}$, H_3PO_4 when dissolved in water (equation 1.2-1.5 and Figure 1.4).

H_3PO_4 (aq) + H_2O (l) ⟷ H_3O^+ + $H_2PO_4^-$ $K_1 = 5.9\times10^{-3}$ (1.2)

H_2PO_4 (aq) + H_2O (l) ⟷ H_3O^+ + HPO_4^{2-} $K_2 = 6.2\times10^{-3}$ (1.3)

HPO_4 (aq) + H_2O (l) ⟷ H_3O^+ + PO_4^{3-} $K_3 = 4.8\times10^{-13}$ (1.4)

PO_4^{3-}(aq) + H_2O (l) ⟷ OH^- + HPO_4^{2-} $K_4 = 2.2\times10^{-2}$ (1.5)

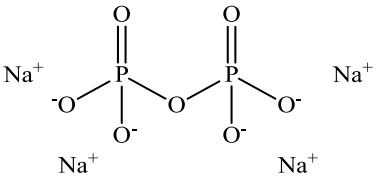


PO_4^{3-} HPO_4^{2-} $H_2PO_4^-$ H_3PO_4

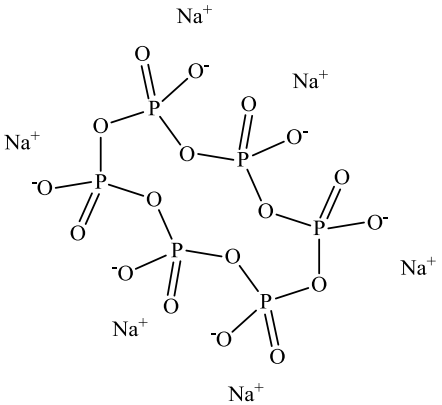
Figure 1.4 Structure of orthophosphate forms.

1.3.1.2 Polyphosphates

Polyphosphates are, for example, sodium hexametaphosphate $Na_3(PO_3)_6$, sodium triphosphate ($Na_5P_3O_{10}$), sodium pyrophosphate ($Na_4P_2O_7$) as shown in Figure 1.5. Polyphosphate is hydrolyzed in water as orthophosphate and its reaction depends on pH and temperature.



Sodium pyrophosphate

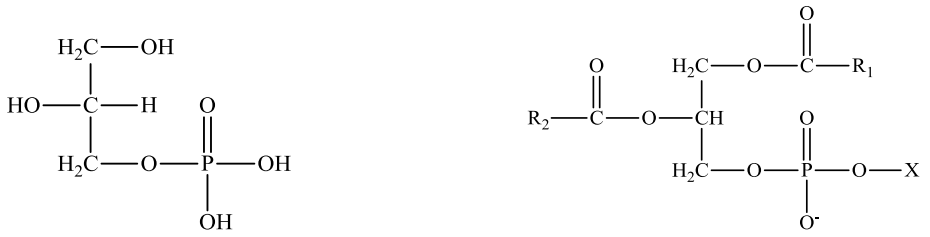


Sodium hexametaphosphate

Figure 1.5 Structure of polyphosphate forms.

1.3.1.3 Organic phosphates

Organic phosphates such as nucleic acid, phospholipids and sugar phosphate, are found in biological components (Csuros, 1997). Phospholipids (about 1%) are the major lipid component of biological membranes. They consist of *sn*-glycerol-phosphate esterified to fatty acid at its C_1 (*sn*-1) and C_2 (*sn*-2) positions and to a polar group (X) at its phosphoryl group (Figure 1.6).



sn-glycerol-phosphate Phospholipids

Figure 1.6 *sn*-Glycerol-3-phosphates and general structure of phospholipids.

1.3.2 Phosphate ions in concentrated latex

Natural latex contains magnesium which its concentration depends on a season and a fertilizer. The removal of magnesium before concentrating latex is performed by the addition of diammonium hydrogen phosphate (DAHP) or diammonium phosphate (DAP). These chemicals will react with ammonia in the latex, resulting in the precipitation of the magnesium ammonium phosphate (equation 1.6). The high quantity of phosphate added will affect the mechanical stability time (MST) of concentrated latex and increase viscosity (Karunanayake and Prerera, 2006; Takhulee *et al.*, 2010).

$$(NH_4)_2HPO_{4\,(aq)} + Mg^{2+}_{(aq)} \longrightarrow Mg(NH_4)PO_{4\,(s)} + H^{+}_{(aq)} + NH_4^{+}{}_{(aq)} \quad (1.6)$$

1.3.3 Toxicity of phosphate compound

The review of the oral toxicity of inorganic phosphates clearly shows that these compounds exhibit very low toxicity (Weiner *et al.*, 2001). The systemic exposure to inorganic phosphates due to consumable product use is likely to be much

lower than the systemic exposure due to food additive use. Therefore, based on the low exposure and low systemic toxicity, very little concern is associated with the systemic toxicity after exposure to inorganic phosphates at levels expected to be present in consumable products.

1.4 Analytical procedures for phosphate determination

1.4.1 Sample digestion

Sample digestion is achieved by the utilization of various acids and oxidizing agents for the complex matrix like latex. The digestion of concentrated latex with concentrated nitric acid (HNO_3) was applied for a preparation step of determination of copper (ISO: 8053). The single acid or combination of acids, such as nitric acid and sulfuric acid (H_2SO_4) were used for an acidic digestion with high temperature.

1.4.2 Complexation methods

1.4.2.1 Ascorbic acid method

Ascorbic acid method is based on the reaction of phosphate and ammonium molybdate in an acidic medium to form antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid and its color is proportional to the phosphorus concentration. The resulting blue complex exhibits maximum absorption at 880-890 nm. The reaction steps for ascorbic acid method are shown in equation 1.7-1.9 (Csuros, 1997).

$$PO_4^{3-} + 12(NH_4)_2MoO_4 + 24H^+ \longrightarrow (NH_4)_3PO_4 \cdot 12MoO_3 + 21NH_4 + 12H_2O \quad (1.7)$$

Ammonium phosphomolybdic acid

$$(NH_4)_3PO_4 \cdot 12MoO_3 + C_8H_6K_2O_{13}Sb_2 \longrightarrow PSb_2Mo_{10}O_{40}^{3-} \quad (1.8)$$

Antimony phospho molybdate

$$PSb_2Mo_{10}O_{40}^{3-} \xrightarrow[C_6H_8O_6]{} \text{Mo (V)} \quad \text{Molybdenum blue} \qquad (1.9)$$

1.4.2.2 Molybdenum blue method

The principle of molybdenum blue method is similar to ascorbic acid method, in which orthophosphate and molybdate ion condense in acidic medium to give molybdophosphoric acid (phosphomolybdic acid) with further reduction by reducing agents to form a blue color. The intensity of the blue color is proportional to the amount of phosphate initially incorporated in the heteropoly acid. The resulting blue complex exhibits maximum absorption at 820-830 nm when using 0.5 M sulfuric acid and hydrazinium sulfate. The steps for molybdenum blue method are shown in equation 1.10-1.11 (Mendham *et al.*, 2000 and Thabano *et al.*, 2004).

$$H_3PO_4 + 12H_2MoO_4 \longrightarrow H_3PO_4(Mo_3O_{10})_4 + 12H_2O \qquad (1.10)$$

Phosphomolybdic acid

$$H_3P(Mo_3O_{10})_4 \longrightarrow \text{Mo (V)} \qquad (1.11)$$

Mo (VI) — Molybdenum blue

1.4.3 Choice of reducing agents of phosphate analysis

Many reducing agents were used for phosphate in various matrices. Ascorbic acid was used for the analysis of phosphorus in the solution of ammonium molybdate and sulfuric acid (Greenfield and Kalber, 1954). The color was developed completely in 24 hours at room temperature and 30 minutes at 60ºC with the stability of color at least 3 days and the concentration of phosphate was detected at least 500 µg P L^{-1}.

To and Randall (1977) used stannous chloride as a reducing agent in the molybdenum blue method for determination of phosphate in seawater, in which phosphate was detected in the range of 0.01-2.00 µg L^{-1}. This complexation is dependent on the concentrations of salt and water temperature. Compared to the ascorbic acid method, phosphate can be analyzed in the concentration range of 0.07-

2.00 µg L^{-1}. The color of solution in ascorbic acid method occurs later than molybdenum blue method, however, its color of solution is stable at room temperature.

The other interesting reducing agent is sodium sulphide. Mahadevaiah *et al.* (2007) used for the determination of phosphate in sugarcane juice, water and detergent samples. The method was preferred by reacting phosphate with molybdate and sodium sulphide in acidic conditions to obtain phosphomolybdate, giving a high absorption at 715 nm.

The Association of Official Analytical Chemists, AOAC (2000) describes a standard method for determination of orthophosphate by reacting ammonium molybdate and potassium antimonyl tartrate in an acidic medium with a dilute solution of phosphate to form the antimonyl phosphomolybdate complex. Upon a reduction with ascorbic acid, this complex forms an intense blue color which absorbs at 660 nm and the analytical concentration range for this method is 0.06-3.00 mg L^{-1} orthophosphate, with a requirement of significant sample dilutions if applied to the analysis of soft drinks.

Murphy and Riley (1958) reported that the disadvantage of using ascorbic acid was the slow color development. However, color development was considerably faster when antimony was introduced as a catalyst. The combination of ascorbic acid as a reductant and antimony as an activator has been used for determination of phosphate (Murphy and Riley, 1962).

Harwood *et al.* (1969) summarized these shortcomings of analysis of phosphate in water as follows

1) Absorbance is temperature-dependent.

2) Accurate time intervals for reading the color are necessary due to relatively unstable of color.

3) There is an appreciable salt error.

4) Arsenate interferes but can be removed chemically.

5) Copper interferes appreciably above 50 pg Cu L^{-1}.

6) The method is unsatisfactory at high concentration levels.

Greenfield and Kalber (1954) reported that the use of ascorbic acid as a reductant decreased these problems.

1.5 Analytical techniques for phosphate

Accurate and sensitive analytical method is required for a quality control of detection of phosphate. UV-Visible spectrophotometry is currently the most inexpensive and simple method. The alternative techniques for the determination of phosphate in samples are ion chromatography and flow injection analysis.

1.5.1 UV-Visible spectrophotometry

Spectrophotometry for ultraviolet (UV) covers the wavelegth from 190 to 350 nm, while visible (VIS) region is from 350 to 800 nm and the near infrared (NIR) is from 800 to 2500 nm. The components of a spectrophotometer depend on the region of the electromagnetic spectrum.

A spectrophotometer contains four basic parts. The source provides radiation over the wavelength range of interest. Light from the source is passed through a wavelength selector that provides a limited band of wavelengths. The radiation exiting the wavelength selector is focused onto a detector which converts the radiation into electrical signals (Brown, 2005).

The relationship between absorption of light absorbed by a solution and the concentration of the solution has been described by Beer's law, as shown in Equation 1.12

$$A = \varepsilon bc \qquad (1.12)$$

Where A is light absorption (absorbance) of the material under test

ε is a molar absorptivity or molar extinction coefficient (cm^2 $mole^{-1}$)

b is a path length (cm)

c is a concentration of the material under test (mol L^{-1})

According to Beer's law, the amount of light transmitted through a colored solution decreases exponentially with increase in a concentration of the colored substance (Skoog, 1996).

All spectrophotometers are designed to measure the absorption of radiant energy and their basic components are shown in Figure 1.7.

1) A light source of radiant energy such as deuterium lamp is the primary source of UV radiation. Tungsten-halogen lamps are used for the visible and NIR regions (Brown, 2005).

2) A wavelength selector such as filter photometers, grating and prism monochromator is used for the isolation of a desired wavelength from the source.

3) Transparent container (cuvette sample) for sample and blank.

4) A detector such as photomultiplier tube and photodiode arrays detector is used to convert the radiant energy to a measurable signal and a readout device that displays the signal from the detector.

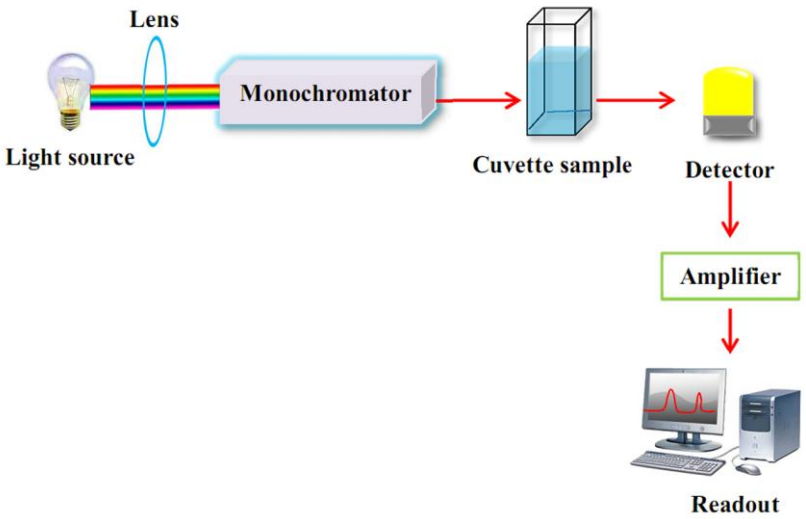


Figure 1.7 Component of spectrophotometer.

Broberg and Pettersson (1988) reported that spectrophotometric methods for phosphate determination were based on the formation of 12-molybdophosphoric acid from phosphate and molybdate in an acidic solution and the subsequent reduction to a blue heteropoly compound.

Ünal *et al.* (2006) applied a modified spectrophotometric ammonium molybdate method based on the analysis of blue color from the reaction of orthophosphates with ammonium molybdate and ascorbic acid. Skrökki (1995) discussed the molybdenum blue method with hydrazine sulphate for the determination of phosphates in meat products. The modified methods described by Lee *et al.* (1998)

and Mendonca *et al.* (2001) were also reported for the determination of soluble orthophosphates in meat samples.

Motomizu and Li (2005) reported the classical spectrophotometric methods based on the reaction of orthophosphate with molybdate, in the absence or in the presence of vanadate or antimonate, in an acidic medium. Heteropoly acids or molybdophosphoric acids, such as phosphomolybdenum yellow or molybdenum yellow and its reduction product called as phosphomolybdenum blue or molybdenum blue can be formed. The molybdenum yellow shows the absorption maximum in UV region where the excess amounts of molybdate also shows light absorption at about 400 nm. In the presence of some reducing agents such as ascorbic acid, hydrazine and tin (II) chloride, molybdenum yellow can be reduced to form molybdenum blue which shows stronger light-absorption than the molybdenum yellow. Table 1.4 summarizes the spectrophotometric method for determination of phosphate in various samples.

1.5.2 Other techniques

1.5.2.1 Ion chromatography (IC)

Ion chromatography is based on a chemical exchange reaction between ions in a solution and a solid substance carrying functional groups which can be fixed as a result of electrostatic forces. For cation chromatography, functional groups are sulfonic acid groups, and for anion chromatography, they are quaternary ammonium groups. Basically, ions with the same charge can be exchanged reversibly between the two phases (López-Ruiz, 2000), as shown in equation 1.13-1.14 (Saari-Nordhaus, 2002).

$$\text{Resin}–SO_3^-H^+ + A^+ \rightleftharpoons \text{Resin}– SO_3^-A^+ + H^+ \qquad (1.13)$$

$$\text{Resin}–N^+R_3\,E^- + B^- \rightleftharpoons \text{Resin}–N^+R_3B^- + E^- \qquad (1.14)$$

When A and B are analytes in eluent

E^- is eluent

A component of ion chromatograph consists of an eluent reservoir, pump, a sample injection valve, a column, a detector and a recording system (Figure 1.9).

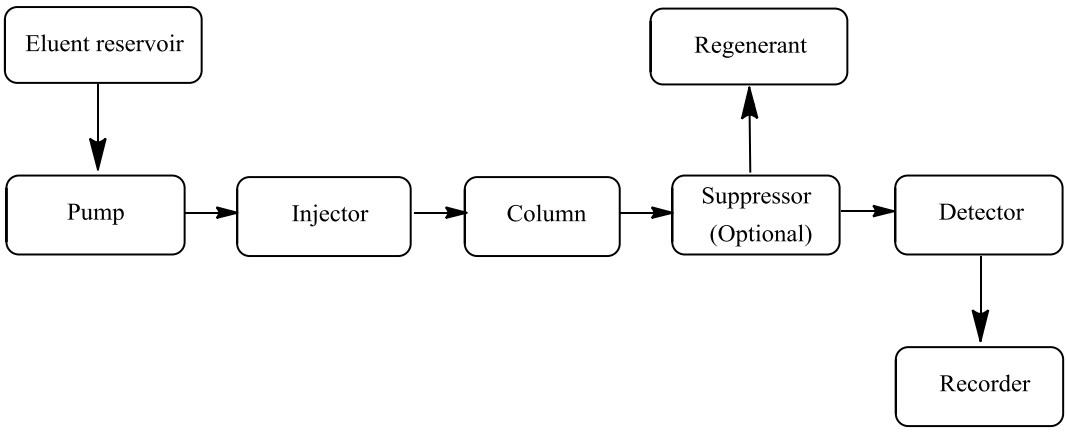


Figure 1.8 Component of ion chromatograph.

1) Eluent

The eluent or mobile phase used in IC generally consists of an aqueous solution of a suitable salt or mixture of salts, with a small percentage of an organic solvent being sometimes added. The salt mixture may itself be a buffer, or separate buffer can be added to the eluent if required. The three foremost properties of the eluent affecting the eluent characteristics of solute ions are the eluent pH, the nature of the competing ion and the concentration of the competing ion. The eluent used for anion analysis and cation analysis is typically a carbonate-bicarbonate solution and dilute sulfuric acid solution, respectively. Eluent is prepared by using deionized water (18 MΩ) and degassed before analysis (López-Ruiz, 2000 and Biesaga *et al*., 2004).

2) Pump

The pump delivers the eluent into the column, the connection tubes and related devices at a constant flow rate.

3) Injector

Injector is used for the sample introduction. Sample in injector is moved by the flowing of the eluent to the column.

4) Column

The column is used for the separation of analyte. It can be a resin column such as polystyrene-divinylbenzene or ethylvinylbenzene crosslinked with 55% divinylbenzene. The anion-exchange layer for anion exchange column is functionalized with quaternary ammonium groups, for example, a commercial IonPac® AS12A. The column resin for cation exchange column is functionalized with a mixture of carboxylic acid and phosphonic acid groups such as IonPac® CS12A.

5) Detector

The detector detects components which are different in property from the eluent and gives signals in proportion to the concentration for a substance of a few micrograms or less. The conductometers and UV spectrophotometers are usually used. When a conductometer is used as the detector, a suppressor can be placed in front of the conductometer. The suppressor is used to reduce the electric conductivity of the eluent and amplifies the ratio of the noises to the signals.

6) Computer system

A computer system is used for recording the intensities of the signals obtained by the detector and evaluating of the signals as an IC chromatogram.

Ion chromatography has been used for the detection of phosphate in many samples.

Sekiguchi *et al.* (2000) applied the ion chromatography with an on-line hydroxide eluent generator to the determination of condensed phosphate in food products extracted by trichloroacetic acid. The precision of the method for the condensed phosphate peak areas obtained by the hydroxide eluent generator was better than that obtained by potassium hydroxide eluents prepared off-line.

Bose *et al.* (2002) used the carbonate/bicarbonate buffer as a mobile phase for a suppressor-based IC because it provided a selectivity for common anion-exchange functional groups and contained both monovalent and divalent ions.

Kapinus *et al.* (2004) evaluated the detection limits for the ions in case of injection of aqueous solution (20-µL sample loop) into the ion chromatograph. Optimum detection limits and separation of anions were obtained when using 3.6 mM $NaHCO_3$/3.75 mM Na_2CO_3 as an eluent with a flow rate of 0.5 mL min^{-1}. The detection limits was 0.8×10^{-5}% for HPO_4^{3-} in aqueous solution samples.

1.5.2.2 Flow injection analysis (FIA)

Flow injection analysis is based on the injection of a liquid sample into a moving, no segmented continuous carrier stream of a suitable liquid. The injected sample forms a zone which is then transported toward a detector that continuously records the changes in absorbance, electrode potential, or other physical parameters, resulting in the passage of the sample material through the flow cell (Kronka *et al*., 1996).

Chen *et al.* (1998) reported the direct determination of phosphate in soil extracts by potentiometric flow injection using a cobalt wire electrode. The method has been applied to soil samples which were extracted by using potassium chloride and potassium chromate solution. Extracts spiked with the standard phosphate solution were diluted in the carrier solution to prevent interferences in the determination of phosphate caused by pH changes. It has been reported that some anions such as carbonate and hydroxide ion can react with cobalt (II) to form precipitates at the electrode surface.

Examples of the flow-based methods for the determination of phosphate in various samples are shown in Table 1.5.

Table 1.4 Spectrophotometric technique for the analysis of phosphate in various samples

Sample	Reducing agent	Wavelength (nm)	Linearity range (mg L^{-1})	LOD/ LOQ*	Interference	Reference
Water	Ascorbic acid	880	0.01-0.50	-	50 mg L^{-1} Fe^{2+} 10 mg L^{-1} Cu^{2+} 10 mg L^{-1} Si^{4+}, AsO_4^{3-}	ASTM D 515-78, 1982
	Amino-naphthol-sulfonic acid	650	0.1-3.0	-	40 mg L^{-1} Fe^{2+} 75 mg L^{-1} Cr^{2+} 50, 000 mg L^{-1} Cl^-	
	Ammonium metavanadate	400-420	1-10	-	Fe^{2+}	
Aqueous solution	Ascorbic acid	820-880	0-100	-	100 mg L^{-1} Si^{4+}	Drummond and Maher, 1995
			0.01-0.25	-	10 mg L^{-1} AsO_4^3	
-	Ascorbic acid	880	0.15-1.30	-	0.10 mg L^{-1} AsO_4^{3-}	Csuros, 1997
Water	Ascorbic acid	880	0.01-2.00	4.0 mg L^{-1}/-	-	Thabano *et al.*, 2004

Table 1.4 Spectrophotometric technique for the analysis of phosphate in various samples (Continued)

Sample	Reducing agent	Wavelength (nm)	Linearity range	LOD/ LOQ*	Interference	Reference
Soil, detergent , water, bone, food sample	Thiourea	840	0.50-10.0	0.0555 mg L^{-1}/-	90 mg L^{-1} Zn^{2+} 209 mg L^{-1} K^{+} 80 mg L^{-1} Mg^{2+} 4 mg L^{-1} Fe^{3+} 2.5 mg L^{-1} V^{5+} 48 mg L^{-1} Cd^{2+} 7 mg L^{-1} Cu^{2+} 638.4 mg L^{-1} SO_4^{2-} 375.2 mg L^{-1} Cl^{-}	Shyla *et al.* 2011
-	Hydrazinium sulfate	820-830	0-2.0 mg L^{-1}	-	-	Mendham *et al.* 2000
-	Hydrazinium sulfate	830	0-200 µg L^{-1}	-	Si^{4+} and AsO_4^{3-}	Bassett *et al.* 1979
Soil	Ascorbic acid	850	5–80 µg L^{-1}	-	Si^{4+} and AsO_4^{3-}	He and Honeycutt, 2005
Meat products	Ascorbic acid	823	0-0.06 mg L^{-1}	-	-	Dušek *et al.* 2003

Table 1.4 Spectrophotometric technique for the analysis of phosphate in various samples (Continued)

Sample	Reducing agent	Wavelength (nm)	Linearity range (mg L^{-1})	LOD/ LOQ*	Interference	Reference
Meat products	Ascorbic acid	823	0-0.06	-	-	Dušek *et al.* 2003
Fertilizers	Quinoline	-	≤ 30	-	-	AOAC 960.02, 2000
-			2-5	-	-	
Meat samples	Ammonium metavanadate	-	4-15	-	-	Jastrzębska *et al.* 2008
Sea water	Ascorbic acid	827	1-25		AsO_4^{3}	Murphy and Riley, 1958
Sugarcane juices, water and detergent	Sodium sulfide	715	0.3-12.24	0.0156 mg L^{-1}/-	120 mg L^{-1} Na^{+} 209 mg L^{-1} K^{+} 400 mg L^{-1} Mg^{2+} 20 mg L^{-1} Fe^{3+} 20 mg L^{-1} Ni^{2+} 12 mg L^{-1} Cu^{2+} 192 mg L^{-1} Cd^{2+} 11 mg L^{-1} Zr^{4+}	Mahadevaiah *et al.* 2007

Table 1.4 Spectrophotometric technique for the analysis of phosphate in various samples (Continued)

Sample	Reducing agent	Wavelength (nm)	Linearity range (mg L^{-1})	LOD/ LOQ* (mg L^{-1})	Interference (mg L^{-1})	Reference
Meat samples	Ascorbic acid	825	0.11-0.87	0.012/ 0.039		Jastrzębska, 2009
	Hydrazine sulfate		0.33-0.87	0.012/ 0.040		
	Mixture of hydroquinone and hydrazine sulfate		0.55-4.37	0.061/ 0.20	-	
	Malachite green		0.02-0.22	0.0021/ 0.0070		
	Ascorbic acid	880	0-0.25	0.005/ 0.020	AsO_4^{3-}, NO_2^- and Cr^{6+}	To and Randall, 1977
River water	Stannous chloride and ascorbic acid	-	5-10	-	AsO_4^{3-}, Cl^-, Si^{4+}, Cr^{6+}, NO_2^-, NO_3^- and S^{2-}	Helen *et al.* 2002

*LOD, limit of detection; LOQ, limit of quantification.

Table 1.5 Flow-based methods for the determination of phosphate in various samples

Sample	**Technique**	**Reagents**	**Linear determination range (mg L^{-1})**	**RSD (%)**	**Detection limit (mg L^{-1})**	**Reference**
Waste water	FIA-Spec	Mo/Sn-Hy	0-18	≤ 2.0	0.15	Benson *et al.* 1996
Synthetic water	SFA-Spec	Mo-Sb/Asc	0.002-0.062	-	0.002	Zhang *et al.* 1999
Lakes waters	MSFA-Spec	Mo-MG	0.005-0.075	2	0.0007	Diniz *et al.* 2004
Drinking, ground and waste water	SIA-Spec	Mo-V-MG	0.05-0.40	18	0.01	Muñoz *et al.* 1997
Waste water	FIA-Spec	Mo-Sb/Asc	0.001-0.05	1.0	0.0006	Korenaga *et al.* 1996
Soils extracts	FIA-Pot	Pht-CoW	0.31-155	3.8	0.031	Chen *et al.* 1998
Hydroponic nutrient	FIA-Pot	Pht-CoW	15.5-310	2-5	0.093	De Marco and Phan, 2003

Table 1.5 Flow-based methods for the determination of phosphate in various samples (Continued)

Sample	Technique	Reagents	Linear determination range (mg L^{-1})	RSD (%)	Detection limit (mg L^{-1})	Reference
Aqueous solutions	FIA-Amp	Trines and P12Dia membrane electrode-AcP	0.16-24.8	< 2.5	0.008	Yao *et al.* 2003
Sea water	CFA-Spec	Mo-MG	0.028-0.102	-	0.019	Mecozzi, 1995
Marsh and river water	FIA-CL	PyrOxG/Lu	0.003-1.0	2.3	0.003	Nakamura *et al.* 1999
Water	MSFA	Asc	0.01–2.00	-	4.0 ppb	Thabano *et al.* 2004

AcP, acid phosphatase; Amp, amperometry; Asc, ascorbic acid; CoW, cobalt wire; CFA, continuous flow analysis; CL, chemiluminescence; FIA, flow injection analysis; ; Hy, hydrazine; Lu, luminol; MG, malachite green; Mo, potassium ammonium molybdate; MSFA, monosegmented flow analysis; Pht, phthalate; Pot, potentiometry; P12Dia, poly(1,2-diaminobenzene) film; PyrOxG, pyruvate oxidase G; Sb, antimony tartrate; SFA, sequential flow analysis; SIA, sequential injection analysis; Spec, spectrophotometric; Sn, tin; V, vanadium.

1.6 Method validation

1.6.1 Linearity dynamic range

The quantitative analysis relates to the relationship between the response measured and the analyte concentration. This relationship is obtained by using either the external calibration curve or the internal standardization calibration curve and is formulated into a mathematical expression which can be used for the calculation of the analyte concentration in the real sample. The external calibration curve was usually used and performed by plotting between absorbance (y axis) and concentration (x axis), as shown in equation 1.15 and Figure 1.9. The relation of absorbance and concentration should not show a significant deviation from a linearity which is considered as the correlation coefficient, r > 0.99. The calibration equation is presented below (Miller, 2005).

$$y = mx + c \qquad (1.15)$$

Where y is measured absorbance
x is concentration
m is slope of calibration curve (sensitivity)
and c is intercept

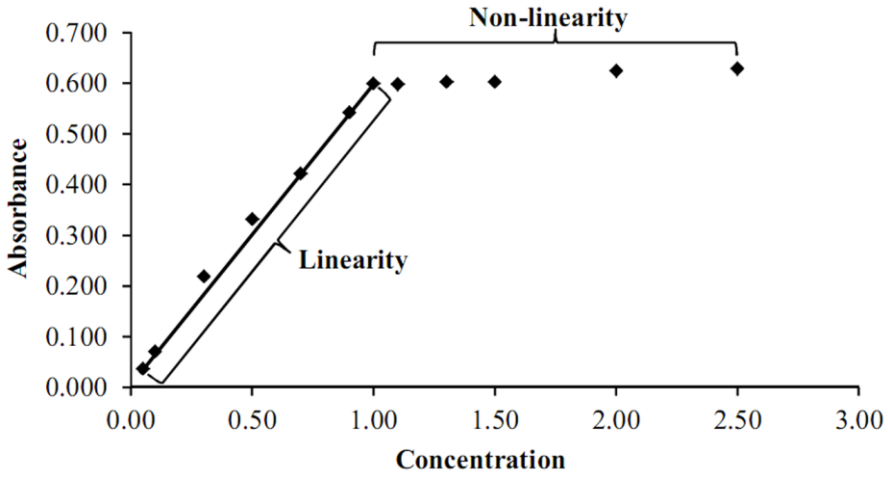


Figure 1.9 Linearity and non-linearity.

1.6.2 Standard addition

The technique of standard addition is widely applied to spectrophotometric, chromatographic and electrochemical methods in order to overcome matrix effect.

The investigation of matrix effect can be carried out by preparing samples spiked with various concentration levels of analyte. A sample and spiked samples are measured and plotted in similar way to standard curve. This plot is called standard addition curve. The results of standard addition curve and standard curve, as shown in Figure 1.10, should be plotted in the same curve. If the slope of these two linear curves are the same, it refers that matrix is not affected. In addition, to perform a statistical analysis of matrix test, the significant difference of the slope of results obtained should be checked (Skoog, 1996 and Miller, 2005).

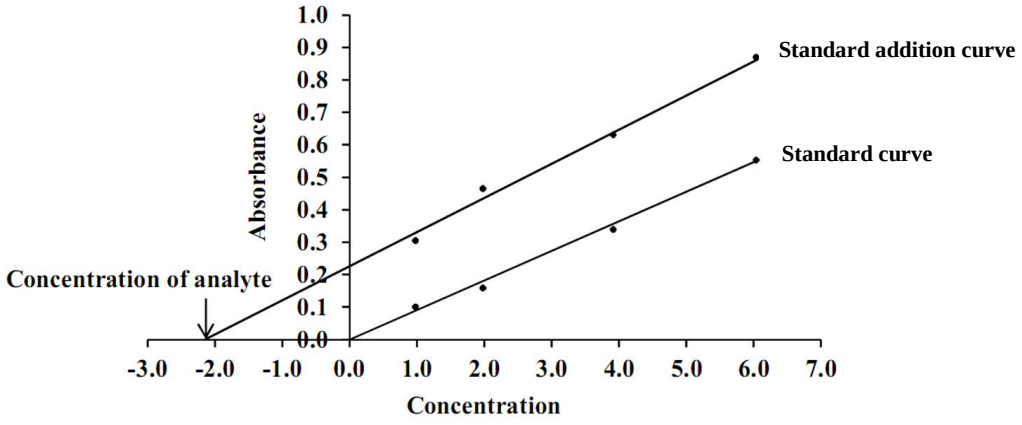


Figure 1.10 Plots of standard addition and standard curves.

1.6.3 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) is the smallest concentration which is possible to deduce the presence of the analyte with reasonable statistical certainty detected, but not necessarily quantities as an exact value. LOD applies to the instrument tested under the optimum conditions and assumes that the sensitivity is constant. LOD must be calculated as the concentration response corresponding to three times signal-to-noise (S/N = 3), as shown in equation 1.16 (Miller, 2005).

$$LOD = \frac{3S_{y/x}}{b} \quad (1.16)$$

The limit of quantification (LOQ) is the lowest amount of the analyte in the sample that can be quantitatively determined with defined precision under the optimum experimental conditions. It is a parameter of quantitative assays for low level of compounds in sample matrices. LOQ can be calculated from equation 1.17 with k = 10 (S/N = 10) (Miller, 2005).

$$LOQ = \frac{10S_{y/x}}{b} \quad (1.17)$$

Where $S_{y/x}$ is the standard deviation of response of blank (equation 1.18)

b is the slope of a calibration curve

and k is a numerical factor chosen according to the confidence level, k = 3 or 3σ (LOD) and k = 10 or 10σ (LOQ) limit of detection corresponds to confidence level of about 99.7%.

$$S_{y/x} = \sqrt{\frac{\sum_i (y_i - y_{ii})^2}{n - 2}} \quad (1.18)$$

Where y_i is absorbance of measurement

y_{ii} is absorbance from calibration curve calculation

n is number of concentration of standard phosphate (mg L^{-1})

1.6.4 Precision and accuracy

1.6.4.1 Precision

All measurements contain random error or noise that cannot be removed. This can be evaluated by repeating measurements of the same sample in 3 to 6 replicates and by calculating the standard deviation (SD) or relative standard deviation (RSD), as described in equation 1.19 and 1.20, respectively (Skoog, 1996 and Miller, 2005).

$$SD = \sqrt{\sum_{i=1}^{n} \frac{(X_i - \bar{X})^2}{(n-1)}} \qquad (1.19)$$

$$\%RSD = \frac{SD}{\bar{X}} \times 100 \qquad (1.20)$$

Where n-1 is degree of freedom

$\bar{x}$ is mean

n is the total number of measurements

The parameter measurements depend on the detection technique. For spectrophotometric technique, concentration and absorbance should be measured. In case of concentration and absorbance, RSD≤ 10% and 0.200 absorbance, respectively. Precision can be the repeatability and reproducibility. Repeatability describes the precision of within-run replicate or intra-day and reproducibility describes the precision of between-run replicate or inter-day.

1.6.4.2 Accuracy

The accuracy describes the experiment value being deviated from the true value and can be expressed as recovery percentage described in equation 1.21.

$$\text{Recovery percentage} = \frac{\text{Phosphate content}_{\text{spiked}} - \text{Phosphate content}_{\text{unspiked}}}{\text{Concentration of standard solution}} \times 100 \qquad (1.21)$$

1.6.5 Sensitivity

Sensitivity is the ability to detect small changes in the concentration of the analyte in the sample. Sensitivity can be expressed as the slope of the linear regression calibration curve, and it is measured of the same time as the linearity test (Miller, 2005).

1.7 Objectives

1.7.1 To develop simple and rapid method for the determination of phosphate in natural rubber concentrated latex

1.7.2 To determine soluble and total phosphates in natural rubber concentrated latex

1.8 Benefits

The developed method will focus on the determination of soluble and total phosphate in natural rubber concentrated latex. The new method is simple, rapid and accurate method and can be applied for phosphate analysis in the concentrated latex.

CHAPTER 2
EXPERIMENTAL

2.1 Overall scope

In this work, the determination of phosphate in concentrated latex was divided into two parts, the analysis of soluble and total phosphates. The optimum conditions with the spectroscopic detection for the analysis of phosphate were investigated in order to obtain a simple and rapid method which was suitable for an industrial application. The scope of this work is shown in Figure 2.1.

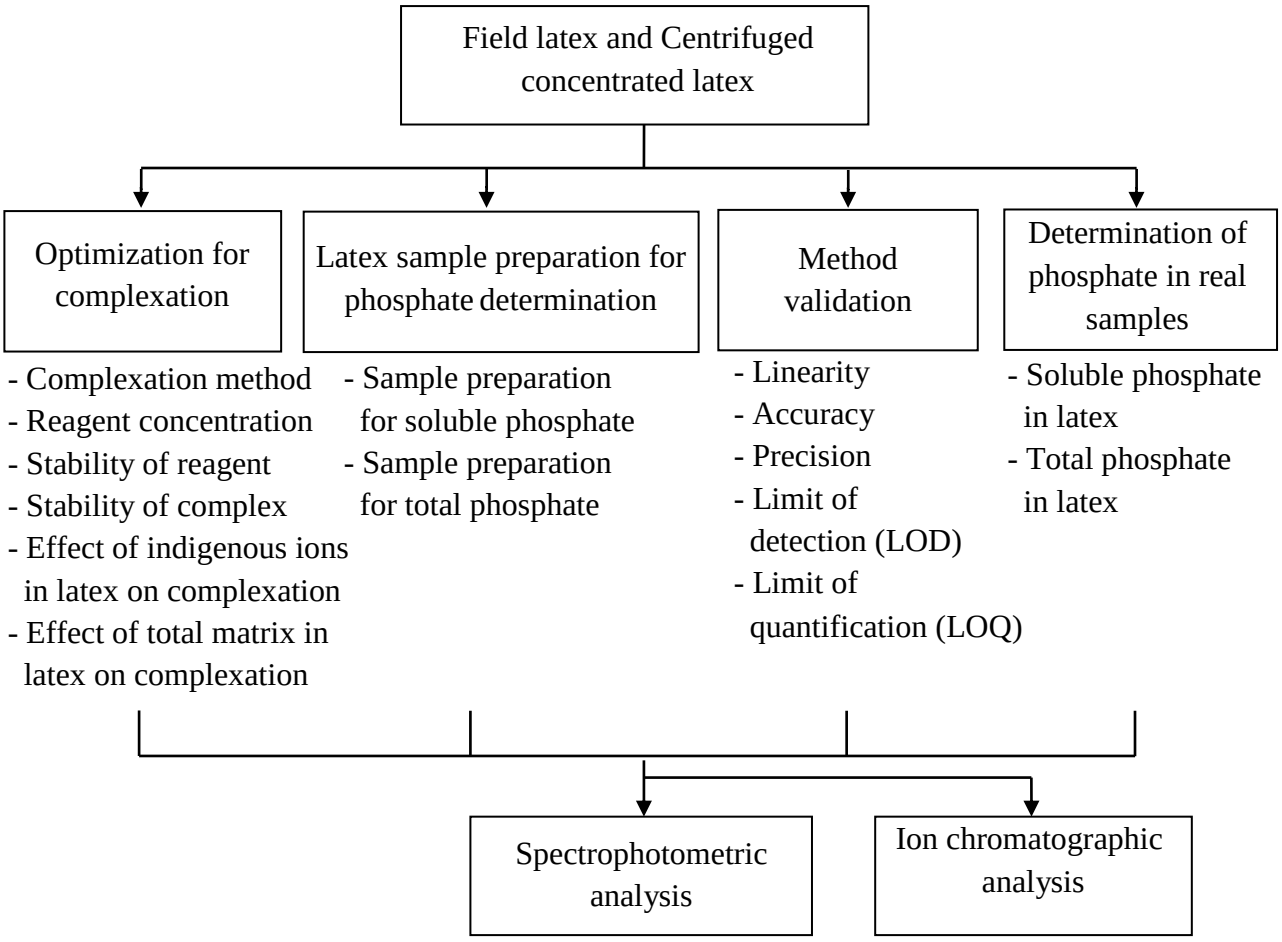


Figure 2.1 Scope of the experiment for the phosphate determination in latex samples.

2.1.1 Chemicals and reagents

All chemicals and reagents were purchased from various companies of analytical grade, as shown in Table 2.1.

Table 2.1 Chemicals and reagents used for determination of phosphate in latex

Chemicals and reagents (AR grade)	**Company**	**Country**
Acetic acid	Merck	Germany
Acetone	LAB-SCAN	Thailand
Ammonium molybdate	Ajax Finechem	Australia
Ammonium sulfate	Ajax Finechem	Australia
Ammonia solution (25%)	J.T. Baker	USA
Ascorbic acid	Riedel-de Haën	witzerland
Calcium sulfate	Ajax Finechem	Australia
Calcium nitrate	Ajax Finechem	Australia
Calcium chloride	Ajax Finechem	Australia
Citric acid	Ajax Finechem	Australia
Copper sulfate	Ajax Finechem	Australia
Deionized water	-	-
Diammonium hydrogen phosphate	Ajax Finechem	Australia
Distilled water	-	-
Dowex 50WX4-50 resin	Sigma-Aldrich	USA
Ethanol	Merck	Germany
Formic acid	LAB-SCAN	Thailand
Glycollic acid	Ajax Finechem	Australia
Hydrazinium sulfate	Ajax Finechem	Australia
Hydrogen peroxide	Merck	Germany
Iron (II) sulfate	Ajax Finechem	Australia
Iron (II) citrate	Fluka	Germany
Liquid paraffin	Vidhyasom	Thailand
Malic acid	Fluka	Germany
Maleic acid	Fluka	Germany
Manganese sulfate	Ajax Finechem	Australia
Magnesium sulfate	Ajax Finechem	Australia
Magnesium ammonium phosphate	Fluka	Germany
Methanol	Merck	Germany
Nitric acid	LAB-SCAN	Thailand
Phenolphthalein	Ajax Finechem	Australia
Potassium dihydrogen phosphate	Merck	Germany

Table 2.1 Chemicals and reagents used for determination of phosphate in latex (continued)

Chemicals and reagents (AR grade)	Company	Country
Potassium antimonyl tartrate	Ajax Finechem	Australia
Potassium sulfate	Ajax Finechem	Australia
Propionic acid	J.T. Baker	USA
Sodium hydroxide	Ajax Finechem	Australia
Sulfuric acid	LAB-SCAN	Thailand
Sodium nitrite	Ajax Finechem	Australia
Sodium nitrate	Ajax Finechem	Australia
Succinic acid	J.T. Baker	USA
Thiourea	Ajax Finechem	Australia
Trichloroacetic acid	Merck	Germany
Zinc sulfate	Ajax Finechem	Australia

2.1.2 Samples

Field latex samples were collected at five different districts located in the Songkhla province, Thailand where were Namom, Hatyai, Meuang, Bangkom and Natrawee districts.

Concentrated latex prepared by only centrifugation method was used in this study. Low ammonium (0.4% v/v) and high ammonium (0.7% v/v) preserved concentrated latex samples were supplied by five different factories in the southern part of Thailand, which were Trang latex Co., Ltd., Tatwin latex Co., Ltd., Chana latex Co., Ltd., Phatthalung Paratex Co., Ltd. and Unimac Rubber Co., Ltd. All samples were collected in nitric acid washed plastic bottles and kept at room temperature.

2.1.3 Glasswares

General glasswares were beaker, volumetric flask, cylinder, dropper, test tube, pipette and glass funnel. The detergent without phosphate compound for cleaning all glassware was used.

2.1.4 Preparation of glasswares

All glasswares were soaked in 10% (v/v) nitric acid for at least 12 hours and rinsed with distilled water. After that, glasswares were dried in the oven at 100ºC, but test tube, glass bead, glass funnel and dropper were dried in the furnace at 250ºC.

2.1.5 Apparatus

All apparatus used for phosphate analysis in this work are shown in Table 2.2.

Table 2.2 Apparatus used for phosphate analysis

Apparatus	Company	Country
Aluminum cup	-	-
Aluminum pot	-	-
Analytical balance	Mettler Toledo, AB204-S	Switzerland
Buchner funnel	-	-
Cuvette (Test tube), 10 mm	Milton Roy	France
Filter paper (No. 42)	Whatman	England
Hot plate	Heidolph, MG3001	Germany
Micropipette	BRAND	Germany
Nylon membrane filter (0.45-µm)	Whatman	England
Oven	Memmert	Germany
pH meter	Mettler Toledo, AG 8603	China
Thermometer	-	UK
Vacuum pump	Jeio Tech, VE-11	Korea
Vacuum flask	-	-
Vortex Mixer	UZUSIO, VTX-3000L	Japan

2.1.6 Instrumental

2.1.6.1 UV-Visible spectrophotometer

UV-Visible spectrophotometer with diode array detector was used to record the spectra of the blue phosphomolybdenum complexes. A Spectronic® 20 with silicon photodiode detector (Genesys™, USA) was used for absorbance measurement at 890 nm.

2.1.6.2 Ion Chromatograph

The ion chromatograph used for the comparison of detection method for soluble phosphate was a Dionex (Sunnyvale, CA, USA) system consisting of an IP25 isocratic pump, a 25-µL sample loop, a CD25 Conductivity detector and a LC20 Chromatography Enclosure. The Dionex IonPac AS12A (4×100 mm) column packed with anion-exchange resin was used as the separation column. The stationary phase was ethylvinylbenzene (EVB) cross-linked with 55% divinylbenzene (DVB) and latex agglomerated column, alkyl quaternary ammonium functionalized latex electrostatically bound to 9-µm sulfonated EVB-DVB. The mobile phase was 2.7 mM sodium carbonate/0.3 mM sodium bicarbonate with a flow rate of 1.25 mL min^{-1}. The injection volume was 25 µL (Biesaga *et al*., 2004).

2.1.7 Preparation of solutions

2.1.7.1 A stock standard solution of phosphate 3000 mg PO_4^{3-} L^{-1}

Potassium dihydrogen phosphate was dried in the oven at 105ºC prior to use. Potassium dihydrogen phosphate (0.4299 g) was dissolved with deionized water, and the solution was transferred into a 100-mL volumetric flask and further diluted to the mark with deionized water. This solution could be used for three months when stored in a glass bottle at 4ºC.

Magnesium ammonium phosphate (0.4337 g) was dissolved with deionized water and 1.0 mL of 2.5 M sulfuric acid, and the solution was transferred into a 100-mL volumetric flask and further diluted to the mark with deionized water. This solution could be used for three months when stored in a glass bottle at 4ºC.

2.1.7.2 Preparation of solution for ascorbic acid method

a) 2.5 M Sulfuric acid

Sulfuric acid (2.5 M) was prepared by diluting 70.0 mL of concentrated sulfuric acid (18 M) with deionized water, and the solution was transferred into a 500-mL volumetric flask and diluted to the mark with deionized water.

b) 4.0% (w/v) Ammonium molybdate solution

Ammonium molybdate (20.00 g) was dissolved in deionized water and the solution was transferred into a 500-mL volumetric flask, and diluted to the mark with deionized water. This solution could be used for three months when stored in a plastic bottle at 4ºC.

c) 0.01 M Potassium antimony tartrate solution

Potassium antimony tartrate (3.07 g) was dissolved in deionized water, and the solution was transferred into a 500-mL volumetric flask and diluted to the mark with deionized water. This solution could be used for three months when stored in a glass bottle at 4ºC.

d) 0.10 M Ascorbic acid solution

Ascorbic acid (1.76 g) was dissolved in deionized water, and the solution was transferred into a 100-mL volumetric flask and diluted to the mark with deionized water. This solution can be used for three months when stored in a brown glass bottle at 4ºC.

e) Combined reagent

The combined reagent used in this work was modified from Csuros (1997), in which 50.00 mL of 2.5 M sulfuric acid, 5.00 mL of 0.01 M potassium antimonyl tartrate, 15.00 mL of 4.0% (w/v) ammonium molybdate and 30.00 mL of 0.10 M ascorbic acid were mixed in a 100-mL volumetric flask, except ascorbic acid which Csuros (1997) used at only 0.01 M. This solution was prepared freshly prior to use and could be stored at room temperature for 4 hours.

2.1.7.3 Preparation of solution for molybdenum blue method

Concentrations of sodium molybdate and hydrazinium sulfate were prepared as mentioned by Libertore (2010).

a) 0.10 M Sodium molybdate

Sodium molybdate (12.50 g) was dissolved in 5.0 M sulfuric acid, and the solution was transferred into a 500-mL volumetric flask and diluted to the mark with 5.0 M sulfuric acid.

b) 0.01 M Hydrazinium sulfate

Hydrazinium sulfate (1.50 g) was dissolved in deionized water, and the solution was transferred into a 1000-mL volumetric flask and diluted to the mark with deionized water.

2.2 Optimization for complexation method

2.2.1 Complexation methods

The determination of phosphate by colorimetric method was performed by using three known methods, ascorbic acid method (ASTM D 515-78, 1982 and Csuros, 1997), molybdenum blue method (Mendham *et al.,* 2000 and Libertore, 2010) and vanadomolybdate method (Muñoz *et al.,* 1997 and Neves *et al.,* 2008).

a) Ascorbic acid method

Concentrated latex (2.5 g) was weighed to the nearest 0.0001 g in an aluminium cup and 5.00 mL of ethanol was added drop-wise. After the coagulation with ethanol, all serum solution was pipetted into a 30-mL test tube and vacuum filtered through a 0.45-µm nylon membrane. The filtrate was collected in a 25-mL volumetric flask, and 4.00 mL of a combined reagent was added, followed by mixing and diluting to volume with deionized water. The reaction was kept for 15 minutes at room temperature to reach the equilibrium, and the blue complex was obtained and further measured the absorbance at 890 nm.

b) Molybdenum blue method

After the coagulation with ethanol as mentioned above, serum solution of 5.00 mL was taken into a 30-mL test tube, and 5.00 mL of 0.1 M sodium molybdate solution and 2.00 mL of 0.01 M hydrazine sulfate solution were added and mixed through, diluted to volume with deionized water and placed into boiling water for 10 minutes to reach the equilibrium. An absorbance of the blue complex obtained was measured at 830 nm.

2.2.2 Reagents concentration for a combined reagent

Parameters of a combined reagent in term of optimal suitable concentration of sulfuric acid, potassium antimonyl tartrate, ammonium molybdate and ascorbic acid were investigated for the maximum color development of phosphate

in concentrated latex. The optimization of parameters was performed by varying one parameter and keeping all others constant. One-way ANOVA at the 95% confidence limit was used for data analysis.

The procedure was performed by pipetting 125 µL of 100 mg PO_4^{3-} L^{-1} stock standard solution into a 25-mL volumetric flask, followed by adding 2.00 mL of a combined reagent and further diluting to the mark with deionized water. The reaction was kept for 15 minutes at room temperature to attain equilibrium, and the blue complex was obtained and further measured the absorbance at 890 nm. Each parameter of each concentration of reagent was prepared in three replicates.

a) Sulfuric acid concentration

Sulfuric acid was prepared in a 100-mL volumetric flask to obtain the final concentration at 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 M with other reagents at constant concentration, *i.e.*, 5.00 mL of 0.01 M potassium antimonyl tartrate, 15.00 mL of 4.0% (w/v) ammonium molybdate and 30.00 mL of 0.10 M ascorbic acid.

b) Potassium antimonyl tartrate concentration

Potassium antimonyl tartrate was prepared in a 100-mL volumetric flask to obtain the final concentration at 0.005, 0.01, 0.03, 0.05, 0.07 and 0.09 M with other reagents at constant concentration, *i.e.*, 50.00 mL of 2.5 M sulfuric acid, 15.00 mL of 4.0% (w/v) ammonium molybdate and 30.00 mL of 0.10 M ascorbic acid.

c) Ammonium molybdate concentration

Ammonium molybdate was prepared in a 100-mL volumetric flask to obtain the final concentration at 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0% (w/v) with other reagents at constant concentration, *i.e.*, 50.00 mL of 2.5 M sulfuric acid, 5.00 mL of 0.01 M potassium antimonyl tartrate and 30.00 mL of 0.10 M ascorbic acid.

d) Ascorbic acid concentration

Ascorbic acid was prepared in a 100-mL volumetric flask to obtain the final concentration at 0.05, 0.10, 0.20, 0.30 and 0.40 M with other reagents at constant concentration, *i.e.*, 50.00 mL of 2.5 M sulfuric acid, 5.00 mL of 0.01 M potassium antimonyl tartrate and 15.00 mL of 4.0% (w/v) ammonium molybdate.

2.2.3 Stability of reagents by ascorbic acid method

a) Stability of 0.01 M potassium antimonyl tartrate

A 0.01 M potassium antimonyl tartrate solution was prepared and stored in a glass bottle at 4ºC. To check its stability, the performance of this compound was checked together with 125 µL of 100 mg PO_4^{3-} L^{-1} and other fresh reagents of 50.00 mL of 2.5 M sulfuric acid, 15.00 mL of 4.0% (w/v) ammonium molybdate and 30.00 mL of 0.10 M ascorbic acid. After reaction was formed, the absorbance of the complex solution was measured at 890 nm. The stability of this reagent was investigated every week up to 15 weeks.

b) Stability of 4.0% (w/v) ammonium molybdate

A 4.0% ammonium molybdate solution was prepared and stored in a plastic bottle at 4ºC. To check its stability, the performance of this compound was checked together with 125 µL of 100 mg PO_4^{3-} L^{-1} and other fresh reagents, 50.00 mL of 2.5 M sulfuric acid, 5.00 mL of 0.01 M potassium antimonyl tartrate and 30.00 mL of 0.10 M ascorbic acid. After reaction was formed, the absorbance of the complex solution was measured at 890 nm. The stability of this reagent was investigated every week up to 15 weeks.

c) Stability of 0.10 M ascorbic acid

A 0.10 M ascorbic acid solution was prepared and stored in a brown glass bottle at 4ºC. To check it stability, the performance of this compound was checked together with 125 µL of 100 mg PO_4^{3-} L^{-1} and other fresh reagents, 50.00 mL of 2.5 M sulfuric acid, 5.00 mL of 0.01 M potassium antimonyl tartrate and 15.00 mL of 4.0% (w/v) ammonium molybdate. After reaction was performed, the absorbance of the complex solution was measured at 890 nm. The stability of this reagent was investigated only up to 6 weeks because ascorbic acid is reactive to ambient environments such as oxygen, light, moisture and temperature (Golubitskii *et al.*, 2007).

d) Stability of a combined reagent

A combined reagent was prepared by mixing 50.00 mL of 2.5 M sulfuric acid, 5.00 mL of 0.01 M potassium antimonyl tartrate, 15.00 mL of 4.0% (w/v) ammonium molybdate and 30.00 mL of 0.10 M ascorbic acid in a 100-mL volumetric flask. This combined reagent was kept in a fridge at 4ºC. The stability of a

combined reagent was investigated by mixing 125 µL of 100 mg PO_4^{3-} L^{-1} and 2.00 mL of a combined reagent in a 25-mL volumetric flask. After dilution to the mark with deionized water, the reaction solution was kept equilibrated for 15 minutes at room temperature to form blue complex and its absorbance was measured at 890 nm. The stability of this reagent was investigated every day up to 3 days.

2.2.4 Stability of phosphate complex by ascorbic acid method

A phosphate standard solution and a latex serum were used to investigate the stability of phosphate complex.

a) Standard chemical reagent

The phosphate standard solution at a final concentration of 0.50 mg PO_4^{3-} L^{-1} was mixed with 8.00 mL of a combined reagent in a 100-mL volumetric flask. After dilution to the mark with deionized water, the reaction solution was kept equilibrated for 15 minutes at room temperature and its color was measured absorbance at 890 nm every five minutes until 2 hours.

b) Latex matrix

Concentrated latex (2.5 g) was weighed to the nearest 0.0001 g into an aluminium cup. Ethanol was added drop-wise onto the latex surface while swirling. After the coagulation, serum was weighted and transferred to a 30-mL test tube with a Teflon-lined screw cap. After an addition of 10.00 mL acetone to coagulate rubber particles, the solution was left for 30 minutes at room temperature. A 5.00 mL of serum solution was pipetted and filtered through a Whatman no. 42 filter paper into a 25-mL volumetric flask. Four milliliters of a combined reagent was added, and the solution was mixed and diluted to 25-mL with deionized water. The reaction solution was kept equilibrated for 15 minutes at room temperature to obtain blue complex and its color was measured absorbance at 890 nm every five minutes until 2 hours.

c) Stability of phosphate complex when using two weeks old ascorbic acid

A 0.10 M ascorbic acid was prepared in a 250-mL volumetric flask and kept in a brown bottle at 4ºC for 2 weeks. The color stability of complexation for a phosphate standard solution and a latex serum was performed as mentioned above.

2.2.5 Effect of indigenous ions in latex on complexation

2.2.5.1 Effect of indigenous ions

Latex is a complicated natural product, therefore, the analysis of other constituents in latex is required for the determination of phosphate in order to determine degree of their interference. Two groups of other constituents, anion and cation, were studied. Phattanakul *et al.* (2009) reported that the carboxylic groups, malate and succinate, were found at the highest concentration apart from phosphate in latex, while Galli *et al.* (2000) purposed these anions as indicators for a quality control of latex. Furthermore, cations or metal ions in the concentration of millimolar level such as magnesium, calcium and potassium, and those in the concentration of micromolar level such as zinc (II), copper (II) and manganese (II) were also investigated (Scott *et al.*, 2003).

The effect of anions and cations in Table 2.3 for the determination of phosphate was investigated at 100 mg L^{-1} of anions and cations and analyzed by ascorbic acid method and molybdenum blue method.

Table 2.3 Anion and cation studied on the determination of phosphate in concentrated latex

Anions	**Chemical form**	**Cations**	**Chemical form**
Formate	Formic acid	Copper ion	Copper sulfate
Propionate	Propionic acid	Magnesium ion	Magnesium sulfate
Glycolate	Glycolic acid	Zinc ion	Zinc sulfate
Citrate	Citric acid	Calcium ion	Calcium sulfate
Nitrate	Sodium nitrite	Manganese ion	Manganese sulfate
Succinate	Succinic acid	Iron (II) ion	Ferrous sulfate
Malate	Malic acid	Calcium ion	Calcium citrate
Maleate	Maleic acid	Magnesium ion	Magnesium citrate

The procedure for the study of interferences was performed by preparing the final concentration standard of anions and cations at 100 mg L^{-1} in a 50-mL volumetric flask, and 4.00 mL of a combined reagent was added. The solution was diluted to the mark with deionized water and kept to reach an equilibrium for 15

minutes. After reaction, a blue complex solution was obtained and its spectrum was measured at 890 nm.

2.2.5.2 Tolerance limits of indigenous ions

Since concentrated latex sample is a complex compound containing some organic compounds, such as proteins, glycolipids, and metals, such as magnesium, iron (II) and manganese (II). Therefore, it is necessary to evaluate the selectivity of coexistence compounds for analysis of phosphate by ascorbic acid method.

Anions and cations listed in Table 2.3 (Section 2.2.5.1) were studied including iron (II) as iron citrate. Three sets of the concentration of 0.50 mg PO_4^{3-} L^{-1} with concentrations of anion and cation standards at 50, 500 and 1000 mg L^{-1} were studied. Briefly, phosphate standard was pipetted into a 50-mL volumetric flask and each anion or cation was added. After the addition of 4.00 mL of a combined reagent, the solution was diluted to the mark with deionized water. The reaction solution was left for 15 minutes at room temperature to reach an equilibrium prior to absorbance measurement at 890 nm.

Both anions and cations were investigated in terms of the tolerance limits which were the maximum concentration of diverse ions produced percentage deviation not more than 5%, being was calculated as equation 2.1 (Khlyntseva *et al.*, 2011).

$$\%\,\text{Deviation} = \frac{\text{Signal of phosphate} - \text{Signal of phosphate with diverse ions}}{\text{Signal of phosphate}} \times 100 \quad (2.1)$$

2.2.6 Effect of total matrix in latex on complexation

The investigation of matrix effect was carried out by spiking with different known concentrations of phosphate compound into samples (Miller, 2005). Eight equal volumes of serum solution were transferred into a 50-mL volumetric flask. All solutions except one were separately spiked with a stock standard solution at 100 mg PO_4^{3-} L^{-1} to obtain the final concentrations of phosphate in solution at 0.05,

0.10, 0.30, 0.50, 0.70, 0.90 and 1.00 mg PO_4^{3-} L^{-1}. After the addition of 4.00 mL of a combined reagent, all eight solutions were diluted to the mark with deionized water. The solutions were kept for 15 minutes at room temperature to obtain a blue complex with absorbance measurement at 890 nm.

2.3 Sample preparation for soluble and total phosphate determination in concentrated latex

2.3.1 Sample preparation for soluble phosphate determination in concentrated latex

Natural rubber latex is a milky colloid containing rubber particles suspended in serum medium. Spectrophotometric detection cannot be applied directly for phosphate analysis due to complex latex compositions. Thus, serum was prepared from latex by the chemical coagulation and analyzed for soluble phosphate determination. Furthermore, serum contains some rubber particles, in which phosphate could probably not dissolve, therefore, the additional step used for the rubber particle precipitation was performed.

2.3.1.1 Effect of reagent on rubber coagulation

It has been reported that 95% (v/v) ethanol, 2% (w/v) calcium chloride and 2% (w/v) calcium nitrate were used for rubber coagulations (Blackley, 1997a, p. 259 and Karunanayake and Perera, 2006). In this study, ethanol was compared to calcium chloride and calcium nitrate in order to obtain the selective coagulation of soluble phosphate. Concentrated latex (2.5 g) was weighed to the nearest 0.0001 g into an aluminium cup. Ethanol or 2% (w/v) calcium chloride or 2% (w/v) calcium nitrate was added drop-wise onto a latex surface while swirling. After the coagulation, serum was weighed and transferred to a 30-mL test tube with a Teflon-lined screw cap. A serum solution was filtered through a 0.45-μm nylon membrane into a 25-mL volumetric flask. Four milliliters of a combined reagent was added, followed by mixing and diluting to 25 mL with deionized water. The reaction solution was left for 15 minutes at room temperature to reach an equilibrium prior to absorbance measurement at 890 nm.

2.3.1.2 Effect of ethanol concentration on rubber coagulation

This study was performed for the coagulation of soluble phosphate by using ethanol. Volume of 95% (v/v) ethanol was investigated in the range of 5.00 to 15.00 mL to achieve a high concentration of soluble phosphate. Concentrated latex (2.5 g) was weighed to the nearest 0.0001 g into an aluminium cup. Ethanol was added drop-wise onto a latex surface while swirling. After coagulation, latex was separated into two parts, solids rubber and light yellow serum. Solids rubber was removed. Serum was transferred to a 30-mL test tube with a Teflon-lined screw cap, vortex mixed and filtered through a 0.45-µm nylon membrane into a 25-mL volumetric flask. Four milliliters of a combined reagent was added, followed by mixing and diluting to volume with deionized water. The reaction solution was left for 15 minutes prior to absorbance measurement at 890 nm.

2.3.1.3 Second coagulant

A previous study (Section 2.3.1.1) was shown that after the coagulation by ethanol, the serum filtered through a 0.45-µm nylon membrane was clear. However, the 0.45-µm nylon membrane is expensive, and a high cost vacuum pump was used for the filtration, resulting that only one sample was filtered each time. Thus, to remove solid particles together with ethanol, trichloroacetic acid (Yeang *et al.*, 2002 and Jiang *et al.*, 2004), acetone, methanol and ammonium sulfate (Jiang *et al.*, 2004) were studied. The procedure was performed by weighing 2.5 g of concentrated latex to the nearest 0.0001 g in an aluminium cup. Five milliliters of ethanol was added drop-wise onto the latex surface while swirling. After coagulation, serum was transferred to a 30-mL test tube with a Teflon-lined screw cap. After adding of 10.00 mL of acetone or trichloroacetic acid or methanol or ammonium sulfate to coagulate any chemicals dissolving in solution, serum was vortex mixed and filtered through a Whatman no. 42 filter paper into a 25-mL volumetric flask. Four milliliters of a combined reagent was added, followed by mixing and diluting to volume with deionized water. The reaction solution was kept for 15 minutes prior to absorbance measurement at 890 nm.

2.3.2 Sample preparation for total phosphate determination in concentrated latex

2.3.2.1 Effect of weight of latex for digestion

Three amounts of latex were optimized for digestion at 0.25, 1.00 and 2.50 g. The procedure was performed by weighing 0.25 or 1.00 or 2.50 g of concentrated latex to the nearest 0.0001 g into a 50-mL test tube with a reagent cap and glass bead was added. Next, 1000 mL of 3000 mg PO_4^{3-} L^{-1} as magnesium ammonium phosphate was spiked into a 0.25 g of concentrated latex to obtain a final concentration at 60.0 mg PO_4^{3-} L^{-1} in a 50 mL of solution. The sample preparation for total phosphate was performed by weighing 0.25 g of concentrated latex to the nearest 0.0001 g into a 50-mL test tube and glass bead was added. Latex was digested with 4.00 mL of concentrate nitric acid in a paraffin oil bath at 160ºC until the mixture darkened and 1.00 mL of concentrate nitric acid was added. After cooling to room temperature, in order to destroy the last organic matters, 0.50 mL of 30% (v/v) hydrogen peroxide and 2 drops of concentrate nitric acid was added into a solution, which was again digested at 160ºC until the solution became colorless or pale yellow. One milliliter of solution was transferred into a 50-mL volumetric flask and diluted to volume with deionized water. One milliliter of the diluted solution was pipetted into a 30-mL test tube and one drop of 1% (w/v) phenolphthalein was added. After the adjustment pH of the solution with 5 M of sodium hydroxide, 2.00 mL of a combined reagent was added, followed by mixing and diluting to a 5 mL with deionized water. The reaction was allowed to settle at room temperature for 15 minutes and formed a blue complex prior to absorbance measurement at 890 nm.

2.3.2.2 Modified standard method

The stability of the phosphate compounds is associated with different metals, therefore, the digestion method relating to various types of acid dissolved metal compound was applied for the determination of total phosphate in concentrated latex. In this study, the digestion standard method was modified from the standard method for the determination of copper content in latex (ISO 8053, 1995).

The procedure was performed by weighing 0.25 g of concentrated latex to the nearest 0.0001 g into a 50-mL test tube and glass bead was added. Latex

was digested with 3.00 mL of concentrate sulfuric acid and 4.00 mL of concentrate nitric acid in a paraffin oil bath at 160ºC until the mixture darkened and 1.00 mL of concentrate nitric acid was added. After cooling to room temperature, in order to destroy the last organic matters, 0.50 mL of 30% (v/v) hydrogen peroxide and 2 drops of concentrate nitric acid were added into a solution, which was again digested at 160ºC until the solution became colorless or pale yellow. One milliliter of solution was transferred into a 50-mL volumetric flask and diluted to volume with deionized water. One milliliter of the diluted solution was pipetted into a 30-mL test tube and one drop of 1% (w/v) phenolphthalein was added. After the adjustment pH of the solution with 5 M of sodium hydroxide, 2.00 mL of a combined reagent was added, followed by mixing and diluting to a 5 mL with deionized water. The reaction was left at room temperature for 15 minutes and formed a blue complex prior to absorbance measurement at 890 nm.

2.3.2.3 Developed digestion method for total phosphate determination in concentrated latex

In this section, three developed methods for the determination of total phosphate was performed with a little modification of the ISO 8053 method. Method A is latex digestion by using nitric acid and hydrogen peroxide, while for Method B, latex digestion was performed by using only nitric acid. Method C is latex digestion by using sulfuric acid and hydrogen peroxide.

a) Method A

The procedure was performed by weighing 0.25 g of concentrated latex to the nearest 0.0001 g into a 50-mL test tube and glass bead was added. Latex was digested with 4.00 mL of concentrate nitric acid in a paraffin oil bath at 160ºC until the mixture darkened and 1.00 mL of concentrate nitric acid was added. After cooling to room temperature, in order to destroy the last organic matters, 0.50 mL of 30% (v/v) hydrogen peroxide and 2 drops of concentrate nitric acid was added into a solution, which was again digested at 160ºC until the solution became colorless or pale yellow. One milliliter of solution was transferred into a 50-mL volumetric flask and diluted to volume with deionized water. One milliliter of the diluted solution was pipetted into a 30-mL test tube and one drop of 1% (w/v) phenolphthalein was added.

After the adjustment pH of the solution with 5 M of sodium hydroxide, 2.00 mL of a combined reagent was added, followed by mixing and diluting to a 5 mL with deionized water. The reaction was allowed to settle at room temperature for 15 minutes and formed a blue complex prior to absorbance measurement at 890 nm.

b) Method B

The procedure was performed by weighing 0.25 g of concentrated latex to the nearest 0.0001 g into a 50-mL test tube and glass bead was added. Latex was digested with 4.00 mL of concentrate nitric acid in a paraffin oil bath at 160ºC until the mixture darkened and 1.00 mL of concentrate nitric acid was added. After cooling to room temperature and 1.00 mL of concentrate nitric acid was added into a solution, which was again digested at 160ºC until the solution became colorless or pale yellow. One milliliter of solution was transferred into a 50-mL volumetric flask and diluted to volume with deionized water. One milliliter of the diluted solution was pipetted into a 30-mL test tube and one drop of 1% (w/v) phenolphthalein was added. After the adjustment pH of the solution with 5 M of sodium hydroxide, 2.00 mL of a combined reagent was added, followed by mixing and diluting to a 5 mL with deionized water. The reaction was allowed to settle at room temperature for 15 minutes and formed a blue complex prior to absorbance measurement at 890 nm.

c) Method C

The procedure was performed by weighing 0.25 g of concentrated latex to the nearest 0.0001 g into a 50-mL test tube and glass bead was added. Latex was digested with 4.00 mL of concentrate sulfuric acid in a paraffin oil bath at 160ºC until the mixture darkened and 4.00 mL of concentrate sulfuric acid was added into a solution, which was again digested at 160ºC. After cooling to room temperature and 1.00 mL of concentrate sulfuric acid was added into a solution, which was again digested at 160ºC. In order to destroy the last organic matters, 0.50 mL of 30% (v/v) hydrogen peroxide and 2 drops of concentrate sulfuric acid was added into a solution, which was again digested at 160ºC until the solution became colorless or pale yellow. One milliliter of solution was transferred into a 50-mL volumetric flask and diluted to volume with deionized water. One milliliter of the diluted solution was pipetted into a

30-mL test tube and one drop of 1% (w/v) phenolphthalein was added. After the adjustment pH of the solution with 5 M of sodium hydroxide, 2.00 mL of a combined reagent was added, followed by mixing and diluting to a 5 mL with deionized water. The reaction was allowed to settle at room temperature for 15 minutes and formed a blue complex prior to absorbance measurement at 890 nm.

2.3.2.4 Comparison between developed method (Method A) and modified standard method

In this study, the developed spectrophotometric method (Method A) for total phosphate as mentioned in 2.3.2.3a was compared to the modified standard method as mentioned in 2.3.2.2.

2.4 Method validation for phosphate determination

2.4.1 Calibration curve of phosphate

Working solutions were obtained by diluting of the stock standard solution of 100 mg PO_4^{3-} L^{-1}. The concentrations of working standard were 0.05, 0.10, 0.30, 0.50, 0.70, 0.90 and 1.00 mg PO_4^{3-} L^{-1} in a 25-mL volumetric flask. Two milliliters of a combined reagent was further added, and the solution was diluted to the mark with deionized water. The solution was kept for 15 minutes at room temperature to obtain a blue complex, which further measured absorbance at 890 nm.

2.4.2 Accuracy

The recovery of soluble phosphate by spectrophotometric detection were assessed by spiking 250, 500, and 750 µL of 3000 mg PO_4^{3-} L^{-1} as potassium dihydrogen phosphate into a 2.5 g of field and concentrated latexes to obtain final concentrations at 30.0, 60.0 and 90.0 mg PO_4^{3-} L^{-1}, respectively, in a 25 mL of solution. The procedure for soluble phosphate was performed as mentioned in section 2.5.1.

The recoveries of total phosphate were assessed by using magnesium ammonium phosphate to obtain an accuracy of the digestion method. The procedure was performed by spiking 500, 1000 and 1500 µL of 3000 mg PO_4^{3-} L^{-1} as magnesium ammonium phosphate into a 0.25 g of field and concentrated latexes to

obtain a final concentration at 30.0, 60.0 and 90.0 mg PO_4^{3-} L^{-1} , respectively, in a 50 mL of solution. The sample preparation for total phosphate was performed as described in section 2.5.2.

2.4.3 Precision

The precisions of the determination of soluble and total phosphates using the developed analytical method were assessed in terms of intra-day (repeatability) and inter-day (reproducibility). Precision is expressed as a coefficient of variation or relative standard deviation (RSD), being calculated as described in equation 1.19-1.20 (Chapter 1).

2.4.4 Limit of detection (LOD) and limit of quantification (LOQ)

LOD is calculated as the phosphate concentration response corresponding to three times signal-to-noise (S/N = 3), being calculated as described in equation 1.16 (Chapter 1).

LOQ is the lowest amount of phosphate in the sample that can be quantitatively determined with a defined precision under the optimum experimental conditions. LOQ can be calculated from equation 1.17 (Chapter 1).

2.5 Determination of phosphate contents in field latex and concentrated latex samples

2.5.1 Determination of soluble phosphate contents in field latex and concentrated latex samples

In this study, concentrations of soluble phosphate in field and concentrated latexes were determined by spectrophotometric detection. The results obtained by spectrophotometric detection were also compared to those obtained by ion chromatographic detection.

a) Field latex

The procedure was performed by weighing 2.5 g of concentrated latex to the nearest 0.0001 g added into an aluminium cup. Ethanol was added drop-wise onto the latex surface while swirling. After the coagulation, serum was weighed and transferred to a 30-mL test tube with a Teflon-lined screw cap. After an addition of

10.00 mL of acetone, the solution was left for 30 minutes at room temperature. A 5.00 mL aliquot of clear serum solution was pipetted and filtered through a Whatman no. 42 filter paper into a 10-mL volumetric flask. Four milliliters of a combined reagent was added, followed by mixing and diluting to 10 mL with deionized water. The reaction solution was left for 15 minutes at room temperature to reach an equilibrium prior to absorbance measurement at 890 nm.

b) Concentrated latex

The procedure was performed as mentioned in 2.5.1a, however, A 5.00 mL aliquot of clear serum solution was pipetted and filtered through a Whatman no. 42 filter paper into a 25-mL volumetric flask. Four milliliters of a combined reagent was added, followed by mixing and diluting to 25 mL with deionized water. The reaction solution was left for 15 minutes at room temperature to reach an equilibrium prior to absorbance measurement at 890 nm.

Another 5.00 mL aliquot of clear serum solution was pipetted and filtered through a Whatman no. 42 filter paper into a 25-mL volumetric flask, followed by diluting to 25 mL with deionized water. This solution was subjected to analyze by ion chromatographic detection.

2.5.2 Determination of total phosphate contents in field latex and concentrated latex samples

The procedure was performed by weighing 0.25 g of field or concentrated latex to the nearest 0.0001 g into a 50-mL test tube and glass bead was added. Latex was digested with 4.00 mL of concentrate nitric acid in a paraffin oil bath at 160ºC until the mixture darkens and 1.00 mL of concentrate nitric acid was further added. After cooling to room temperature, 0.50 mL of 30% (v/v) hydrogen peroxide and 2 drops of concentrate nitric acid was added into a solution, which was again digested at 160ºC until the solution became colorless or pale yellow. One milliliter of solution was transferred into a 50-mL volumetric flask and diluted to volume with deionized water. One milliliter of the diluted solution was pipetted into a 30-mL test tube and one drop of 1% (w/v) phenolphthalein was added. After the adjustment pH of the solution with 5 M of sodium hydroxide, 2.00 mL of a combined

reagent was added, followed by mixing and diluting to a 5 mL with deionized water. The reaction was settle for 15 minutes at room temperature and formed a blue complex prior to absorbance measurement at 890 nm.

2.6 Statistics

In this work, One-way ANOVA, *F*-test and paired *t*-test at the 95% confidence limit in Microsoft Excel 2010 version program were used for data analysis.

CHAPTER 3
RESULTS AND DISCUSSION

The spectrophotometric method for determination of phosphate content in concentrated latex was developed. Phosphate in latex could be two forms. One is soluble phosphate obtained after coagulation and the other is total phosphate obtained from latex digestion. Therefore, this work was divided into two parts. The first part was to develop method for determination of soluble phosphate and the result was compared with ion chromatographic method. The second part was to develop method for determination of total phosphate and the result obtained was compared with the standard method (ISO 8053, 1995).

In order to develop a rapid method for determination of soluble and total phosphate in concentrated latex, the important parameters affecting the development studied were: (1) choice of complexation method, (2) anion and cation interferences, (3) type of chemical coagulation for soluble phosphate and (4) solvent for digestion. The validation of the developed method, precision and accuracy were also performed. Three complexation methods, phosphovanadomolybdate method, ascorbic acid method and molybdenum blue method were considered. Phosphovanadomolybdate method was not chosen since its yellow-colored solution obtained after reaction was similar to a yellow-colored latex serum, resulting in a low sensitivity for detection of phosphate by colorimetry. In this study, ammonium molybdate method based on ascorbic acid as a reducing agent was called ascorbic acid method, while that based on hydrazine sulfate was called molybdenum blue method.

3.1 Optimization for complexation method

3.1.1 Complexation methods

The ascorbic acid method and molybdenum blue method were studied for the determination of phosphate in latex. Figure 3.1 shows the absorption spectra of phosphate standard solution obtained by ascorbic acid method and molybdenum blue method, providing the maximum absorbance at 890 and 830 nm, respectively. Compared to the molybdenum blue method, ascorbic acid method provided better

advantages such as its simple reaction method and low cost for total analysis of phosphate due to no heat consumed and its blue-colored complex could be developed at room temperature. Furthermore, some cations and anions reported by Galli *et al.* (2000), Phattanakul *et al.* (2009) and Scott *et al.* (2003), namely, propionic acid, citric acid, maleic acid, magnesium ion, zinc ion and calcium ion, were initially studied for both ascorbic acid method and molybdenum blue method. The result showed that most cations and anions studied appeared to interfere only blue-colored of solution detected by molybdenum blue method. Therefore, ascorbic acid method was preferred and used throughout the experiment.

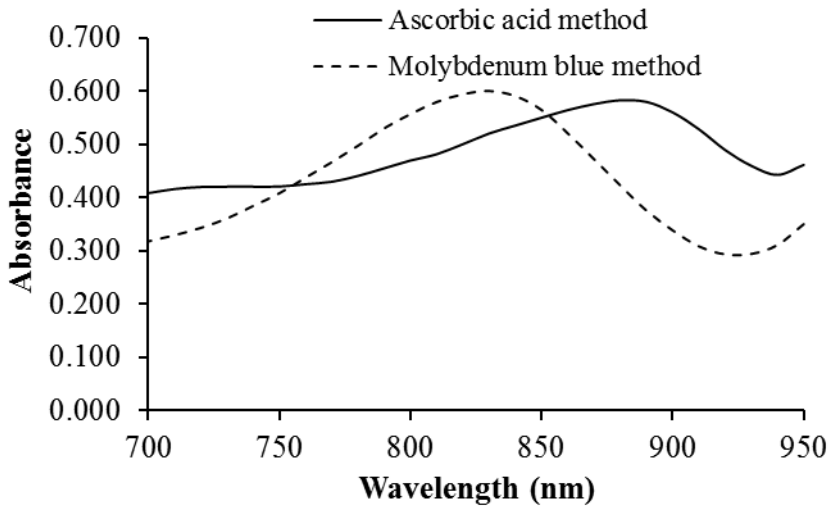


Figure 3.1 Absorption spectra of standard solution at 1.00 mg PO_4^{3-} L^{-1} obtained by ascorbic acid method and molybdenum blue method.

3.1.2 Reagent concentration for a combined reagent

The concentration of reagents, sulfuric acid, potassium antimonyl tartrate, ammonium molybdate and ascorbic acid were optimum to obtain the maximum color development for phosphate in latex sample. According to Beer's law, the absorbance of phosphate obtained depends on the phosphate concentration. Thus, the absorbance of phosphate measured increases when phosphate concentration increases.

a) Effect of sulfuric acid concentration

The absorbance of phosphate complex greatly depends on the pH values of the acidic medium (Pai *et al.,* 1990; Drummond *et al.,* 1995). The concentration of sulfuric acid in a combined reagent was investigated at 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 M (Figure 3.2). The result showed that the absorbance of phosphate increased with sulfuric acid concentration up to 2.5 M and decreased when increasing sulfuric acid concentration. This was because pH of soluble was not suitable for color development. It was found that the optimum color development contained a $[H^+]$ / $[MoO_4^{2-}]$ ratio of 78.1 within a pH ranged from 0.39-0.42. Therefore, 2.5 M of sulfuric acid was chosen for the combined reagent because it provided the maximum absorbance of phosphate, resulting in the high concentration of phosphate detected.

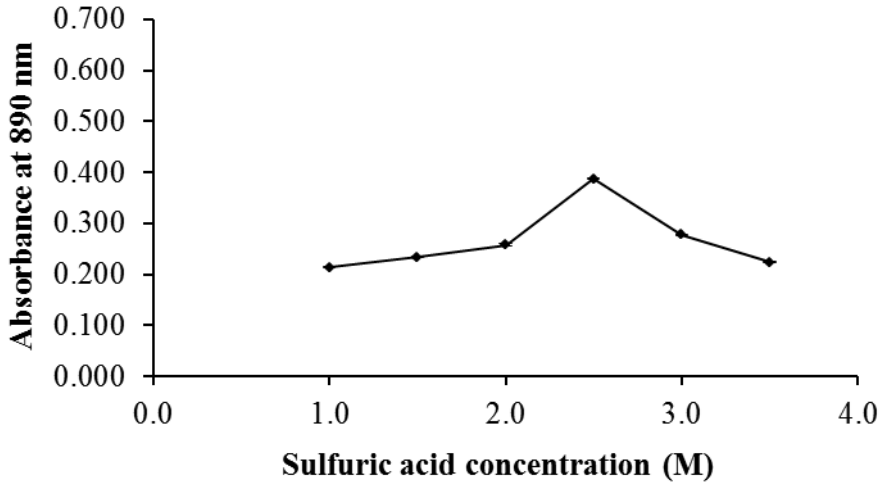


Figure 3.2 Effect of sulfuric acid concentration for 0.50 mg PO_4^{3-} L^{-1}. Conditions: 5.00 mL of 0.01 M potassium antimonyl tartrate, 15.00 mL of 4.0% ammonium molybdate and 30.00 mL of 0.10 M ascorbic acid.

b) Effect of potassium antimonyl tartrate concentration

Potassium antimonyl tartrate was used for increasing rate of reduction with ascorbic acid (Going and Eisenreich, 1974), therefore, the effects of its concentration in a combined reagent for phosphate complex absorbance and reaction time of the maximum color development were studied at 0.005, 0.010, 0.030, 0.050, 0.070 and 0.090 M. The phosphate complex absorbance increased with potassium antimonyl tartrate concentration up to 0.01 M and then was stable after increasing

potassium antimonyl tartrate concentration up to 0.050 M with slightly decreased after 0.070 M (Figure 3.3). When the concentration of potassium antimonyl tartrate increased to 0.01 M, the reaction time of colored development was 2 min. Hence, 0.01 M of potassium antimonyl tartrate was chosen for a combined reagent due to the highest absorbance obtained and short reaction time.

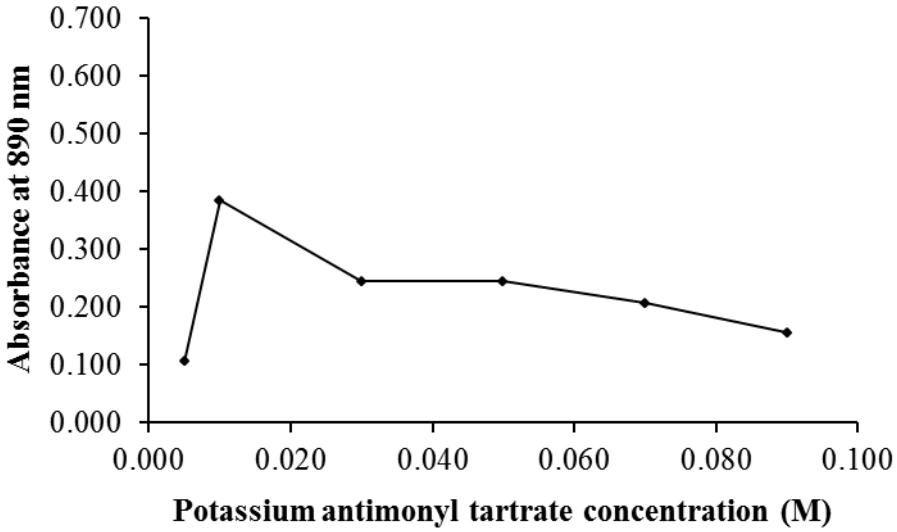


Figure 3.3 Effect of potassium antimonyl tartrate concentration for 0.50 mg PO_4^{3-} L^{-1}. Conditions: 50.00 mL of 2.5 M sulfuric acid, 15.00 mL of 4.0% ammonium molybdate and 30.00 mL of 0.10 M ascorbic acid.

c) Effect of ammonium molybdate concentration

Ammonium molybdate affects the color intensity of the complex. The concentrations of ammonium molybdate in a combined reagent were investigated at 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0%. As shown in Figure 3.4, the phosphate absorbance increased when concentration of ammonium molybdate was increased up to 4.0% and decreased after ammonium molybdate concentration more than 4.0%. Therefore, 4.0% ammonium molybdate was chosen in order to obtain the highest sensitivity of phosphate concentration in samples.

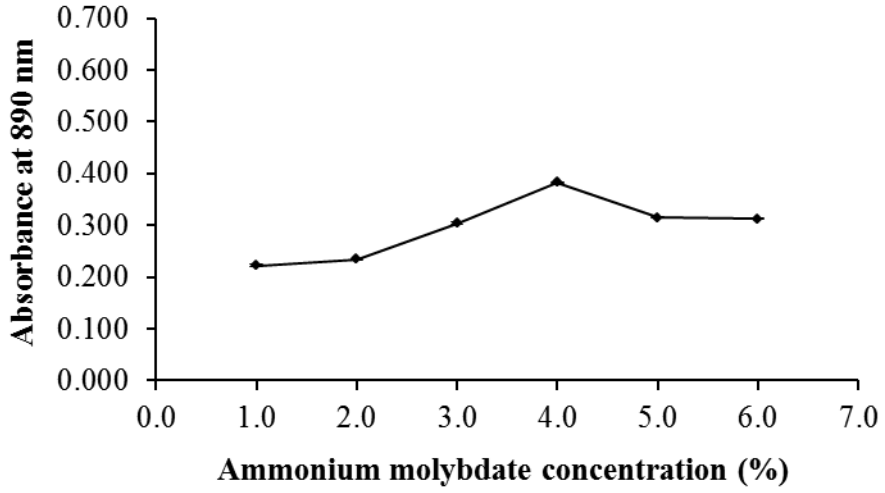


Figure 3.4 Effect of ammonium molybdate concentration for 0.50 mg PO_4^{3-} L^{-1}. Conditions: 50.00 mL of 2.5 M sulfuric acid, 5.00 mL of 0.01 M potassium antimonyl tartrate and 30.00 mL of 0.10 M ascorbic acid.

d) Effect of ascorbic acid concentration

In the acidic medium, the interaction of potassium dihydrogen phosphate with ammonium molybdate and potassium antimonyl tartrate resulted in phosphorus molybdenum heteropoly acid which was further reacted with ascorbic acid, resulting in molybdenum blue complex. Thus, the content of ascorbic acid strongly affects the color development (Csuros, 1997).

The concentrations of ascorbic acid in a combined reagent were investigated at 0.05, 0.10, 0.20, 0.30 and 0.40 M. The highest phosphate absorbance increased with ascorbic acid concentration up to 0.10 M (Figure 3.5). The concentration of ascorbic acid at 0.10 M reduced the reaction time to 2 min. Going and Eisenreich (1974) reported that it was necessary to use an ascorbic acid concentration at least twenty times of the maximum phosphate content present in samples in order to obtain full color development within 10-30 min. In our results, 0.10 M ascorbic acid was found to be excess enough to reach equilibrium within 10 min, therefore, it was chosen.

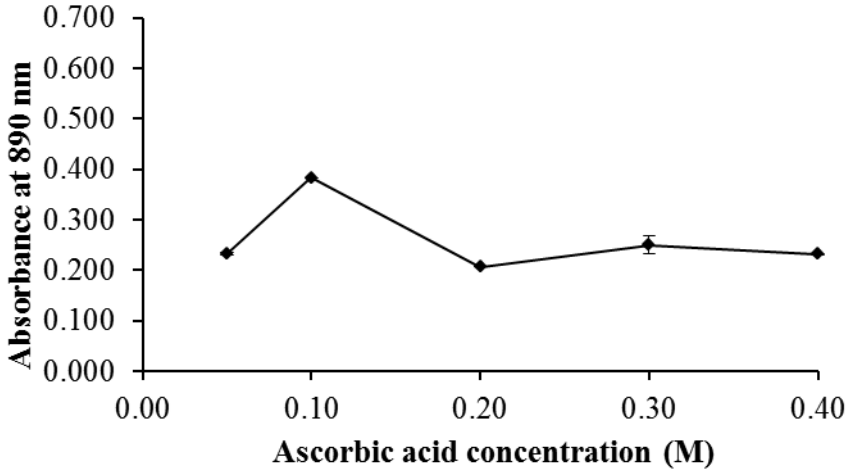


Figure 3.5 Effect of ascorbic acid concentration for 0.5 mg PO_4^{3-} L^{-1}. Conditions: 50.00 mL of 2.5 M sulfuric acid, 5.00 mL of 0.01 M potassium antimonyl tartrate and 15.00 mL of 4.0% ammonium molybdate.

3.1.3 Stability of reagent by ascorbic acid method

The stability of each reagent in a combined reagent was studied in order to obtain the reproducible results of phosphate determination.

a) Stability of 0.01 M potassium antimonyl tartrate

Potassium antimonyl tartrate prepared in deionized water was stored in a glass bottle at 4ºC and allowed to return to room temperature before use. Its stability was checked together with phosphate standard solution and other fresh reagents in a combined reagent. As shown in Figure 3.6, this solution was stable for 12 weeks. The result was obtained in good agreement with the value reported by Csuros (1997).

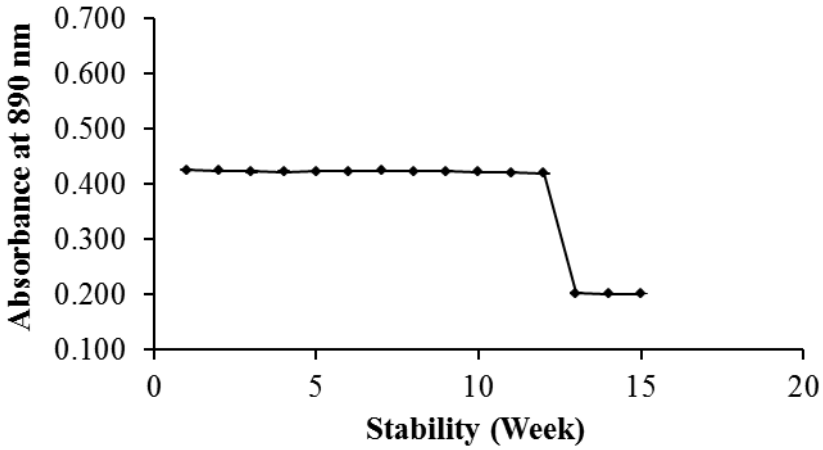


Figure 3.6 Stability of 0.01 M potassium antimonyl tartrate.

b) Stability of 4.0% ammonium molybdate

Figure 3.7 shows the stability of 4.0% ammonium molybdate prepared in deionized water. It was shown that ammonium molybdate was stable for 12 weeks which was in good agreement with the study reported by Csuros (1997).

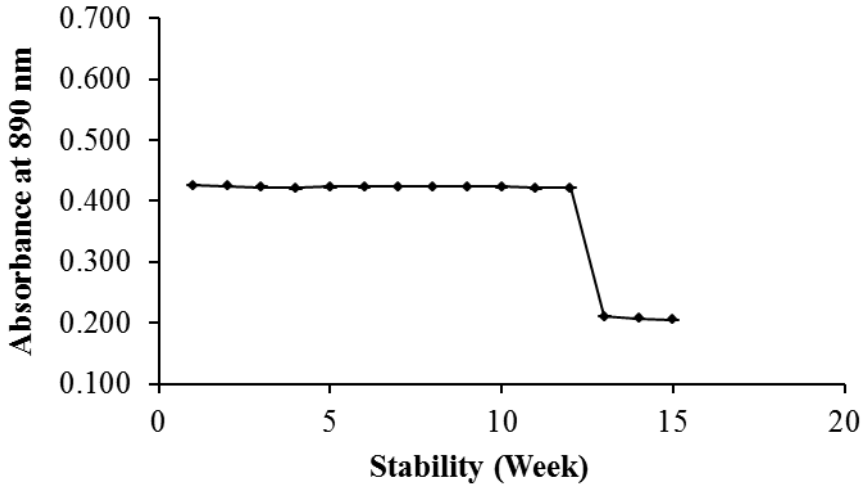


Figure 3.7 Stability of 4.0% ammonium molybdate.

c) Stability of 0.10 M ascorbic acid

It can be seen in Figure 3.8 that ascorbic acid solution was stable for two weeks. This result was different from values reported by Murphy and Riley (1958) and Csuros (1997). Murphy and Riley (1958) reported that acidified molybdate solution was stable for only 4-24 hours, while Csuros (1997) reported that ascorbic acid in distilled water was stable up to one week.

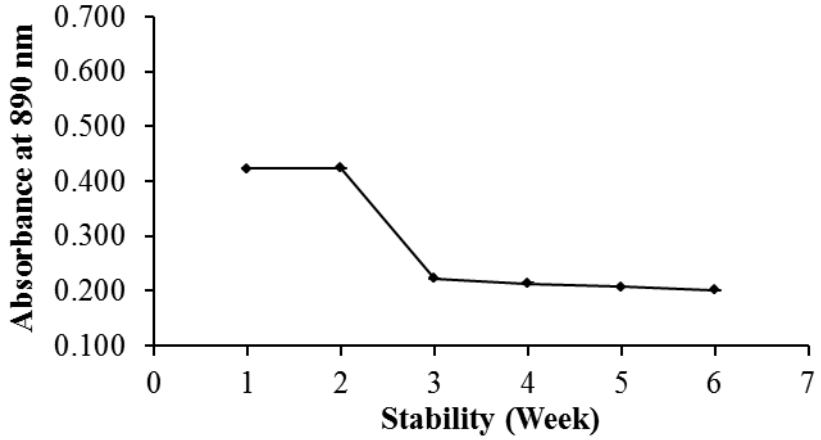


Figure 3.8 Stability of 0.10 M ascorbic acid.

d) Stability of a combined reagent

A combined reagent was prepared and its stability was investigated every day until 3 days. This solution was stored at 4ºC when not in use. It can be seen in Figure 3.9 that a combined reagent used for color development was stable for 2 days. Therefore, a combined reagent in this work could be used for only 2 days after prepared. A result obtained was contrast to a value reported by Drummond *et al.* (1995), in which it was stable for 15 days. This difference in stability was because Drummond *et al.* (1995) used ascorbic acid prepared in sulfuric acid for a combined reagent. In this work, a combined reagent used for color development was recommend to be used within 2 days and stored at 4ºC.

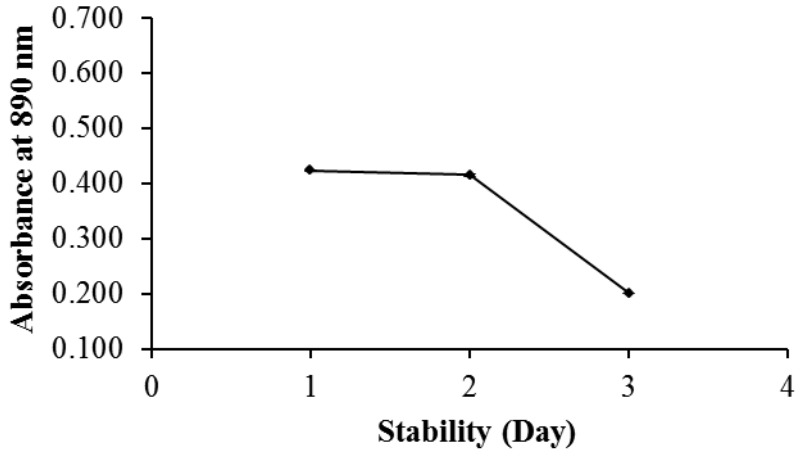


Figure 3.9 Stability of a combined reagent.

3.1.4 Stability of phosphate complex by ascorbic acid method

The stability of phosphate complex obtained by using the optimum combined reagent was studied in order to investigate the reproducibility of the phosphate complex measurement if the measurement of absorbance could not be conducted continuously. The color stability was observed after complexation of phosphate in a standard chemical reagent and a latex serum. It can be seen in Figure 3.10 that color development obtained by phosphate in a standard reagent and a serum was stable up to 130 min. The phosphate complex absorbance in a standard solution was found to be higher than that in a serum solution because concentration of phosphate used was in a standard solution higher than that in sample.

The advantage of long stability of phosphate complexation in this work was that after reaction, samples could be analyzed at least 20 samples per min with good reproducibilities.

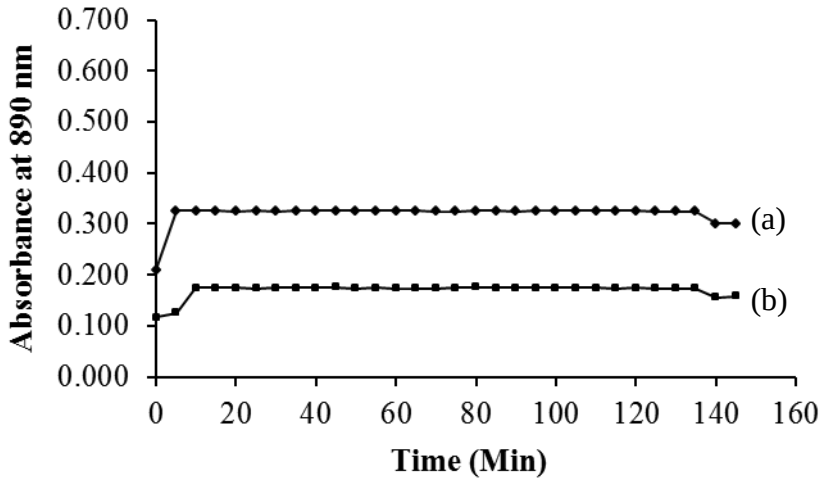


Figure 3.10 Stability of complex obtained by using (a) 500 mg PO_4^{3-} L^{-1} in a standard chemical reagent (b) phosphate in a sample solution. Conditions were: 50.00 mL of 2.5 M sulfuric acid, 5.00 mL of 0.01 M potassium antimonyl tartrate, 15.00 mL of 4.0% ammonium molybdate and 30.00 mL of 0.10 M ascorbic acid.

Stability of phosphate complex when using two weeks old ascorbic acid for a combined reagent was investigated together with phosphate in a standard chemical reagent and latex serum. As shown in Figure 3.11, phosphate complex obtained by using two weeks old ascorbic acid was stable up to 130 min which was the same result obtained by a method using fresh ascorbic acid. This result presented that two weeks old ascorbic acid could be used for complexation.

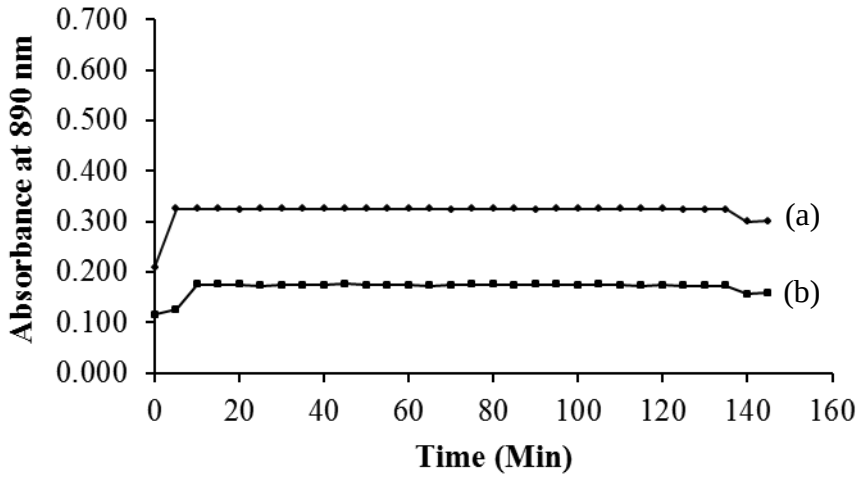


Figure 3.11 Stability of complex when using two weeks old ascorbic acid obtained by using (a) 500 mg PO_4^{3-} L^{-1} in a standard chemical reagent (b) phosphate in a sample solution. Conditions were: 50.00 mL of 2.5 M sulfuric acid, 5.00 mL of 0.01 M potassium antimonyl tartrate, 15.00 mL of 4.0% ammonium molybdate and 30.00 mL of 0.10 M ascorbic acid.

3.1.5 Effect of indigenous ions in latex on complexation

3.1.5.1 Effect of indigenous ions

It is known that *Hevea brasiliensis* latex is a complicated natural product (Blackley, 1997, Vol. 2, p. 1-5). Therefore, other constituents in latex were investigated for the determination of phosphate content in order to know their degree of interference. Cation and anion constituents as mentioned by Galli *et al.* (2000), Galli *et al.* (2003) and Phattanakul *et al.* (2009) were studied for ascorbic acid and molybdenum blue method. As shown in Figure 3.12, both cations and anions did not interfere the blue color of complexation obtained by ascorbic acid method.

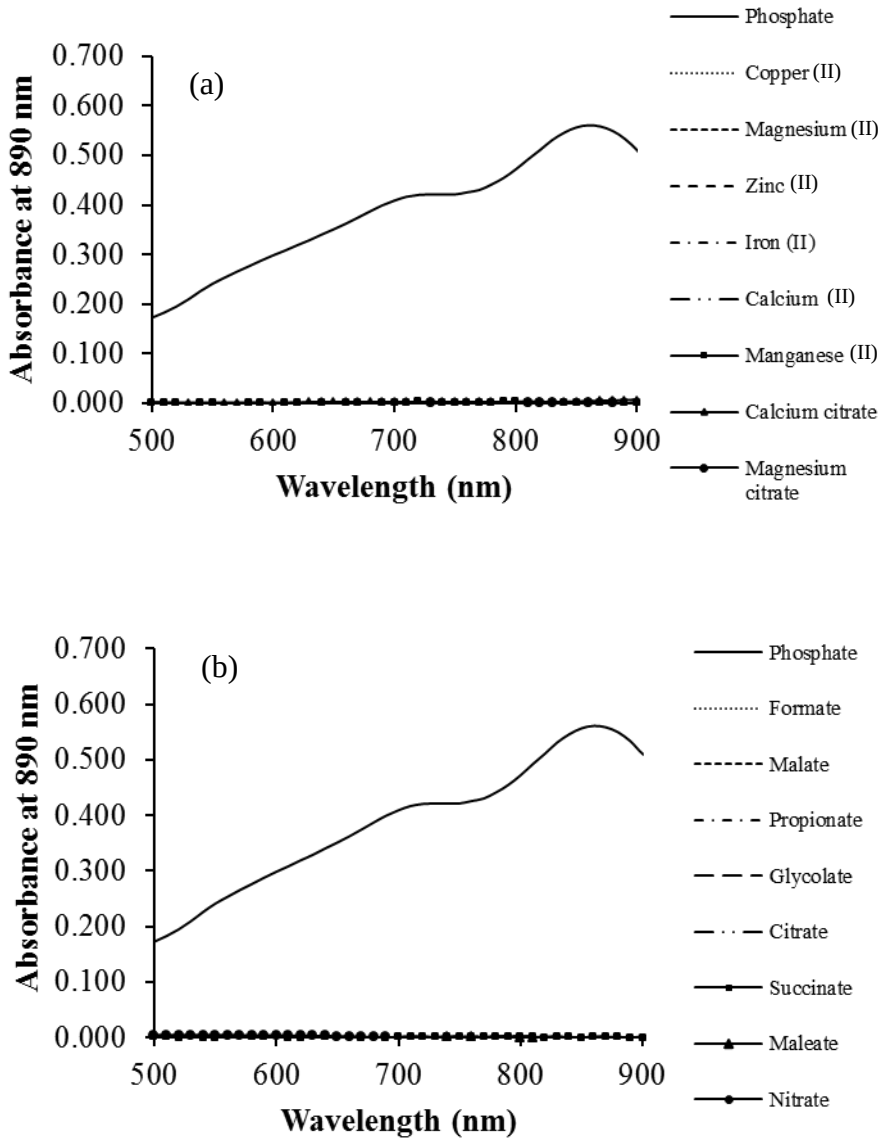


Figure 3.12 Spectra of (a) cations and (b) anions at 100 mg L^{-1} studied by ascorbic acid method for 0.5 mg PO_4^{3-} L^{-1}.

For molybdenum blue method, cations and anions affected the color development of phosphate complex by one and two times when compared to the colored of complex of standard phosphate solution, respectively (Figure 3.13). Therefore, ascorbic acid method was chosen for spectrophotometric determination of phosphate content in latex.

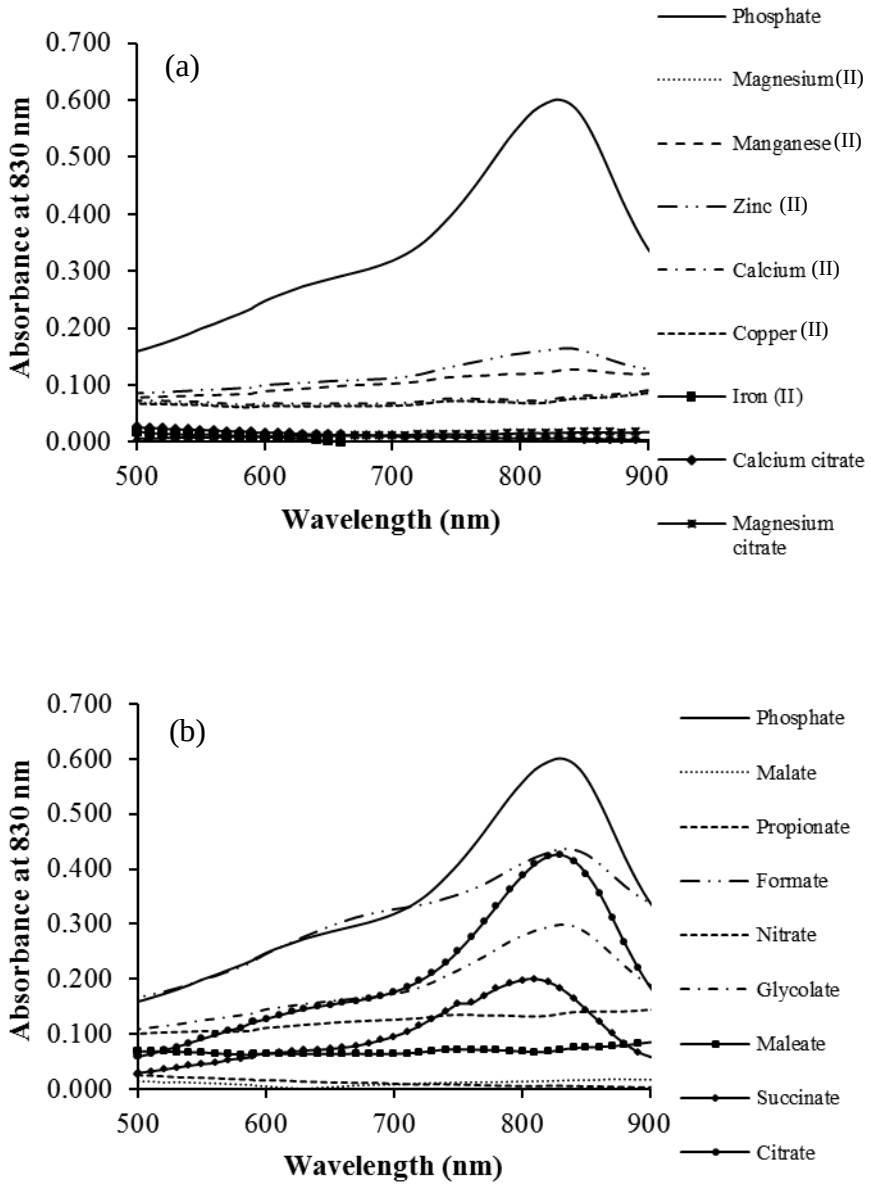


Figure 3.13 Spectra of (a) cations and (b) anions at 100 mg L^{-1} studied by molybdenum method for 0.5 mg PO_4^{3-} L^{-1}.

3.1.5.2 Tolerance limits of indigenous ions

It is necessary to evaluate the effect of coexistence ions on the color reaction of phosphate ion due to complicated matrix concentrated latex. Both anions and cations were investigated in terms of the tolerance limits which were the maximum concentration of ion causing an error of ≤ 5% in the absorbance measurement (Khlyntseva *et al.,* 2011). The results are shown in Tables 3.1 and 3.2. It

indicated that most cations and anions did not interfere the determination of phosphate. Phosphate complexation could tolerance most of studied ions except that citrate, iron (II), iron (III) and calcium ion as calcium citrate were lesser tolerated than others in the range of 470 and 750 mg L^{-1} for phosphate determination obtained by the developed method.

Table 3.1 Tolerance limit of anions for the determination of 0.50 mg PO_4^{3-} L^{-1} by ascorbic acid method (n=6)

Ion name	**Compound**	**Tolerance limits (mg L^{-1})**
Formate	Formic acid	>1000
Propionate	Propionic acid	>1000
Glycolate	Glycollic acid	>1000
Citrate	Citric acid	500
Nitrate	Sodium nitrate	>1000
Succinate	Succinic acid	>1000
Malate	Malic acid	>1000
Maleate	Maleic acid	>1000

Table 3.2 Tolerance limit of cations for the determination of 0.50 mg PO_4^{3-} L^{-1} by ascorbic acid method (n=6)

Ion name	**Compound**	**Tolerance limits (mg L^{-1})**
Copper (II)	Copper sulfate	965
Magnesium (II)	Magnesium sulfate	>1000
Zinc (II)	Zinc sulfate	>1000
Calcium (II)	Calcium sulfate	>1000
Manganese (II)	Manganese sulfate	>1000
Iron (II)	Iron (II) sulfate	650
Iron (III)	Iron (III) citrate	470
Calcium (II)	Calcium citrate	750
Magnesium (II)	Magnesium citrate	950

3.1.6 Effect of total matrix in latex on complexation

Standard solution at different amount was added into latex sample in order to perform standard addition curve. Figure 3.14 shows the comparison of standard addition curve and standard curve for analysis of soluble phosphate. It was found that a sensitivity of standard addition curve (slope) was not significantly different from that of standard curve (One-way ANOVA at a 95% confidence limit). This presented that no matrix of latex affected the quantitation of soluble phosphate in latex.

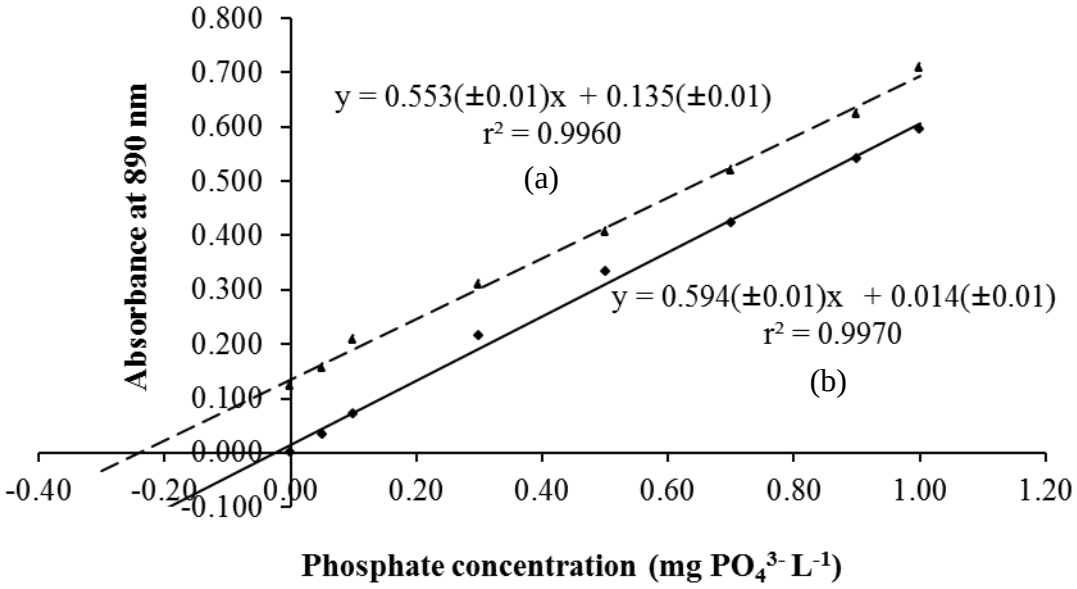


Figure 3.14 Comparison of (a) standard addition curve and (b) standard curve in the range of 0.05-1.00 mg PO_4^{3-} L^{-1} for analysis of soluble phosphate.

For analysis of total phosphate content, the result as shown in Figure 3.15 indicated that sensitivity of standard addition curve prepared by adding of magnesium ammonium phosphate solution was comparable to that of standard curve. This also presented that no latex matrix affected the quantitation of total phosphate in latex.

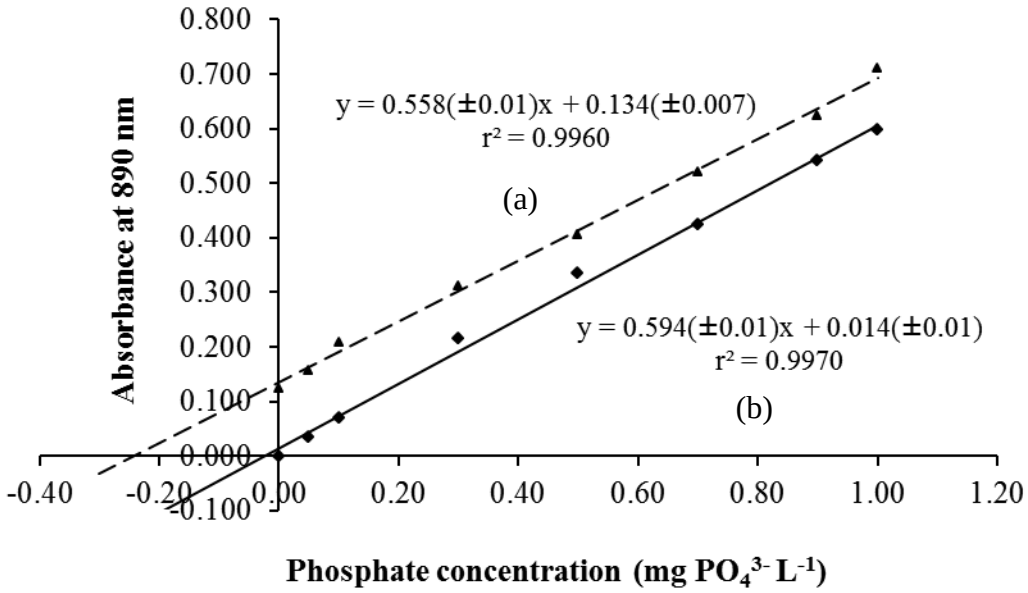


Figure 3.15 Comparison of (a) standard addition curve and (b) standard curve in the range of 0.05-1.00 mg PO_4^{3-} L^{-1} for analysis of total phosphate.

3.2 Sample preparation for soluble phosphate determination in concentrated latex

In this work, soluble phosphate in latex which was chemically coagulated by different reagents. The studied reagents of coagulation were 95% (v/v) ethanol, 2% (w/v) calcium chloride and 2% (w/v) calcium nitrate.

3.2.1 Effect of reagent on rubber coagulation

Apart from ethanol, calcium chloride and calcium nitrate as reported by Blackley (1997, Vol. 1, p. 259) and Karunanayake and Perera (2006) were also studied for their coagulation efficiency. As shown in Figure 3.16, ethanol provided much higher 2 times absorbance of soluble phosphate than calcium chloride and calcium nitrate. Calcium chloride and calcium nitrate could cause colloidal particle agglomeration of the latex which was a white precipitated form in serum (Galli *et al.*, 2000), resulting in very low absorbance measurement of soluble phosphate. Thus, ethanol was suitable for the latex coagulation because it provided the highest concentration of soluble phosphate detected.

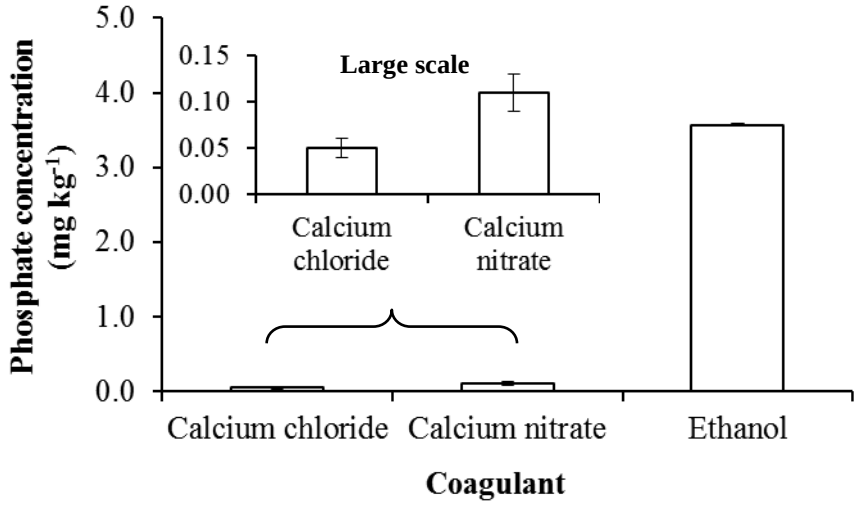


Figure 3.16 Effect of chemicals on rubber coagulation.

3.2.2 Effect of ethanol concentration on rubber coagulation

Ethanol was studied as the latex coagulation reagent for soluble phosphate analysis. As can be seen in Figure 3.17, different volume of ethanol provided the same level of soluble phosphate detected. In order to reduce cost for the analysis, 5.00 mL of ethanol was chosen for the latex coagulation.

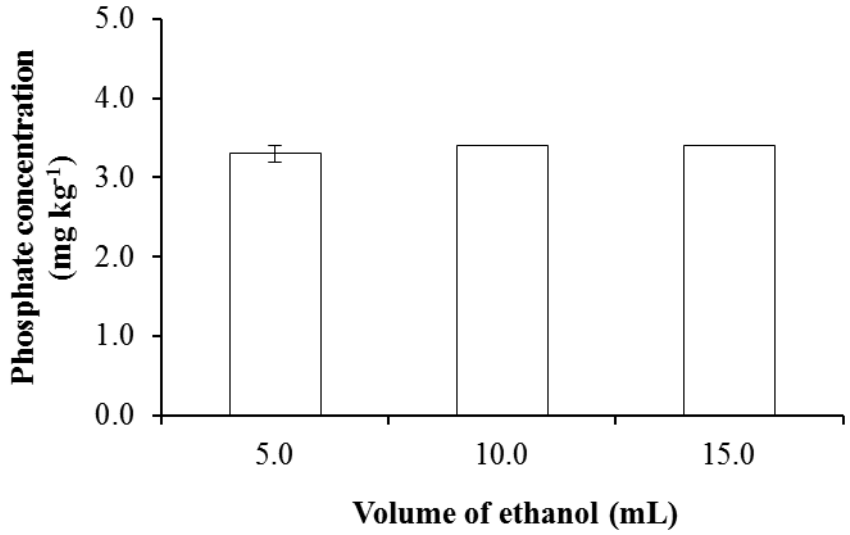


Figure 3.17 Effect of volume of ethanol for latex coagulation.

3.2.3 Second coagulant

Soluble phosphate was initially analyzed by coagulation with ethanol together with filtration *via* a nylon membrane. However, the cost of analysis is high due to the cost of a nylon membrane. Therefore, second coagulants, *i.e.,* trichloroacetic acid (Yeang *et al.,* 2002 and Jiang *et al.,* 2004), acetone, methanol and ammonium sulfate (Jiang *et al.,* 2004) were studied together with a Whatman no. 42 filter paper to remove of solid particles. The results showed that after second coagulation with trichloroacetic acid, methanol and ammonium sulfate and filtration, the serum solutions were turbid. In contrast to other reagents, only acetone provided a clear serum solution and the highest concentration of soluble phosphate. Thus, acetone was chosen for the solid particles coagulation after the latex coagulation with ethanol.

3.3 Sample preparation for total phosphate determination in concentrated latex

3.3.1 Effect of weight of latex for digestion

The amount of natural rubber latex 2-10 g is required for ISO 8053 method, therefore, the investigation of weight of latex samples for the development method is important. The weights of 0.25, 1.00 and 2.50 g of latex were studied for latex digestion. As shown in Figure 3.18, the percentage of recovery obtained from three weights were significantly different (*F*-test and paired *t*-test at 95% confident limit). It was found that 1.00 and 2.50 g of concentrated latex provided lower recoveries with the RSD higher than 10% because latex sample was bounced out of the test tube during digestion. Therefore, 0.25 g of concentrated latex was chosen for total phosphate determination.

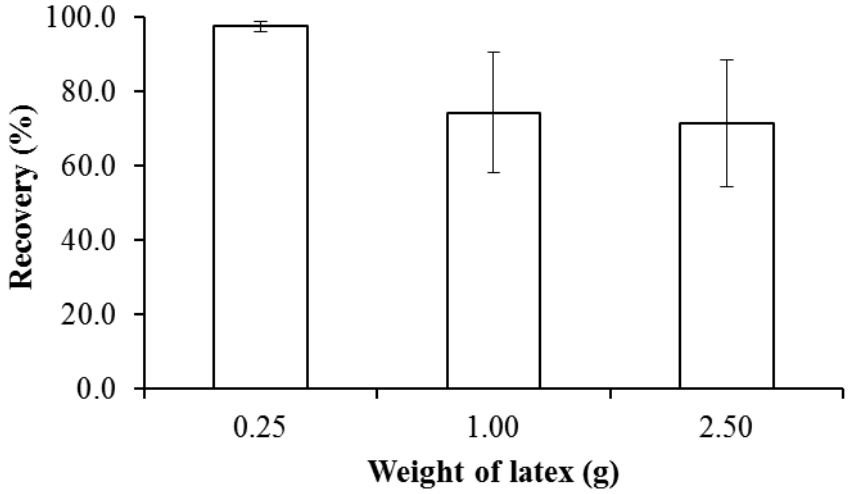


Figure 3.18 Effect of different amounts of concentrated latex sample for total phosphate determination.

3.3.2 Method for latex digestion

Different digestion methods, namely Method A using nitric acid and 30% hydrogen peroxide, Method B using nitric acid and Method C using sulfuric acid and 30% hydrogen peroxide, were compared in order to obtain a suitable digestion method for determination of total phosphate in latex. Each method provided clear solution after an acidic digestion. Concentrations of total phosphate in latex were 269.1 ± 2.3, 266.3 ± 2.6 and 264.7 ± 2.7 mg kg^{-1} based on %TSC for Method A, Method B and method C, respectively, with no differences in concentrations obtained from three methods (Figure 3.19). Method A provided faster digestion and less volume use of acid than Method B, while Method C took the longest time for digestion. Using volume of sulfuric acid in Method C was higher 2 times than Method A and B. Thus, latex digestion by using nitric acid with 30% hydrogen peroxide (Method A) was chosen for the determination of total phosphate in concentrated latex.

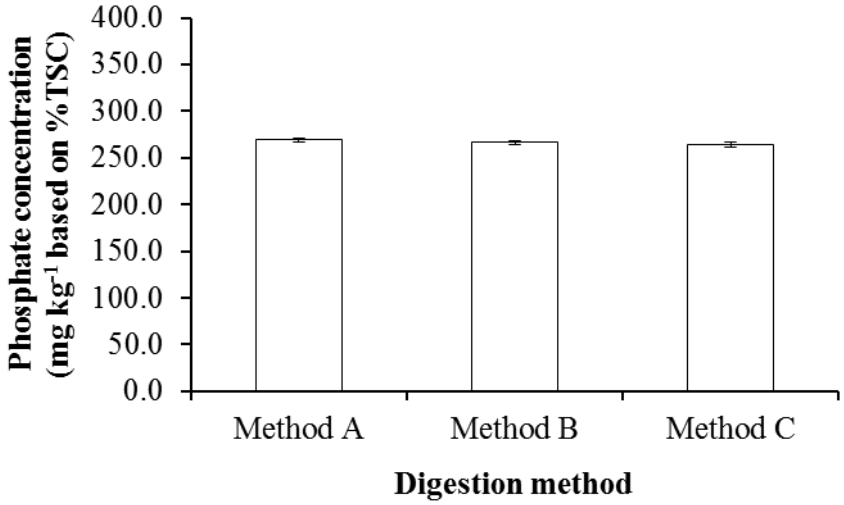


Figure 3.19 Total phosphate concentrations obtained by three developed methods; Method A: HNO_3 + 30% H_2O_2, Method B: HNO_3 only and Method C: H_2SO_4 + 30% H_2O_2 (n=6).

The concentration of total phosphate in latex samples obtained by the developed method (Method A) was also compared with the modified standard method (ISO 8053, 1995). As can be seen in Figure 3.20, the results obtained by two methods were not significantly different (*F*-test and paired *t*-test at a 95% confident limit). These results suggested that the sample preparation and spectrophotometric method developed could be applied effectively for the determination of total phosphates in concentrated latex.

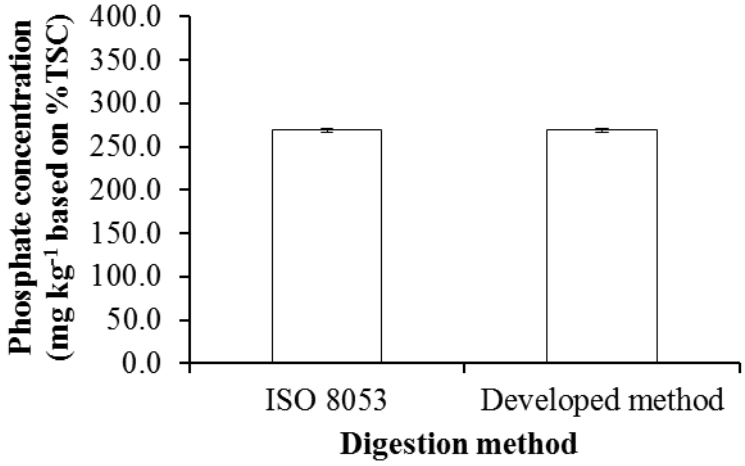


Figure 3.20 Total phosphate concentration obtained by modified standard method: HNO_3 + H_2SO_4 + 30% H_2O_2 and developed method: HNO_3 + 30% H_2O_2 (n=6).

3.4 Method validation for phosphate determination

3.4.1 Calibration curve of phosphate

As shown in Figure 3.21, the standard calibration curve performed by using an optimum condition and spectrophotometric detection based on ascorbic acid method was found to give a linear response in the concentration range of 0.05-1.00 mg PO_4^3 L^{-1}, with a good correlation coefficient of 0.9970. The molar absorptivity (ε) was found equal 8.93×10^4 L mol^{-1} cm^{-1} which was similar to that of malachite green method (ε = 8.98×10^4 L mol^{-1} cm^{-1}) (Jastrzębska, 2009) and higher than the values reported by Khlyntseva *et al.* (2011) and Mahadevaiah *et al.* (2007) at 3.70×10^4 L mol^{-1} cm^{-1} and 6.10×10^3 L mol^{-1} cm^{-1}, respectively. Mathematical limit of detection and limit of quantification were found to be 0.03 mg PO_4^{3-} L^{-1} at the signal to noise ratio of 3 and 0.05 mg PO_4^{3-} L^{-1} at the signal to noise ratio of 10, respectively.

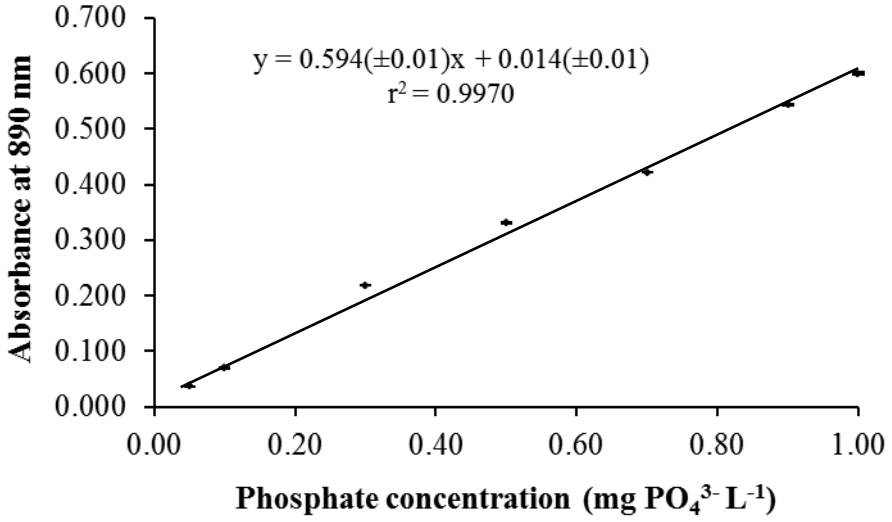


Figure 3.21 Calibration curve for the determination of phosphate in the range of 0.05-1.00 mg PO_4^{3-} L^{-1}.

The comparison of the proposed method with other reports for phosphate analysis was shown in Table 3.3. It was found that our proposed method provided higher sensitivity, faster reaction and lower detection limit of phosphate analysis than other reports.

Table 3.3 Comparison of the proposed method with other reports for phosphate analysis

Parameter	**This work**	**Szlyk *et al.* (2004)**	**Jastrzębs-ka (2009)**	**Hrynczyszyn *et al.* (2010)**	**Shyla *et al.* (2011)**
Sample	Concen-trated latex	Soya food	Meat	Meat	Soil, water detergent, bone and food
Complexation method	Ascorbic acid	Malachite green	Ascorbic acid	Hydrazine sulfate	Thiourea
λ max (nm)	890	640	730	820	840
Linear concentration range (ppm)	0.05-1.00	0.02-0.22	0.11-0.87	50-750	0.50-10.00
Slope (m)	0.594	2.8994	0.2823	0.0011	0.0126
Standard deviation of slope	0.01	0.0107	0.0025	0.15×10^{-5}	-[a]
Intercept (c)	0.014	0.0627	0.0206	0.0004	0.0135
Standard deviation of intercept	0.01	0.0027	0.00146	0.16×10^{-6}	-
Regression coefficient (R^2)	0.9970	0.9995	0.9988	0.9994	0.9769
Detection limit, LOD (mg L^{-1})	0.03	0.021	0.012	16	-
Quantification limit, LOQ (mg L^{-1})	0.05	0.0070	0.040	53	-
Molar absorptivity, Ɛ (L mol^{-1} cm^{-1})	8.93×10^{4}	8.98×10^{3}	8.72×10^{4}	-	1.712×10^{3}
Sandell's sensitivity (μL/cm^2/0.001 Abs)[b]	1.50×10^{-3}	-	-	-	0.0555
Color development time (min)	1-2	20	10	-	>5

[a]not indicated. [b]calculated as reported by Agrawal *et al.* (2010).

3.4.2 Accuracy and precision

The recoveries of soluble phosphate content were assessed by spiking potassium dihydrogen phosphate into concentrated latex samples to obtain the final concentration of soluble phosphate at 30.0, 60.0 and 90.0 mg PO_4^{3-} L^{-1}. As can be seen in Table 3.4, the mean percentage recoveries were found to be in the good range at 87.6-107.2%, with the RSD less than 5% for intra-day and 82.6-107.7%, with the RSD less than 5% for inter-day.

For total phosphate, it is known that phosphate is added into latex to remove magnesium which is precipitated as magnesium ammonium phosphate. Therefore, magnesium ammonium phosphate was spiked into concentrated latex in order to check whether phosphate in a form of magnesium ammonium phosphate was digested completely. The recoveries were assessed by spiking magnesium ammonium phosphate into concentrated latex samples to obtain the final concentration of total phosphate at 30, 60.0 and 90.0 mg PO_4^{3-} L^{-1}. As can be seen in Table 3.5, the mean percentage recoveries for low and high ammonium preserved latexes were found to be in the good range at 87.9-103.1%, with the RSD less than 5% for intra-day and 87.2-102.8%, with the RSD less than 5% for inter-day. Thus, this result suggested that a completed digestion was obtained for the determination of total phosphate in concentrated latex.

Furthermore, recoveries for the developed method were carried out by determining soluble and total phosphate contents in field latex before latex centrifugation and in concentrated latex samples after precipitating as magnesium ammonium phosphate followed by latex centrifugation. The samples were collected from five different districts located in the Songkhla province of Thailand. The recoveries of soluble phosphate contents were assessed by spiking potassium dihydrogen phosphate into field latex and concentrated latex samples to obtain the final concentration of soluble phosphate at 30.0, 60.0 and 90.0 mg PO_4^{3-} L^{-1}. As can be seen in Table 3.6, the mean percentage recoveries of soluble phosphates were found to be in the good range between 82.0-105.2%, with the RSD less than 2% for field latex and 84.1-102.3%, with the RSD less than 5% for concentrated latex.

For total phosphate, the recoveries were assessed by spiking magnesium ammonium phosphate into field latex and concentrated latex samples to obtain the final concentrations of total phosphate at 30.0, 60.0 and 90.0 mg PO_4^{3-} L^{-1}. As can be seen in Table 3.6, the mean percentage recoveries of total phosphate in field and concentrated latexes were found to be in the good range at 81.5-105.9%, with the RSD less than 5% and 82.9-103.9%, with the RSD less than 5%, respectively.

Table 3.4 Recovery and precision for soluble phosphate in concentrated latex samples (Phosphate spiked as potassium dihydrogen phosphate)

Sample[a]	Added PO_4^{3-} as KH_2PO_4 (mg PO_4^{3-} L^{-1})	Found soluble PO_4^{3-} (mg kg^{-1}) (%RSD[b]) intra-day		Recovery (%RSD)		Found soluble PO_4^{3-} (mg kg^{-1}) (%RSD) inter-day		Recovery (%RSD)	
		LA[c]	HA[d]	LA	HA	LA	HA	LA	HA
Hb1	0.0	10.0 (0.3)	11.6 (0.4)	-	-	9.9 (1.6)	10.0 (0.4)	-	-
	30.0	42.2 (0.6)	40.4 (0.1)	107.2 (1.1)	96.1 (0.2)	37.5 (1.5)	36.8 (1.3)	91.9 (1.4)	89.4 (1.2)
	60.0	58.6 (0.3)	67.3 (0.6)	97.6 (0.4)	92.8 (0.7)	60.1 (1.9)	61.1 (0.5)	83.6 (1.2)	85.2 (0.3)
	90.0	89.5 (0.5)	97.5 (0.1)	97.2 (0.6)	95.5 (0.1)	84.3 (1.4)	89.9 (0.4)	82.6 (1.5)	88.8 (0.7)
Hb2	0.0	14.1 (0.4)	14.9 (0.3)	-	-	11.2 (1.5)	14.1 (0.6)	-	-
	30.0	43.5 (0.4)	44.6 (0.2)	97.7 (0.5)	98.9 (0.1)	37.2 (2.4)	39.3 (1.9)	86.8 (3.4)	100.8 (4.4)
	60.0	70.6 (0.2)	71.8 (0.2)	94.1 (0.3)	94.8 (0.3)	68.1 (2.2)	66.4 (1.9)	94.99 (2.2)	93.9 (2.0)
	90.0	97.5 (0.1)	97.7 (0.2)	97.7 (0.2)	91.9 (0.3)	91.3 (1.7)	90.7 (1.8)	89.1 (1.7)	89.6 (2.1)
Hb3	0.0	10.8 (0.9)	11.1 (0.4)	-	-	11.3 (1.4)	11.3 (0.3)	-	-
	30.0	43.7 (0.4)	39.4 (0.4)	103.1 (0.6)	92.5 (0.6)	43.6 (1.0)	41.9 (2.1)	107.7 (1.5)	102.2 (1.9)
	60.0	69.1 (0.5)	70.1 (0.3)	93.9 (0.5)	97.5 (0.4)	63.6 (1.5)	64.2 (1.5)	87.2 (1.9)	88.3 (0.8)
	90.0	100.3 (0.2)	94.3 (0.2)	98.3 (0.3)	91.9 (0.3)	91.6 (0.6)	88.3 (0.8)	89.2 (0.7)	85.6 (0.6)

Table 3.4 Recovery and precision for soluble phosphate in concentrated latex samples (Phosphate spiked as potassium dihydrogen phosphate) (Continued)

Sample[a]	Added PO_4^{3-} as KH_2PO_4 (mg PO_4^{3-} L^{-1})	Found soluble PO_4^{3-} (mg kg^{-1}) (%RSD[b]) intra-day		Recovery (%RSD)		Found soluble PO_4^{3-} (mg kg^{-1}) (%RSD) inter-day		Recovery (%RSD)	
		LA[c]	HA[d]	LA	HA	LA	HA	LA	HA
Hb4	0.0	12.8 (0.5)	11.6 (0.4)	-	-	12.3 (0.4)	11.3 (0.4)	-	-
	30.0	43.7 (0.4)	39.4 (0.4)	103.1 (0.6)	92.5 (0.6)	38.7 (1.1)	39.0 (0.9)	87.4 (1.6)	92.6 (3.1)
	60.0	69.1 (0.5)	70.1 (0.3)	93.9 (0.5)	97.5 (0.4)	64.9 (1.7)	62.3 (1.8)	87.6 (1.9)	85.1 (1.3)
	90.0	100.3 (0.2)	94.3 (0.2)	97.3 (0.3)	91.9 (0.3)	91.7 (0.6)	89.1 (1.2)	88.3 (1.4)	86.5 (1.4)
Hb5	0.0	11.9 (0.5)	12.2 (0.3)	-	-	11.9 (0.6)	12.1 (0.8)	-	-
	30.0	39.6 (0.7)	40.7 (1.3)	91.9 (0.9)	95.2 (1.9)	40.3 (1.2)	42.3 (1.9)	94.7 (1.7)	100.5 (2.5)
	60.0	71.4 (0.2)	71.5 (0.2)	99.0 (0.2)	98.9 (0.3)	64.1 (1.7)	63.2 (1.2)	86.9 (1.8)	85.1 (1.3)
	90.0	90.7 (0.1)	98.8 (0.2)	87.6 (0.1)	96.3 (0.2)	94.3 (1.6)	94.3 (1.5)	91.6 (1.7)	91.3 (1.7)

[a]Hb is *Hevea brasiliensis* latex, [b]RSD is relative standard deviation (n=6), [c]LA is low ammonia preserved concentrated latex, [d]HA is high ammonia preserved concentrated latex.

Table 3.5 Recovery and precision for total phosphate in concentrated latex samples (Phosphate spiked as magnesium ammonium phosphate)

Sample[a]	Added PO_4^{3-} as $Mg(NH_4)PO_4$ ($mg\ PO_4^{-3}\ L^{-1}$)	Found total PO_4^{3-} ($mg\ kg^{-1}$) (%RSD[b]) intra-day		Recovery (%RSD)		Found total PO_4^{3-} ($mg\ kg^{-1}$) (%RSD) inter-day		Recovery (%RSD)	
		LA[c]	HA[d]	LA	HA	LA	HA	LA	HA
Hb1	0.0	137.7 (0.8)	134.3 (0.9)	-	-	137.7 (0.6)	133.2 (0.7)	-	-
	30.0	165.6 (0.3)	161.4 (0.7)	93.0 (1.2)	94.1 (1.5)	160.3 (1.2)	165.8 (1.9)	92.3 (1.0)	102.3 (1.8)
	60.0	201.9 (1.4)	195.7 (1.2)	102.1 (1.3)	98.3 (1.4)	189.2 (0.7)	191.8 (1.4)	89.3 (0.9)	99.1 (1.3)
	90.0	221.1 (0.8)	220.6 (1.5)	92.6 (1.6)	96.9 (2.0)	222.3 (1.2)	219.6 (0.8)	98.9 (1.1)	89.9 (0.7)
Hb2	0.0	158.7 (0.5)	149.7 (0.5)	-	-	157.8 (1.0)	147.9 (1.0)	-	-
	30.0	179.2 (1.8)	180.5 (1.5)	94.2 (0.9)	96.5 (1.1)	182.3 (1.2)	182.1 (0.1)	91.2 (1.2)	103.8 (0.2)
	60.0	220.3 (0.9)	215.6 (1.0)	102.6 (1.3)	98.9 (1.3)	210.9 (0.4)	205.8 (1.4)	92.1 (1.1)	98.4 (1.3)
	90.0	241.6 (1.2)	245.4 (0.9)	97.5 (1.2)	98.1 (2.1)	239.1 (1.1)	229.6 (1.8)	87.2 (1.0)	89.7 (1.6)
Hb3	0.0	143.4 (0.8)	145.3 (0.7)	-	-	145.2 (0.5)	145.8 (0.7)	-	-
	30.0	170.5 (0.6)	171.8 (1.1)	97.5 (1.0)	98.9 (1.3)	172.1 (1.7)	173.7 (1.4)	93.4 (1.5)	95.1 (1.3)
	60.0	199.8 (1.2)	196.7 (1.5)	97.8 (1.3)	96.1 (0.9)	207.1 (1.8)	208.2 (0.9)	102.1 (1.3)	102.3 (1.2)
	90.0	239.4 (1.4)	229.8 (0.3)	102.7 (1.5)	96.9 (1.1)	229.3 (0.8)	231.9 (1.1)	85.5 (0.6)	94.8 (1.3)

Table 3.5 Recovery and precision for total phosphate in concentrated latex samples (Phosphate spiked as magnesium ammonium phosphate) (Continued)

Sample[a]	Added PO_4^{3-} as $Mg(NH_4)PO_4$ ($mg\ PO_4^{-3}\ L^{-1}$)	Found total PO_4^{3-} ($mg\ kg^{-1}$) (%RSD[b]) intra-day		Recovery (%RSD)		Found total PO_4^{3-} ($mg\ kg^{-1}$) (%RSD) inter-day		Recovery (%RSD)	
		LA[c]	HA[d]	LA	HA	LA	HA	LA	HA
Hb4	0.0	128.6 (0.7)	148.6 (1.0)	-	-	129.7 (1.3)	149.7 (2.2)	-	-
	30.0	129.9 (0.5)	171.3 (2.1)	98.5 (1.1)	97.3 (1.4)	155.6 (1.0)	176.2 (1.3)	93.2 (1.1)	97.9 (1.2)
	60.0	185.7 (0.4)	210.3 (1.6)	97.7 (0.4)	103.1 (1.3)	179.1 (0.8)	211.4 (0.3)	88.8 (1.0)	102.8 (0.5)
	90.0	220.5 (1.2)	229.7 (1.4)	102.3 (0.9)	91.1 (1.4)	216.3 (1.0)	231.2 (1.2)	98.3 (1.0)	92.1 (1.2)
Hb5	0.0	145.3 (0.4)	144.2 (0.4)	-	-	145.3 (0.4)	144.1 (0.4)	-	-
	30.0	169.9 (0.8)	172.9 (0.9)	87.9 (1.9)	98.3 (0.7)	177.2 (1.1)	170.7 (0.4)	101.8 (1.0)	96.3 (0.8)
	60.0	199.8 (1.2)	208.7 (1.1)	96.3 (1.3)	101.9 (1.2)	200.6 (0.2)	203.8 (1.2)	95.8 (0.4)	98.5 (1.2)
	90.0	234.4 (1.0)	230.8 (1.2)	99.8 (2.0)	95.2 (1.8)	230.8 (0.5)	228.4 (1.9)	96.2 (1.0)	91.3 (1.7)

[a]Hb is *Hevea brasiliensis* latex, [b]RSD is relative standard deviation (n=6), [c]LA is low ammonia preserved concentrated latex, [d]HA is high ammonia preserved concentrated latex.

Table 3.6 Recovery and precision for soluble and total phosphates in field and concentrated latex samples collected from five different districts in south of Thailand (Phosphate spiked as potassium dihydrogen phosphate for soluble phosphate and magnesium ammonium phosphate for total phosphate)

Sample[a]	Added PO_4^{3-} (mg PO_4^{3-} L^{-1})	Found soluble PO_4^{3-} mg kg^{-1} (%RSD[b])		Recovery (%RSD)		Found Total PO_4^{3-} mg kg^{-1} (%RSD)		Recovery (%RSD)	
		Field latex	Concentrated latex	Field latex	Concentrated latex	Field latex	Concentrated latex	Field latex	Concentrated latex
Hb6	0.0	9.5 (0.4)	11.1 (0.7)	-	-	66.7 (0.4)	168.0 (0.3)	-	-
	30.0	36.4 (0.1)	38.2 (0.4)	89.5 (0.1)	90.3 (0.5)	97.8 (0.2)	194.5 (0.5)	103.4 (0.6)	88.6 (3.8)
	60.0	61.0 (0.6)	68.7 (0.5)	85.8 (0.7)	95.9 (0.6)	117 (1.0)	227.6 (0.7)	85.2 (2.3)	99.4 (2.1)
	90.0	96.9 (0.1)	103.2 (0.2)	97.1 (0.1)	102.3 (0.2)	141.8 (0.2)	261.5 (0.2)	83.4 (0.5)	103.9 (0.8)
Hb7	0.0	9.6 (1.0)	16.0 (1.2)	-	-	87.5 (0.9)	175.9 (0.9)	-	-
	30.0	34.4 (0.1)	42.0 (0.7)	82.6 (0.3)	86.4 (1.0)	115.6 (0.7)	201.2 (0.2)	93.7 (3.5)	84.5 (0.7)
	60.0	58.8 (0.6)	66.5 (0.3)	82.0 (0.9)	84.1 (0.7)	141.2 (0.8)	233.6 (0.3)	89.5 (0.9)	96.2 (2.4)
	90.0	94.9 (0.1)	94.5 (0.3)	94.8 (0.2)	87.2 (0.5)	160.8 (0.4)	263.3 (1.4)	81.5 (1.9)	97.2 (2.2)
Hb8	0.0	9.7 (1.5)	12.5 (2.2)	-	-	80.7 (4.3)	168.6 (0.9)	-	-
	30.0	39.6 (0.3)	38.3 (0.6)	99.9 (0.6)	85.9 (1.7)	107.8 (0.6)	199.6 (1.1)	90.3 (0.9)	103.5 (2.6)
	60.0	62.2 (0.3)	64.9 (0.5)	87.5 (0.4)	87.3 (0.9)	134.1 (0.3)	225.4 (0.3)	89.0 (0.6)	94.7 (2.6)
	90.0	88.5 (0.3)	92.8 (0.6)	87.7 (0.5)	89.2 (0.9)	158.5 (0.9)	258.2 (0.9)	86.5 (1.5)	99.6 (1.6)

Table 3.6 Recovery and precision for soluble and total phosphates in field and concentrated latex samples collected from five different districts in south of Thailand (Phosphate spiked as potassium dihydrogen phosphate for soluble phosphate and magnesium ammonium phosphate for total phosphate) (Continued)

Sample[a]	Added PO_4^{3-} (mg PO_4^{3-} L^{-1})	Found soluble PO_4^{3-} mg kg^{-1} (%RSD[b])		Recovery (%RSD)		Found Total PO_4^{3-} mg kg^{-1} (%RSD)		Recovery (%RSD)	
		Field latex	Concentrated latex	Field latex	Concentrated latex	Field latex	Concentrated latex	Field latex	Concentrated latex
Hb9	0.0	8.1 (0.6)	11.1 (0.7)	-	-	101.0 (2.5)	192.9 (1.0)	-	-
	30.0	39.6 (0.4)	41.3 (0.4)	105.2 (0.5)	100.7 (0.5)	131.5 (0.9)	218.1 (0.2)	101.7 (0.7)	84.1 (2.3)
	60.0	57.8 (0.3)	64.1 (1.5)	82.8 (0.4)	88.4 (1.6)	157.1 (0.7)	242.7 (0.4)	93.5 (0.5)	83.0 (2.7)
	90.0	99.5 (0.6)	96.3 (0.1)	101.6 (0.6)	94.6 (0.2)	190.4 (0.9)	267.5 (0.6)	99.4 (4.5)	82.9 (4.7)
Hb10	0.0	8.5 (2.0)	10.6 (1.4)	-	-	72.7 (0.9)	132.9 (0.9)	-	-
	30.0	40.1 (0.2)	37.9 (1.6)	105.2 (0.6)	91.2 (2.7)	104.5 (0.9)	160.8 (0.1)	105.9 (3.2)	92.8 (3.2)
	60.0	59.1 (0.6)	63.3 (1.0)	84.3 (0.8)	87.9 (0.9)	130.5 (0.9)	184.2 (0.7)	96.2 (2.1)	85.5 (2.8)
	90.0	86.7 (0.1)	95.1 (0.3)	86.9 (0.1)	93.9 (0.3)	156.1 (0.8)	210.7 (1.4)	92.6 (1.8)	86.4 (0.8)

[a]Hb is *Hevea brasiliensis* latex, [b]RSD is relative standard deviation (n=5).

3.5 Determination of soluble and total phosphate contents in concentrated latex samples collected from five different latex factories

Applications of the developed method were carried out by determining soluble and total phosphate contents in low and high ammonia preserved concentrated latex samples collected from five different latex factories located in the southern part of Thailand. The concentrations of soluble phosphates detected in latex samples were in the range of 21.6-28.3 mg kg^{-1} based on %TSC, with the standard deviation less than 1 for intra-day and 22.2-27.9 mg kg^{-1} based on %TSC, with the standard deviation less than 1 for inter-day (Table 3.7 and Figure 3.22).

Table 3.7 Concentration of soluble phosphate based on %TSC in concentrated latex samples (n=6)

Sample	Mean phosphate in latex (mg kg^{-1} on %TSC ± SD[a]) intra-day		Mean phosphate in latex (mg kg^{-1} on %TSC ± SD) inter-day	
	Low ammonia	High ammonia	Low ammonia	High ammonia
Hb1	25.0 ± 0.8	23.1 ± 0.1	24.9 ± 0.4	25.0 ± 0.1
Hb2	28.3 ± 0.1	25.4 ± 0.1	27.9 ± 0.4	25.2 ± 0.2
Hb3	21.6 ± 0.2	22.2 ± 0.1	22.6 ± 0.3	22.5 ± 0.1
Hb4	25.5 ± 0.1	23.3 ± 0.1	24.5 ± 0.1	22.2 ± 0.1
Hb5	23.9 ± 0.1	24.3 ± 0.1	23.8 ± 0.1	24.3 ± 0.2

[a]is standard deviation.

The concentrations of total phosphate contents detected in latex samples were in the range of 257.2-318.8 and 259.4-317.3 mg kg^{-1} based on %TSC for intra- and inter-day, respectively with the standard deviation less than 5% (Table 3.8 and Figure 3.22).

Table 3.8 Concentration of total phosphate based on %TSC in concentrated latex samples (n=6)

Sample	Mean total phosphate in latex (mg kg^{-1} on %TSC ± SD[a]) intra-day		Mean total phosphate in latex (mg kg^{-1} on %TSC ± SD) inter-day	
	Low ammonia	High ammonia	Low ammonia	High ammonia
Hb1	275.4 ± 1.6	266.4 ± 1.9	275.4 ± 1.6	266.4 ± 2.0
Hb2	318.8 ± 1.7	299.5 ± 1.4	317.3 ± 1.7	296.7 ± 2.9
Hb3	290.3 ± 1.5	290.7 ± 2.1	290.3 ± 1.5	290.7 ± 2.1
Hb4	257.2 ± 1.9	297.1 ± 2.9	259.4 ± 3.3	299.5 ± 2.2
Hb5	286.5 ± 1.1	288.7 ± 1.1	290.5 ± 1.1	288.3 ± 1.1

[a]is standard deviation.

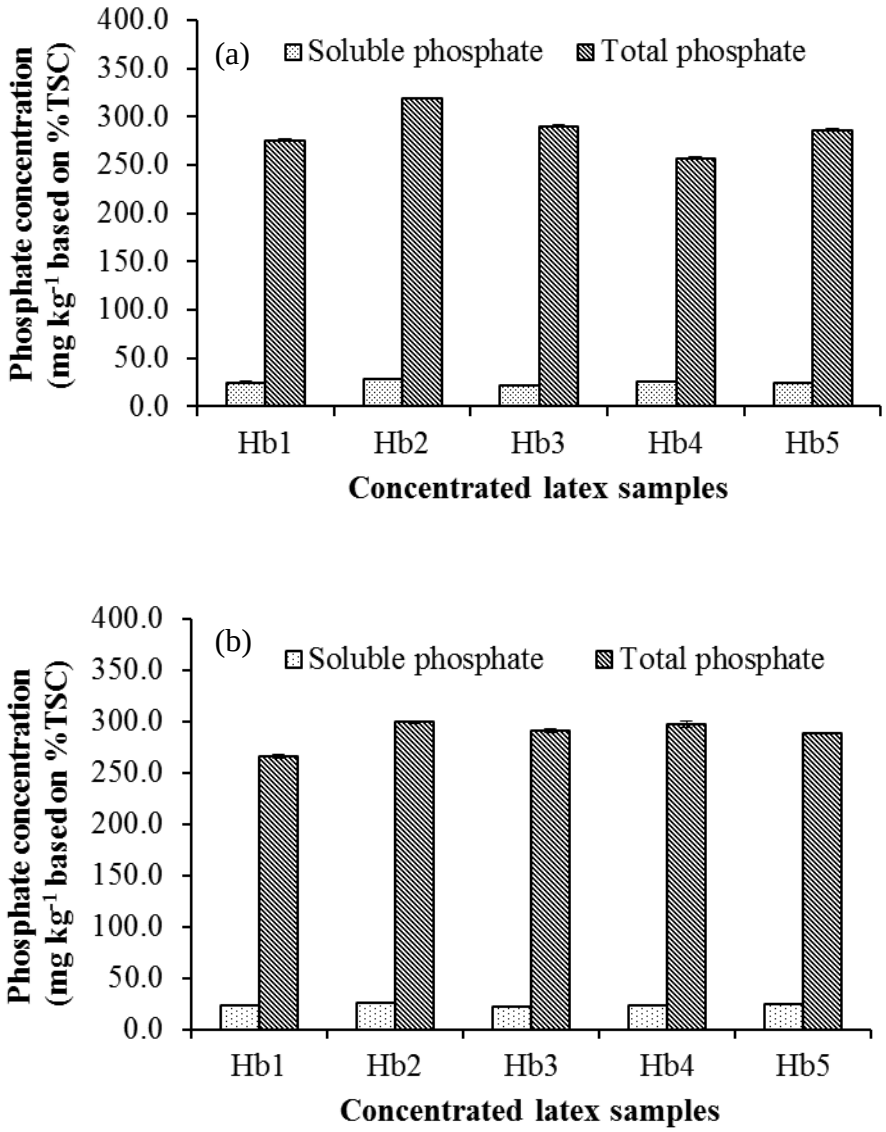


Figure 3.22 The soluble and total phosphate concentration based on %TSC in (a) low ammonia preserved latex and (b) high ammonia preserved latex (intra-day determination).

Concentrations of soluble phosphates obtained by the developed spectrophotometric method were compared with those obtained by ion chromatographic method. As shown in Table 3.9, those values between spectrophotometric and ion chromatographic method were not significantly different (paired *t*-test at 95% confident limit). The comparative linear regression of ion chromatographic (X axis) versus spectrophotometric (Y axis) for phosphate in low and high ammonia latex samples showed that spectrophotometric method provided

good correlation coefficients (r^2 > 0.8) (Figure 3.23). Thus, either the developed spectrophotometric method or ion chromatographic method could be used for the determination of phosphate in concentrated latex.

Table 3.9 Comparison of soluble phosphate concentration between spectrophotometry and ion chromatography (IC) in concentrated latex samples (n=6)

Sample	Mean soluble phosphate in latex[a] (mg kg^{-1} on %TSC ± SD[c])		Mean soluble phosphate in latex[b] (mg kg^{-1} on %TSC ± SD)	
	Spectrophotometry	IC	Spectrophotometry	IC
Hb1	25.0 ± 0.8	25.5 ± 3.7	23.1 ± 0.1	24.3 ± 2.5
Hb2	28.3 ± 0.1	27.7 ± 2.0	25.4 ± 0.1	28.5 ± 2.6
Hb3	21.6 ± 0.2	21.4 ± 0.5	22.2 ± 0.1	22.5 ± 1.4
Hb4	25.5 ± 0.1	25.7 ± 1.6	23.3 ± 0.1	23.4 ± 2.0
Hb5	23.9 ± 0.1	23.0 ± 2.1	24.3 ± 0.1	23.9 ± 2.0

[a]from low ammonia preserved concentrated latex, [b]from high ammonia preserved concentrated latex, [c]is standard deviation (n=6).

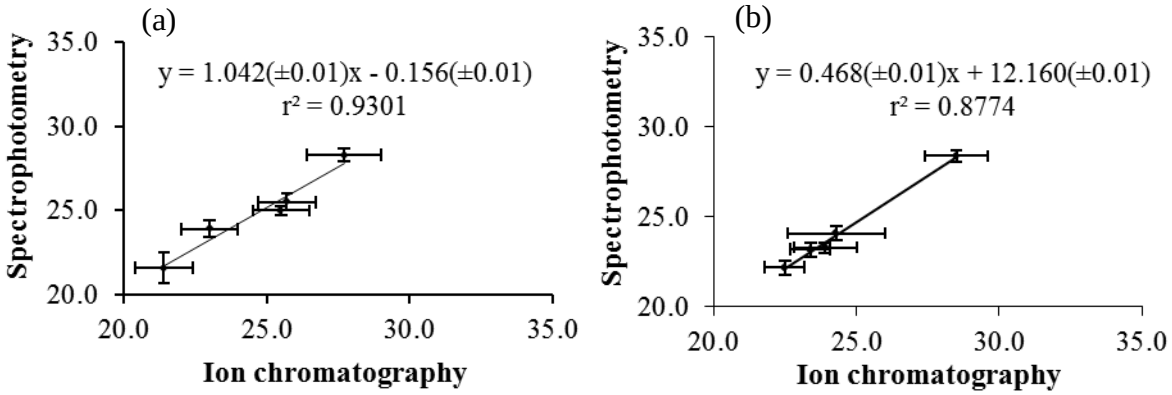


Figure 3.23 The relationship between soluble phosphate concentration obtained by spectrophotometry (Y axis) and that obtained by ion chromatography (X axis) in (a) low ammonia preserved concentrated latex and (b) high ammonia preserved concentrated latex.

3.6 Determination of soluble and total phosphate contents in field and concentrated latex samples collected from different districts in Songkhla province of Thailand

Known magnesium contents in field latex were removed by adding phosphate in the form of diammonium hydrogen phosphate before latex centrifugation. The developed method was also carried out by determining soluble and total phosphate contents in field latex before latex centrifugation and concentrated latex after latex centrifugation collected from five different districts located in the Songkhla province of Thailand. Table 3.10 and Figure 3.24 show the concentrations of soluble and total phosphates in field latex and concentrated latex based on total solids content (%TSC).

The concentrations of soluble phosphates detected in field and concentrated latex samples were in the range of 17.8-22.2 mg kg^{-1} based on %TSC, with the standard deviation less than 2 and 25.1-35.3 mg kg^{-1} based on %TSC, with the standard deviation less than 1, respectively.

The concentrations of total phosphates detected in field and concentrated latex samples were in the range of 133.5-201.9 mg kg^{-1} based on %TSC with the standard deviation less than 5 and 251.9-385.7 mg kg^{-1} based on %TSC with the standard deviation less than 4, respectively.

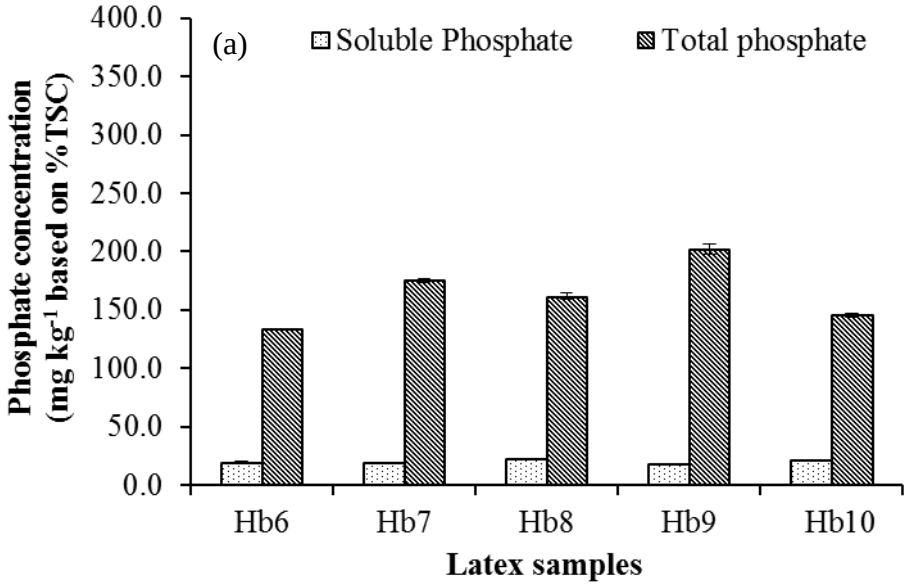
Table 3.10 Concentration of soluble and total phosphates based on %TSC in field latex and concentrated latex samples (n=5)

Sample	Mean soluble phosphate in latex (mg kg^{-1} on %TSC ± SD[a])		Mean total phosphate in latex (mg kg^{-1} on %TSC ± SD)	
	Field latex	Concentrated latex	Field latex	Concentrated latex
Hb6	19.0 ± 1.1	27.8 ± 0.2	133.5 ± 0.4	251.9 ± 0.6
Hb7	19.2 ± 0.2	35.3 ± 0.4	174.9 ± 1.6	351.8 ± 3.2

Hb8	22.2 ± 0.3	25.1 ± 0.5	161.5 ± 2.8	337.1 ± 3.0
Hb9	17.8 ± 0.1	33.4 ± 0.2	201.9 ± 4.1	385.7 ± 3.9
Hb10	21.1 ± 0.4	31.7 ± 0.4	145.5 ± 1.3	265.9 ± 3.5

[a]is standard deviation.

It was found that the concentration of soluble and total phosphates in field latex were less than those in the concentrated latex about 2 and 2 times, respectively.



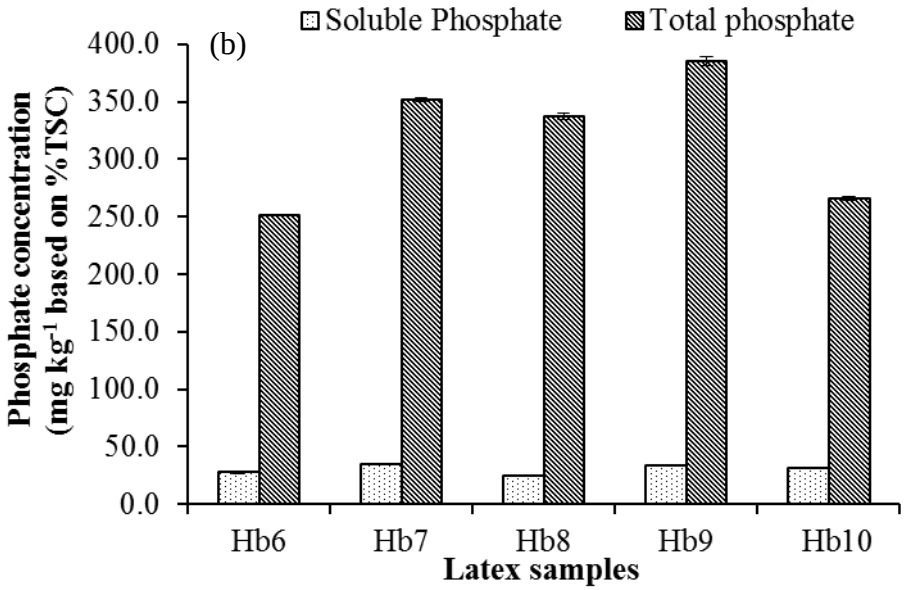


Figure 3.24 The soluble and total phosphate contents in (a) field latex and (b) concentrated latexes.

The concentration of phosphates in concentrated latex is higher than those in field latex because of the residues of phosphate ion obtained after precipitation of magnesium as magnesium ammonium phosphate.

The latex industry and manufacturers in Songkhla, Suratthani and Trang provinces of Thailand were interviewed for the effect of phosphate ion in the concentrated latex. It is found that too high concentration of phosphate ion residues causes the destabilization of concentrated latex, resulting in the destabilization of compounded latex with low adhesive when molding in the dipping process. Mostly, the phosphate content is obtained from the addition of diammonium hydrogen phosphate in field latex to remove magnesium in latex. The manufacturers of the rubber industry such as glove, toys and condoms conclude that the concentration of phosphate less than 100 mg PO_4^{3-} L^{-1} is found to be suitable for film properties of dipped products. Other sources of phosphate residue could be from natural environment such as season, type of soil and the addition of fertilizer by agriculturers.

Karunanayake and Perera (2006) reported that the best quality of latex and dipped products should contain phosphate at a concentration of 30 mg PO_4^{3-} L^{-1}. In this work, the concentration of soluble phosphate in field and concentrated latex

samples studied over a sampling period were likely to represent a good quality for industry use. It could be seen that soluble phosphates presented in concentrated latex were approximately 8.2-9.0% of total phosphate content. In contrast to our result, a study reported by Tunnicliffe (1956) showed that the free phosphate in rubber latex presented at approximately 16.7-17.9% of total phosphorus content, which were higher than the content observed herein. Differences in the concentration of soluble phosphate up to a factor of about 2 from Tunnicliffe (1956) might be influenced by several factors. Tunnicliffe (1956) used trichloroacetic acid as a latex coagulation agent, probably resulting in a dissolvation of total phosphate in a serum. Furthermore, the sample preparation protocol employed in this work included latex coagulation by ethanol, solid particles coagulation with acetone and filtration by using a Whatman no. 42 filter paper, while Tunnicliffe (1995) used trichloroacetic acid for latex coagulation and removed interfering substances from the serum by cation exchange column.

CHAPTER 4
CONCLUSIONS

4.1 Optimal method for the determination of phosphate in concentrated latex

The spectrophotometric method for the determination of phosphate content in concentrated latex was developed. This work was studied two parts. The first part was to develop method for the determination of soluble phosphate and compared with ion chromatographic method. The second part was to develop method for the determination of total phosphate and compared with modified ISO 8053 standard method. The developed method was based on the formation of phosphomolybdate complex obtained after the reaction between molybdate and phosphate, followed by its reduction with ascorbic acid in aqueous sulfuric acid medium. The absorbance of complex was measured at 890 nm.

An optimum combined reagent for the ascorbic acid method contained 50.00 mL of 2.5 M sulfuric acid, 5.00 mL of 0.01 M potassium antimonyl tartrate, 15.00 mL of 4.0% (w/v) ammonium molybdate and 30.00 mL of 0.10 M ascorbic acid. This combined reagent was stable for 2 days, and the color development of phosphate complex occurred within 1-2 minutes and was stable up to 130 minutes. It was found that optimum color development contained a $[H^+]$ / $[MoO_4^{2-}]$ ratio of 78.1 within a pH ranged from 0.39-0.42. The long stability of phosphate complex in this work was advantageous because samples could be analyzed at least 20 samples per minute with good reproducibilities. It was also indicated that most cations and anions did not interfere the ascorbic acid method for the determination of phosphate in concentrated latex. Moreover, no matrices affected the quantification of soluble and total phosphates in latex.

The sample preparation of soluble phosphate included latex coagulation by ethanol and solid particles coagulation with acetone, followed by a serum filtration by using a Whatman no. 42 filter paper. The sample preparation of total phosphate was conducted by using nitric acid with hydrogen peroxide for latex digestion. Under the optimum combined reagent with the spectrophotometric detection based on ascorbic acid method, the standard calibration curve was found to

be a linear response in the concentration range of 0.05-1.00 mg PO_4^{3-} L^{-1}, with a good correlation coefficient of 0.9970.

The developed spectrophotometric method for determination of phosphate in concentrated latex provides many advantages compared to other results obtained by Jastrzębska (2009) and Liberatore (2010), as shown in Table 4.1.

Table 4.1 The advantages of ascorbic acid method for the determination of phosphates in real samples

Developed method	**Molybdenum blue method***
1. Chemicals used are available and inexpensive	1. Toxic chemicals used such as hydrazine sulfate are carcinogenic
2. Chemicals used are low toxicity	2. The chemical reactions occur at high temperature, resulting in high electric power consumption
3. The reaction of phosphate complex is obtained within 1-2 minutes which is about 5 times faster than molybdenum blue method	3. The color developed occurs within 15 minutes (Ascorbic acid method faster than approximately 5 times).
4. The reactions occurs at room temperature, which no any electric power is consumed	4. Phosphate complex should be analysis within 30 minutes because the blue complexes are rapidly degraded
5. The color development is very stable for 130 minutes	5. The process of sample preparation and analysis is about 2 hours
6. The process of sample preparation and analysis is about 30 and 60 minutes for the determination of soluble and total phosphates, respectively	6. Matrices affect for phosphate analysis
7. Samples can be analyzed at least 20 samples per minute	
8. No matrices affect for phosphate analysis	
9. The apparatus for the experiment are available in routine laboratories	
10. Overall, cost of analysis is about 10 baht per sample	

*Jastrzębska (2009) and Liberatore (2010).

4.2 Recovery and precision for phosphates analysis

4.2.1 Soluble and total phosphates in concentrated latex samples collected from five different latex factories

The recoveries of soluble phosphate were assessed by spiking potassium dihydrogen phosphate into concentrated latex samples. The mean percentage recoveries were found to be in the range between 87.6-107.2%, with the RSD less than 5% for intra-day and 82.6-107.7%, with the RSD less than 5% for inter-day.

For total phosphate, the recoveries were assessed by spiking magnesium ammonium phosphate into concentrated latex samples. The mean percentage recoveries when spiking magnesium ammonium phosphate were found to be in the range of 87.9-103.1%, with the RSD less than 5% for intra-day and 87.2-102.8%, with the RSD less than 5% for inter-day.

4.2.2 Soluble and total phosphates in field and concentrated latex samples collected from different districts in Songkhla province of Thailand

The recoveries of soluble phosphate content were assessed by spiking potassium dihydrogen phosphate into field latex and concentrated latex samples. The mean percentage recoveries were found to be in the good range at 82.0-105.2%, with the RSD less than 2% for field latex and 84.1-102.3%, with the RSD less than 5% for concentrated latex.

For total phosphate, the recoveries were assessed by spiking magnesium ammonium phosphate into field latex and concentrated latex. The mean percentage recoveries for field and concentrated latexes were found to be in the good range at 81.5-105.9%, with the RSD less than 5% and 82.9-103.9%, with the RSD less than 5%, respectively.

4.3 Phosphate contents based on %TSC in latex samples

4.3.1 Soluble and total phosphate contents in concentrated latex samples collected from five different latex factories

Five different ammonia preserved concentrated latex sample were used for analysis of soluble and total phosphates. The concentrations of soluble phosphates

detected in latex samples were in the range of 21.6-28.3 mg kg^{-1} based on %TSC, with the standard deviation less than 1 for intra-day and 22.2-27.9 mg kg^{-1} based on %TSC, with the standard deviation less than 1 for inter-day. The results of soluble phosphates obtained in this work were not significantly different from those obtained by ion chromatography. This indicated that the developed method could be used for the analysis of soluble phosphates in real samples. The concentrations of total phosphates detected in latex samples were in the range between 257.2-318.8 and 259.4-317.3 mg kg^{-1} based on %TSC for intra- and inter-day, with the standard deviation less than 5.

4.3.2 Soluble and total phosphate contents in field and concentrated latex samples collected from different districts in Songkhla province of Thailand

The concentrations of soluble phosphates detected in latex samples were in the range of 17.8-22.2 mg kg^{-1} based on %TSC, with the standard deviation less than 2 for field latex and 25.1-35.3 mg kg^{-1} based on %TSC, with the standard deviation less than 1 for concentrated latex.

The concentrations of total phosphates detected in latex samples were in the range of 133.5-201.9 mg kg^{-1} based on %TSC with the standard deviation less than 5 for field latex and 251.9-385.7 mg kg^{-1} based on %TSC with the standard deviation less than 4 for concentrated latex.

Thus, the developed spectrophotometric method could be used for the determination of soluble and total phosphates in field and concentrated latexes.

In conclusion, the developed sample preparation and spectrophotometric method for the determination of soluble and total phosphates in field latex and ammonia preserved concentrated latex was found to be a validate and effective method. This developed method offers a wide linear range, high sensitivity, good precision and accuracy, and a high tolerance of coexistence anions and cations appeared in latex samples. It has been successfully applied to determine soluble and total phosphates in latex samples.

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Appendices

Appendix A

The analysis of natural rubber latex quality

A1. Magnesium analysis

A1.1 Chemicals and reagents

(1) 0.005 Ethylenediaminetetraacetic acid (EDTA)

EDTA (1.8612 g) was dissolved in deionized water, and the solution was transferred into a 1000–mL volumetric flask and diluted to the mark with deionized water. This solution can be used for three months when stored in a brown glass bottle at 4ºC.

(2) 0.6 M Potassium cyanide (KCN)

KCN (19.53 g) was dissolved in deionized water, and the solution was transferred into a 500–mL volumetric flask and diluted to the mark with deionized water. This solution can be used for three months when stored in a glass bottle at 4ºC.

(3) 25% (v/v) Acetic acid

Acetic acid (25% v/v) was prepared by diluting 250 mL of concentrated acetic acid (99.8% v/v) with deionized water, and the solution was transferred into a 1000–mL volumetric flask and diluted to the mark with deionized water.

(4) Eriochrome Black T

Eriochrome Black T was prepared by grinding of Eriochrome Black T (0.3 g) with sodium chloride (100 g) until it becomes homogeneous. This Eriochrome Black T can be used for three months when stored in a brown bottom at room temperature.

(5) Ammonium/Ammonium chloride buffer pH 10.5 (NH_4Cl/NH_4OH)

Ammonium/Ammonium chloride buffer solution was prepared by mixing ammonium chloride (5.35 g) with 7.43 mL of 28% (v/v) ammonium hydroxide. The solution was transferred into a 1000–mL volumetric flask and diluted to the mark with deionized water.

(6) 0.005 M Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)

Magnesium sulfate heptahydrate (1.2325 g) was dissolved in deionized water, and the solution was transferred into a 1000–mL volumetric flask and diluted to the mark with deionized water. This solution can be used for three months when stored in a plastic bottle at 4ºC.

A1.2 Standardization of EDTA

EDTA was standardized to obtain the exact concentration prior to titration. The step of standardization can be performed by pipetting 5.00 mL of 0.02 M $Mg(SO_4)$ into the erlenmeyer flask. The pH of solution was adjusted to 10.3 to 10.8 by adding NH_4Cl/NH_4OH buffer pH 10.0. Five milliliters of 0.6 M KCN which acts as a masking agent and 0.1 g Eriochrome Black T were added into the solution. The solution was titrated with EDTA until the end point (blue color) was reached. The concentration of magnesium (mg kg^{-1} on solids) was calculated as equation A1.

$$C_1V_1 = C_2V_2 \qquad \text{(A1)}$$

Where C_1 is concentration of EDTA

C_2 is concentration of $Mg(SO_4)$

V_1 is volume of EDTA

V_2 is volume of $Mg(SO_4)$

A1.3 Determination of magnesium by titration method

Determination of magnesium by titrimetric method based on complex formation between Mg(II) and ethylenediaminetetraacetic acid (EDTA) was employed. Eriochrome Black T was used as an indicator (TIS 980, 2009).

The procedure was performed by weighing 2.0 g of field latex into the conical flask. Latex was diluted with 100.0 mL of deionized water and pH of the solution was adjusted to 10.0-10.3 by adding NH_4Cl/NH_4OH buffer pH 10.5. Five milliliters of 0.6 M KCN and 0.1 g Eriochrome Black T were added into a solution. The solution was titrated with 0.005 M EDTA until the blue color was reached.

For concentrated latex sample, 10.0 g of concentrated latex was weighted into the beaker. Latex was diluted with 10.0 mL of deionized water and coagulated with 5.0 mL of 25% (v/v) acetic acid. A 10.0 mL of a serum solution was pipetted into the conical flask and pH of the solution was adjusted to 10.0-10.3 by adding NH_4Cl/NH_4OH buffer pH 10.5. Five milliliters of 0.6 M KCN and 0.1 g Eriochrome Black T were added into a solution. The solution was titrated with 0.005 M EDTA until the blue color was reached.

The concentration of magnesium (mg kg^{-1} on solids) was calculated as equation A2 (TIS 980: 2009).

$$\text{Magnesium content} = \frac{24.31 \times C_{EDTA} \times V_{EDTA} \times 1000}{A} \quad \text{(A2)}$$

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

V_{EDTA} is volume of EDTA (mL)

A is weight of latex (g) or volume of a serum solution (mL) (density of serum is 1.021 g dm^{-3})

24.31 is molecular weight of magnesium (g mol^{-1})

A2. Total solids content (%TSC) analysis (ISO 124: 1997)

The total solids content is all rubber contents in latex including solid and non-rubber substances.

The procedure was performed by weighing aluminium cup and 2.0 g of latex samples was weighed in the pre-weighted aluminium cup. One milliliter of deionized water was added and the aluminium cup was swirled for latex dispersion. Latex samples was incubated at 70 ± 2ºC for 16 hours or until the whiteness of the test portion was disappeared. After that, sample was left to settle at room temperature in a desiccators. The aluminum cup contained latex a sample was weighed, and it was incubated one more time for 15 minutes, placed to cool in a desiccators and weighed again. The weight of each time must not be different more than 0.5 mg. The six replicates were performed at each sample and the total solids content (%TSC) was calculated by using equation A3.

$$\%\text{TSC} = \frac{m_1}{m_0} \times 100 \qquad \text{(A3)}$$

Where m_0 is the mass, in grams, of the test portion

m_1 is the mass, in grams, of the dried material

A3. Dry rubber content (%DRC) analysis (ISO 126: 2005)

Dry rubber content (DRC) is all rubber in latex which obtains from latex coagulation by acetic acid.

The procedure was performed by weighing 10.0 g of latex sample into the beaker. Latex was diluted with 2.00 mL of deionized water and coagulated by adding 5.00 mL of 25% (v/v) acetic acid. A sample was placed in the water bath at 70 ± 5ºC for 15-30 minutes. The coagulated latex was pressed to obtain a latex sheet which is not exceeded 2 mm in thickness. The sheet was rinsed thoroughly with running water for at least 5 minutes in the case of concentrate latex preserved with ammonia. A sheet latex was dried at a temperature of 70 ± 5ºC until there is no white patches and the weight is constant. If the sheet was dried on a watch glass, it should be careful when turning over two or three times during the first few hours. The sheet latex was allowed to be cool in a desiccators for 30 minutes and weighed. Drying is repeated again, allowed to be cool and weighed until the loss of mass is less than 1 mg after heating for 30 minutes. The six replicates were performed at each sample and the dry rubber content was calculated by using equation A4.

$$\%\text{DRC} = \frac{m_1}{m_0} \times 100 \qquad \text{(A4)}$$

Where m_0 is the mass, in grams, of the test portion

m_1 is the mass, in grams, of the dry sheet

Table A1. Chemical and physical properties of low and high ammonia preserved concentrated latex samples collected from five different latex factories located in the southern part of Thailand

A1.1 Hb latex 1

Property	Test results		Limits[a]
	LA latex	HA latex	
Total solids content (%)	61.65	61.63	61.50 Min[b]
Dry rubber content (%)	60.21	60.45	60.00 Min
pH	10.36	10.7	10.50 Min
Mg^{2+} (mg L^{-1})	21.36	21.39	30.00 Max[c]

[a] is limits of standard, [b] is minimum value and [b] is maximum value.

A1.2 Hb latex 2

Property	Test results		Limits[a]
	LA latex	HA latex	
Total solids content (%)	61.62	61.69	61.50 Min[b]
Dry rubber content (%)	60.19	60.2	60.00 Min
pH	10.35	10.51	10.50 Min
Mg^{2+} (mg L^{-1})	20.56	20.6	30.00 Max[c]

[a] is limits of standard, [b] is minimum value and [b] is maximum value.

A1.3 Hb latex 3

Property	Test results		Limits[a]
	LA latex	HA latex	
Total solids content (%)	61.65	61.57	61.50 Min[b]
Dry rubber content (%)	60.21	60.28	60.00 Min
pH	10.26	10.47	10.50 Min
Mg^{2+} (mg L^{-1})	20.36	20.48	30.00 Max[c]

A1.4 Hb latex 4

Property	Test results		Limits[a]
	LA latex	HA latex	
Total solids content (%)	61.65	61.63	61.50 Min[b]
Dry rubber content (%)	60.21	60.45	60.00 Min
pH	10.36	10.7	10.50 Min
Mg^{2+} (mg L^{-1})	21.36	21.39	30.00 Max[c]

[a] is limits of standard, [b] is minimum value and [b] is maximum value.

A1.5 Hb latex 5

Property	Test results		Limits[a]
	LA latex	HA latex	
Total solids content (%)	61.65	62.27	61.50 Min[b]
Dry rubber content (%)	60.21	60.91	60.00 Min
pH	10.16	10.47	10.50 Min
Mg^{2+} (mg L^{-1})	19.36	18.51	30.00 Max[c]

[a] is limits of standard, [b] is minimum value and [b] is maximum value.

Table A2. Chemical and physical properties of field and concentrated latexes samples collected from five different latex districts located in the Songkhla province of Thailand

A1.6 Hb latex 6

Property	Test results	
	Field latex	**Concentrated latex**
Total solids content (%)	38.39	62.60
Dry rubber content (%)	34.62	60.27
pH	10.44	10.32
Mg^{2+} (mg L^{-1})	316.92	5.40

A1.7 Hb latex 7

Property	Test results	
	Field latex	**Concentrated latex**
Total solids content (%)	28.83	62.92
Dry rubber content (%)	26.76	60.54
pH	10.52	10.39
Mg^{2+} (mg L^{-1})	414.45	5.62

A1.8 Hb latex 8

Property	Test results	
	Field latex	**Concentrated latex**
Total solids content (%)	34.15	63.51
Dry rubber content (%)	31.32	61.36
pH	10.34	10.45
Mg^{2+} (mg L^{-1})	396.22	9.59

A1.9 Hb latex 9

Property	Test results	
	Field latex	**Concentrated latex**
Total solids content (%)	32.44	62.90
Dry rubber content (%)	31.41	61.42
pH	10.37	10.27
Mg^{2+} (mg L^{-1})	441.32	0.90

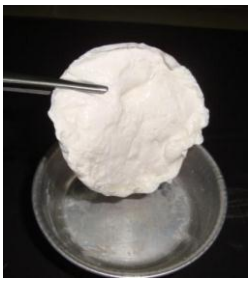
A1.10 Hb latex 10

Property	Test results	
	Field latex	**Concentrated latex**
Total solids content (%)	32.10	63.37
Dry rubber content (%)	30.72	63.00
pH	10.09	10.01
Mg^{2+} (mg L^{-1})	14.37	2.58

Appendix B

Latex coagulation for soluble phosphate analysis

(1)

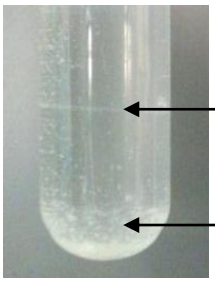


Latex coagulation with ethanol

(2)

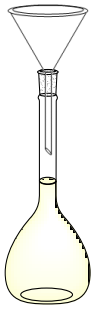
Serum solution

(3)



Second coagulation with acetone

(4)



Filtration of 5.00 mL with Whatman no. 42

(5)

Color of complexation

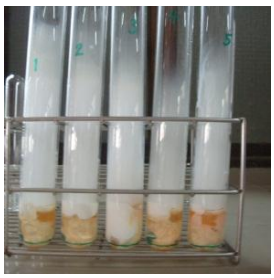
Appendix C

Latex digestion for total phosphate analysis

(1)

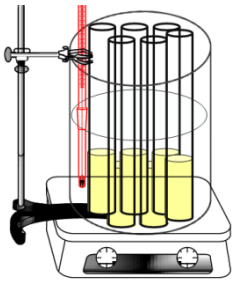
Concentrated latex

(2)



Addition of 4.00 mL nitric acid

(3)



Digestion at 160ºC

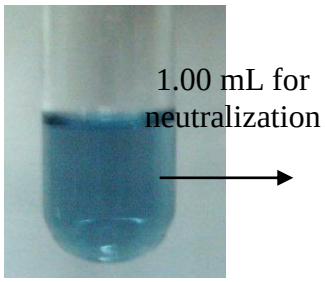
(4)

Latex digestion with nitric acid and hydrogen peroxide

(5)

Dilution to 50.00 mL with DI water

(6)



Color of complexation

Appendix D

Chromatograms and calibration curve for ion chromatography

D1. Chromatogram of standard solution

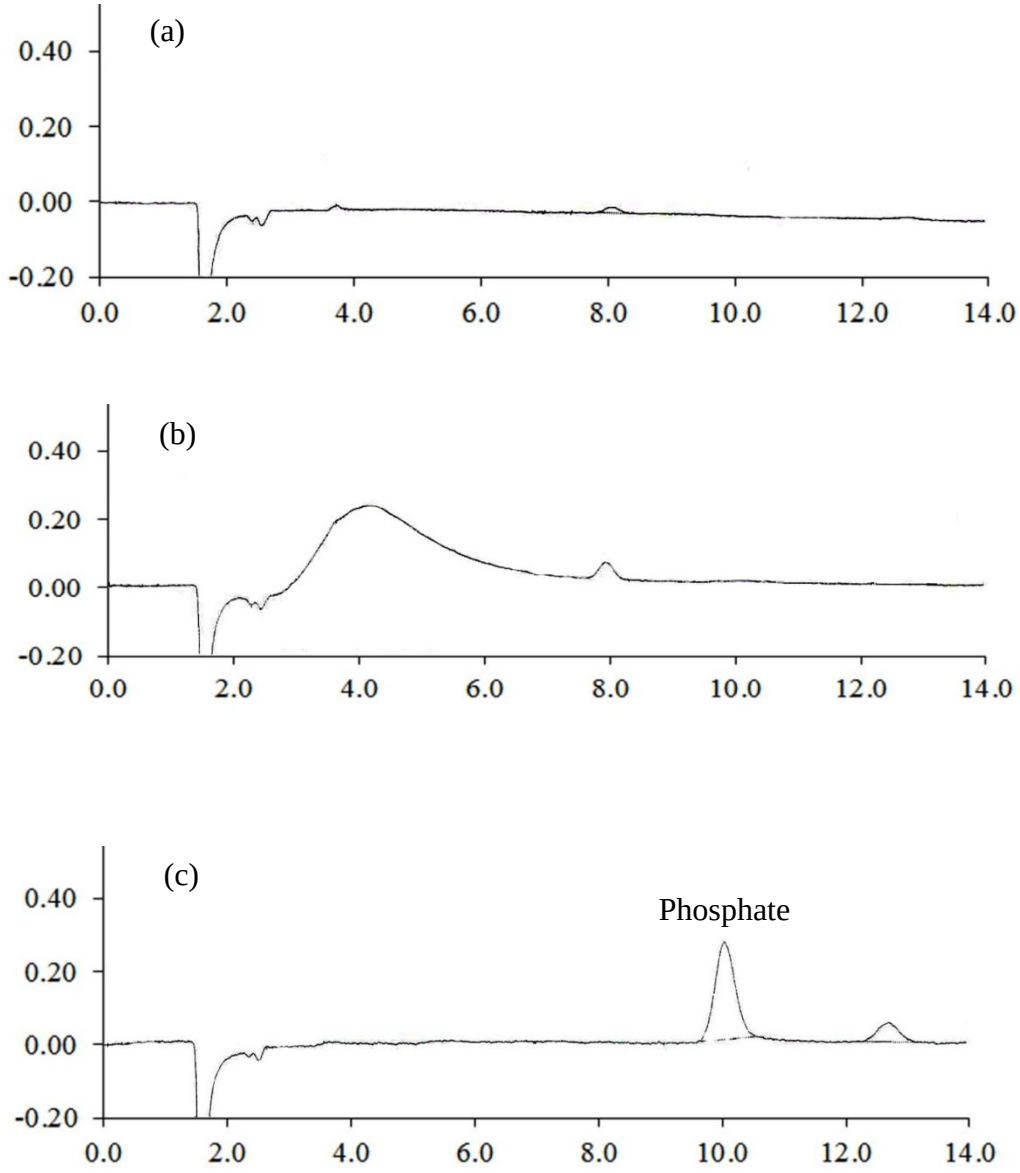


Figure D.1 Chromatograms of (a) deionized water, (b) blank sample solution (the mixture ethanol and acetone) and (c) 1.00 mg PO_4^{3-} L^{-1} in deionized water.

D2. Ion chromatographic detection

As shown in Figure D2., the standard calibration curve performed using ion chromatographic detection was found to be a linear response in the concentration range of 0.05-5.00 mg PO_4^{3-} L^{-1}, with a good correlation coefficient of 0.9992. Mathematical limit of detection and limit of quantification were found to be 0.01 mg PO_4^{3-} L^{-1} at the signal to noise ratio of 3 and 0.03 mg PO_4^{3-} L^{-1} at the signal to noise ratio of 10, respectively.

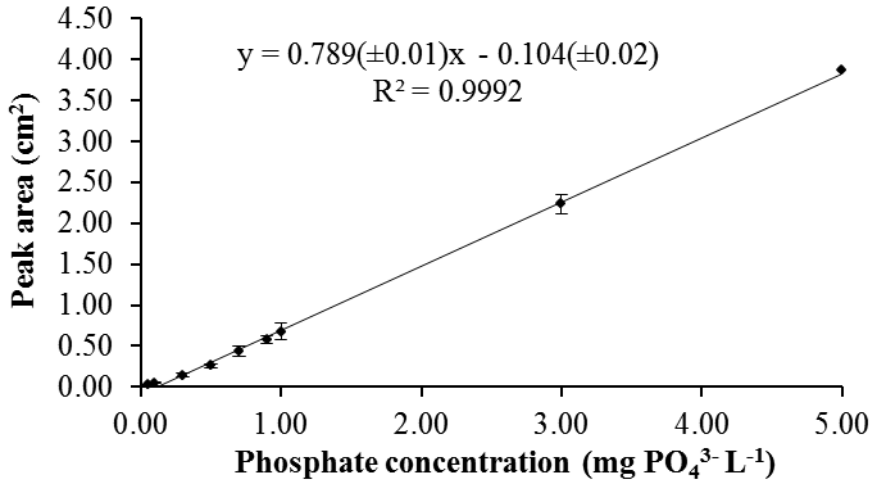


Figure D2. Calibration curve for the determination of phosphate in the range 0.05-5.00 mg PO_4^{3-} L^{-1} by using ion chromatography.

D3. Chromatogram of soluble phosphate in five different concentrated latex samples

D3.1 Hb latex 1

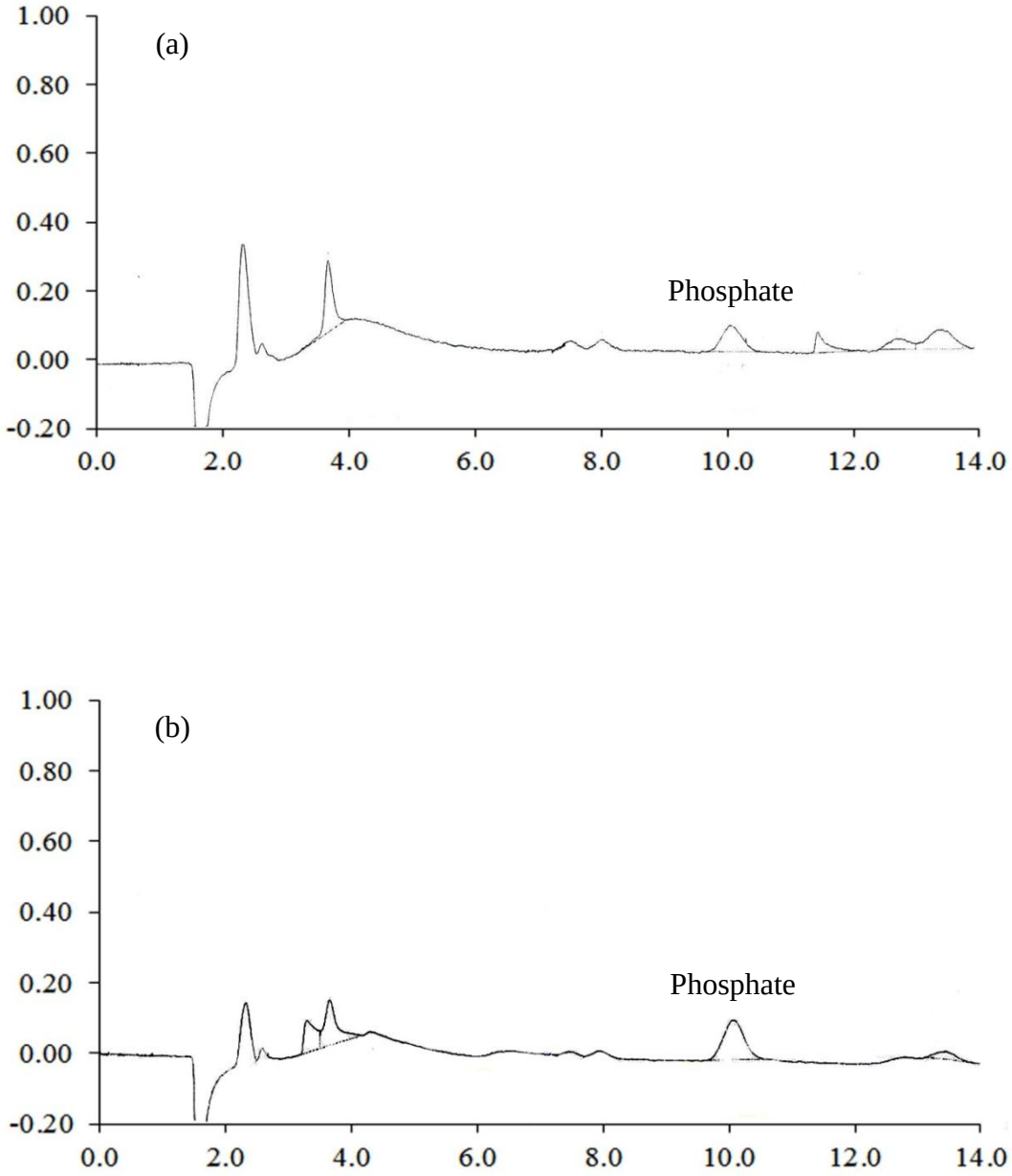


Figure D3. Chromatograms of Hb latex 1 in (a) high ammonia and (b) low ammonia samples.

D3.2 Hb latex 2

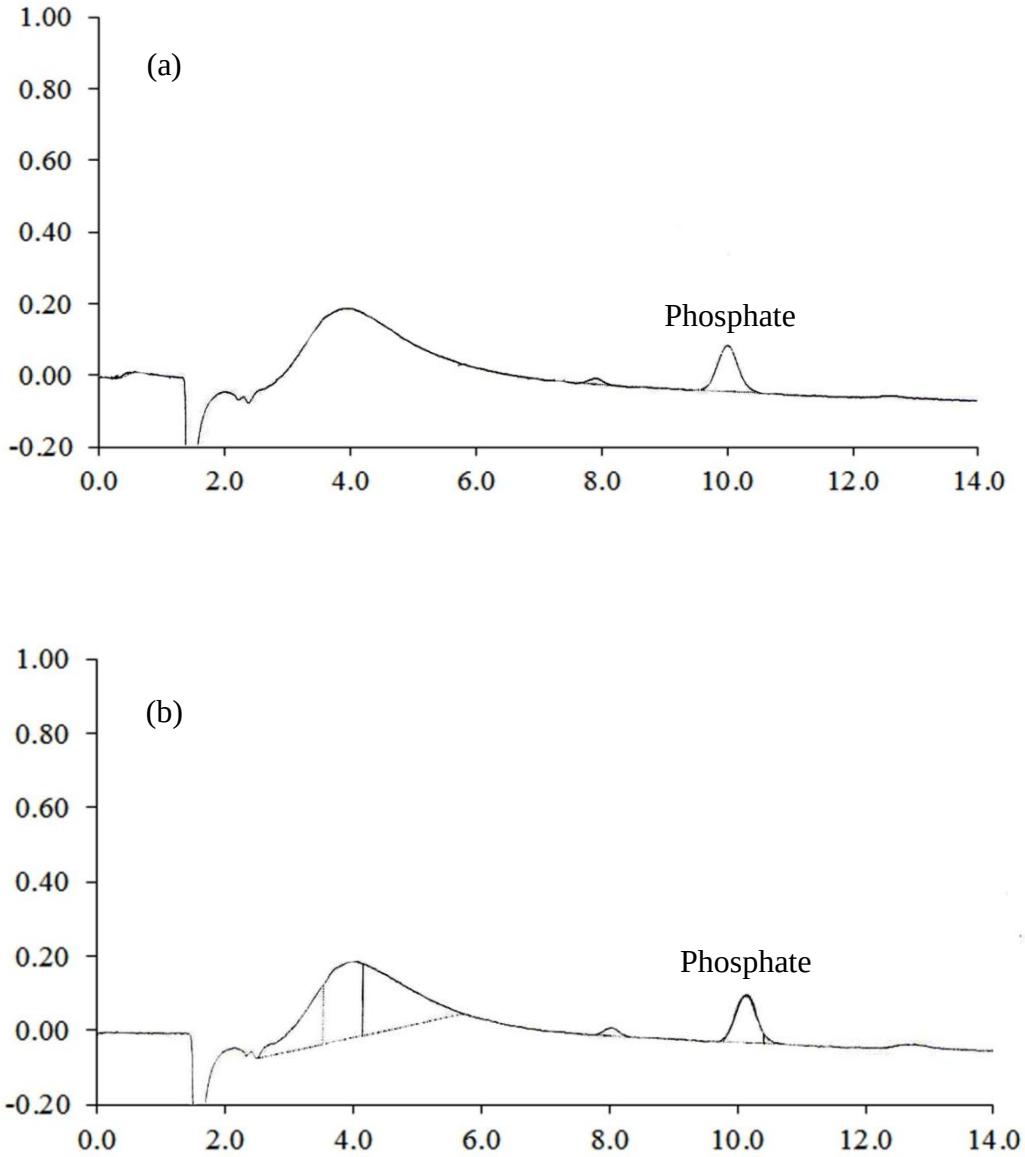


Figure D4. Chromatograms of Hb latex 2 in (a) high ammonia and (b) low ammonia samples.

D3.3 Hb latex 3

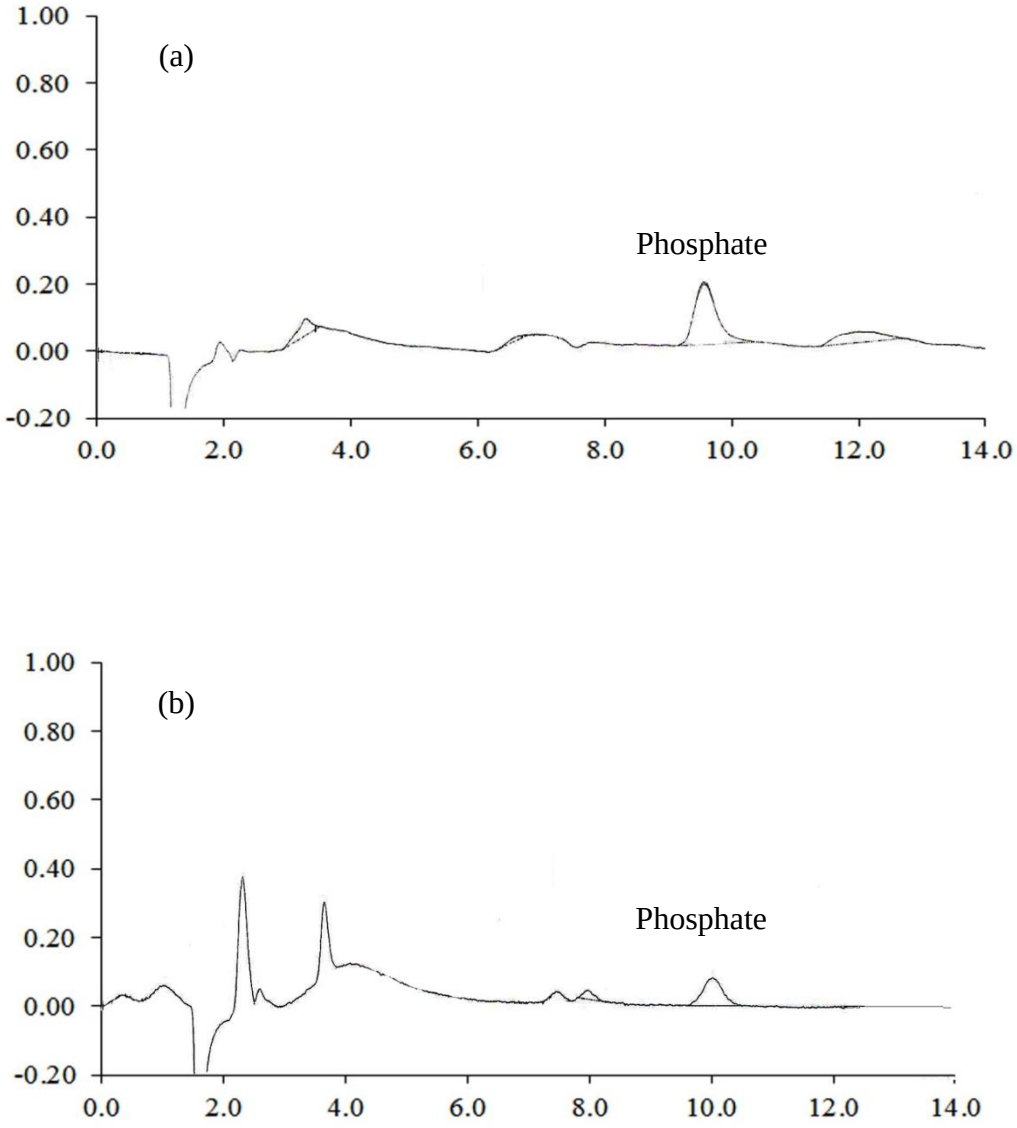


Figure D5. Chromatograms of Hb latex 3 in (a) high ammonia and (b) low ammonia samples.

D3.4 Hb latex 4

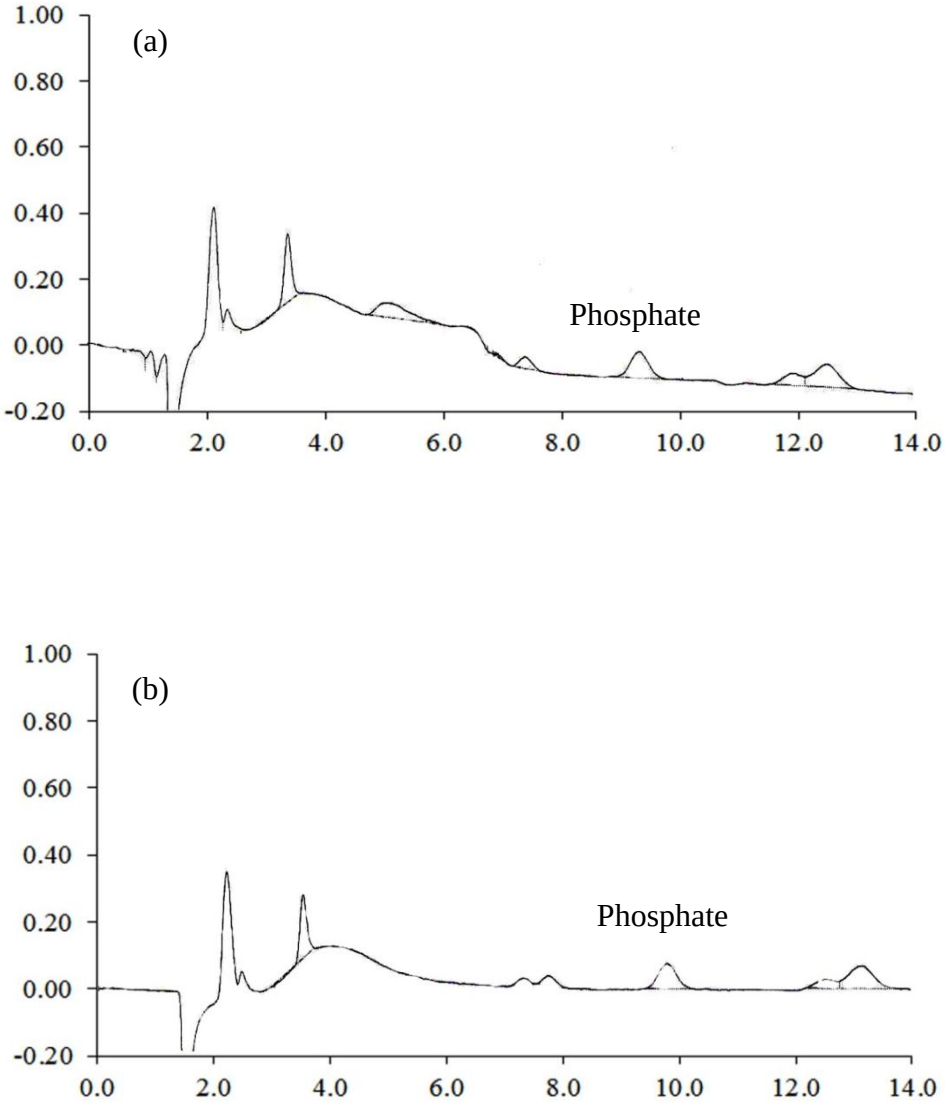


Figure D6. Chromatograms of Hb latex 4 in (a) high ammonia and (b) low ammonia samples.

D3.5 Hb latex 5

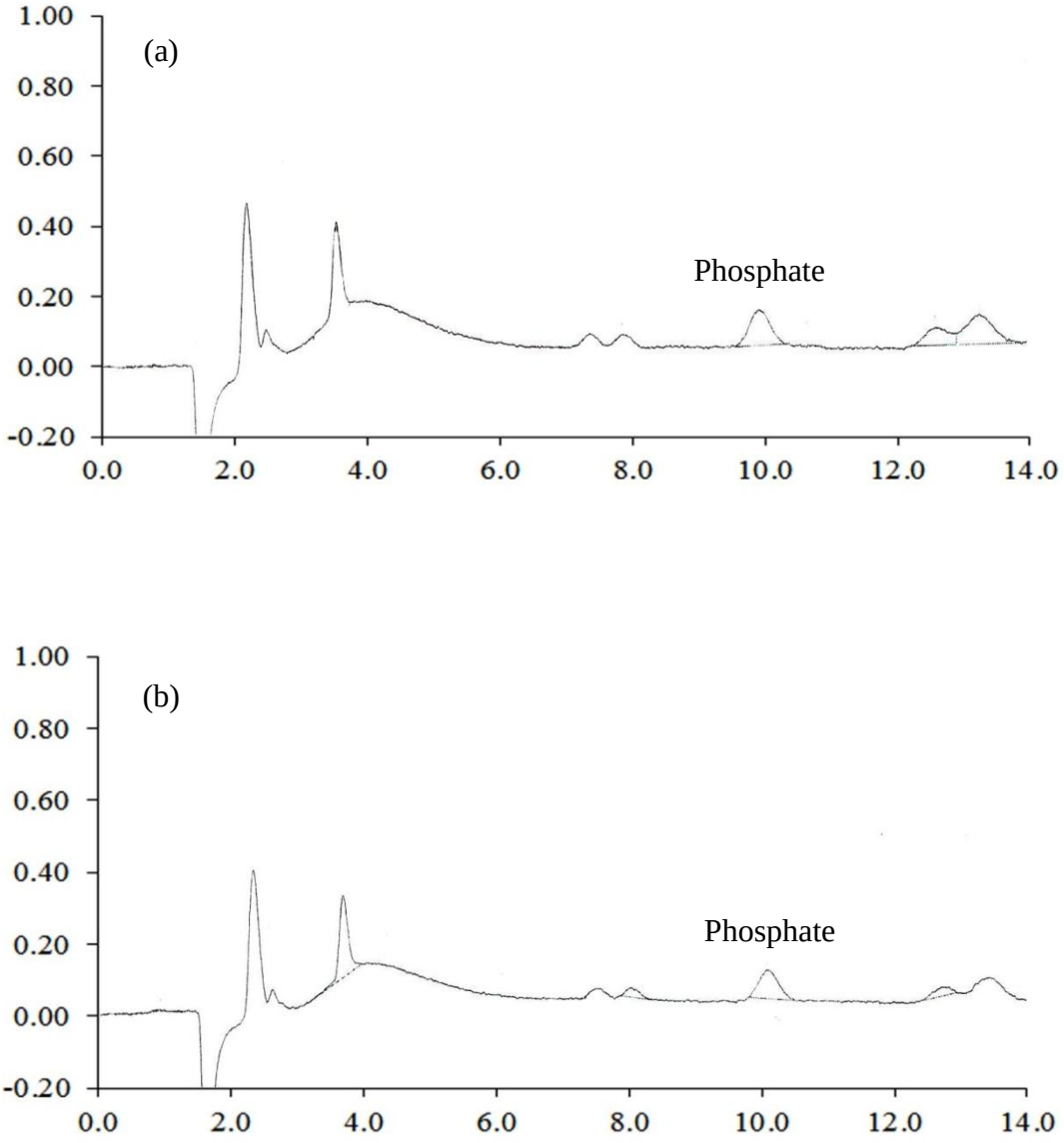


Figure D7. Chromatograms of Hb latex 5 in (a) high ammonia and (b) low ammonia samples.

Appendix E

Calculation of magnesium precipitation

Magnesium precipitation in field latex is performed by using diammonium hydrogen phosphate (DAHP) before latex centrifugation, resulting in a precipitation as magnesium ammonium phosphate.

The calculation of magnesium precipitation can be described;

Magnesium (Mg^{2+}) 1.0 g $\longrightarrow$ DAHP 5.43 g

Magnesium (Mg^{2+}) A g $\longrightarrow$ DAHP $A \times 5.43$ g in 1000 mL

Latex 1000 mL $\longrightarrow$ DAHP $A \times 5.43$ g

Latex B mL $\longrightarrow$ DAHP $\frac{A \times 5.43 \times B}{1000}$ g

Where A is magnesium concentration in field latex samples (g L^{-1})

B is volume of latex sample (10000 mL)

Appendix F

Calculation of phosphate concentration

Phosphate concentration obtained by Visible-spectrophotometric detection is converted from mg L^{-1} to mg kg^{-1} by this equation

$$\text{Equation} = \frac{A \times 2500}{B \times C}$$

F1. Calculation of soluble phosphate concentration

The constant value of 2500 is obtained from 4 steps as followed;

1) Converting mg L^{-1} to mg/ 25 mL

Volume 1000 mL $\longrightarrow$ Phosphate A mg

Volume 25 mL $\longrightarrow$ Phosphate $\frac{A \times 25}{1000}$ mg

Where A is concentration in mg L^{-1} from a calibration curve at 890 nm

Volume 5 mL $\longrightarrow$ Phosphate $\frac{A \times 25}{1000}$ mg

Volume 15 mL $\longrightarrow$ Phosphate $\frac{A \times 25 \times 15}{1000 \times 5} = \frac{A \times 25 \times 3}{1000}$ mg

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

Rubber B g $\longrightarrow$ Phosphate $\frac{A \times 25 \times 3}{1000}$ mg

Rubber 1000 g $\longrightarrow$ Phosphate $\frac{A \times 25 \times 3}{1000} \times \frac{1000}{B} = \frac{A \times 25 \times 3}{B}$ mg

Where A is concentration in mg L^{-1} from a calibration curve at 890 nm

B is weight of latex sample (2.5 g)

The calculation of phosphate in mg kg^{-1} based on %TSC

3) Converting %TSC to g in NR latex

Rubber 100 g ⟶ solids content C g

Rubber B g ⟶ solids content $\frac{C \times B}{100}$ g

Where C is total solids content (from calculation in latex samples)

B is weight of latex sample (2.5 g)

4) Converting based on solids content

Solids content $\frac{C \times B}{100}$ g ⟶ Phosphate $\frac{A \times 25 \times 3}{B}$

Solids content 1 kg ⟶ Phosphate $\left(\frac{A \times 25 \times 3}{B} \times B\right) \times \left(\frac{100}{C \times B}\right)$

F2. Calculation of total phosphate concentration

The constant value of 2500 is obtained from 4 steps as followed;

1) Converting mg L^{-1} to mg/ 5 mL

Volume 1000 mL ⟶ Phosphate A mg

Volume 5 mL ⟶ Phosphate $\frac{A \times 5}{1000}$ mg

Where A is concentration in mg L^{-1} from a calibration curve at 890 nm

2) Converting mg L^{-1} to mg/ 50 mL

Volume 1 mL $\longrightarrow$ Phosphate $\frac{A \times 5}{1000}$ mg

Volume 50 mL $\longrightarrow$ Phosphate $\frac{A \times 5 \times 50}{1000}$ mg

3) Converting mg/50 mL to mg kg^{-1}

Rubber B g $\longrightarrow$ Phosphate $\frac{A \times 5 \times 50}{1000}$ mg

Rubber 1000 g $\longrightarrow$ Phosphate $\frac{A \times 5 \times 50}{1000} \times \frac{1000}{B} = \frac{A \times 5 \times 50}{B}$ mg

Where A is concentration in mg L^{-1} from a calibration curve at 890 nm

B is weight of latex sample (2.5 g)

The calculation of phosphate in mg kg^{-1} based on %TSC

4) Converting %TSC to g in NR latex

Rubber 100 g $\longrightarrow$ solids content C g

Rubber B g $\longrightarrow$ solids content $\frac{C \times B}{100}$ g

Where C is total solids content (from calculation in latex samples)

B is weight of latex sample (0.25 g)

5) Converting based on solids content

Solids content $\frac{C \times B}{100}$ g $\longrightarrow$ Phosphate $\frac{A \times 5 \times 50}{B}$

Solids content 1 kg $\longrightarrow$ Phosphate $\left(\frac{A \times 5 \times 50}{B} \times B\right) \times \left(\frac{100}{C \times B}\right)$

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Simple Spectrophotometric Determination of Phosphate in Concentrated Latex

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Abstract

A simple preparation with spectrophotometric detection was developed for the determination of phosphate in both high ammonia and low ammonia preserved concentrated latex. The developed method is based on an ethanolic coagulation for soluble phosphate and an acidic digestion for total phosphate, with detection involving the ascorbic acid method at 890 nm. Under the optimum conditions, a calibration was linear over the concentration range of 0.05-1.00 mg PO_4^{3-}/L, with a good correlation coefficient of 0.9970. Limit of detection and limit of quantification were found to be 0.03 and 0.05 mg PO_4^{3-}/L, respectively. Mean percentage recoveries were 82.3-106.7% for soluble phosphate and 86.6-105.1% for total phosphate, with the RSD below 8.0%. It was found to be a very simple method, which proved to be accurate, reliable and was successfully applied to determine soluble and total phosphates in concentrated latex.

Keywords: Phosphate, Concentrated latex, Ascorbic acid, Spectrophotometry

1. Introduction

Concentrated latex has been widely used in many rubber industries involving dipping process, latex foam process and coating (Blackley, 1997a). Generally, the concentrated latex is prepared by the centrifugation method of field latex in order to obtain at least 60% dry rubber contents in latex (Blackley, 1997b). To obtain a good quality control, e.g., 0.2-0.7% ammonia, 60% dry rubber contents and 61% total solids content of concentrated latex, it is necessary to reduce the quantity of magnesium which is found to be a major cause of the coagulation and destabilization of latex (Blackley, 1997a; Karunanayake and Perera et al, 2006). Magnesium is principally removed by adding phosphate in the form of either diammonium hydrogen phosphate or diammonium phosphate into the field latex before the latex centrifugation, resulting in the precipitation as magnesium ammonium phosphate (Blackley,

1997a). The amount of phosphate added needs to be calculated in order to reduce magnesium in latex lower than 20 mg/L (ISO 11852, 2009). However, sometimes adding too much phosphate according to the estimated calculation can cause problems to latex products. Phosphate content in latex for dipped products is suggested to be lower than 30 mg PO_4^{3-}/L in latex (Karunanayake and Perera, 2006). Pendle and Gorton (1978) reported that in contrast to latexes containing fillers, the stability of uncompounded latexes was reduced by the presence of complex phosphates as trisodium polyphosphate and tetrasodium pyrophosphate since the remaining phosphate in the bulk aqueous phase of latex contributed to increased ionic strength. Karunanayake and Perera (2006) suggested that the concentration of 30 mg PO_4^{3-}/L was found to produce the highest latex stability during storage and better film properties of dipped products. Furthermore, they reported the stability of latex was changed during the storage and processing by the addition of excess magnesium and phosphate into latex (Karunanayake and Perera, 2006). Takhulee et al (2010) have also reported that higher than 30 mg PO_4^{3-}/L content in latex could cause the destabilization of concentrated latex, compounded latex and dipping process, and also reduce the strength of glove products.

The classical analysis for phosphate determination involves the gravimetric method related to the precipitation of phosphate as, for example, magnesium pyrophosphate, magnesium ammonium phosphate hexahydrate or ammonium phosphomolybdate, and the volumetric method related to the titration of ammonium phosphomolybdate with sodium hydroxide (Estela and Cerdà, 2005). Due to a lack of the sensitivity of classical methods, most samples are analyzed by instrumental techniques including flow injection analysis with photometric detection (Jing-fu and Gui-bin, 2000), high performance liquid chromatography with spectrophotometric detection (Haberer and Brandes, 2003), spectrophotometry (Takhulee et al, 2010; Shyla et al, 2009; Jastrzębska, 2009; Csuros, 1997; Muñoz et al, 1997; Tunnicliffe, 1956; Liberatore, 2009), colorimetry (Van der Bie, 1947) and ion chromatography (AOAC, 2000). Compared to other techniques, spectrophotometric detection involving ammonium molybdate method (Takhulee et al, 2010; Jing-fu and Gui-bin, 2000; Haberer and Brandes, 2003; Shyla et al, 2009; Jastrzębska, 2009; Csuros, 1997; Muñoz et al, 1997; Tunnicliffe, 1956; Liberatore, 2009) and phosphovanadomolybdate method appears to be the most practical method for phosphate. The ammonium molybdate method is frequently used and more sensitive than the phosphovanadomolybdate method (Muñoz et al, 1997). It relates to the reaction between phosphate and molybdate in acidic solution, in which molybdophosphoric acid is formed and further reduced by various reducing agents, i.e., ascorbic acid (Jing-fu and Gui-bin, 2000; Jastrzębska, 2009; Csuros, 1997), thiourea (Shyla et al, 2009), hydrazine sulfate (Jastrzębska, 2009; Liberatore, 2009), the mixture of hydrazine sulfate and hydroquinone (1:1) (Jastrzębska, 2009), stannous chloride (Muñoz et al, 1997; Van der Bie, 1947) and ferrous sulfate (Tunnicliffe, 1956). It seems that ascorbic acid is the most promising agent with the presence of antimony serving as a catalyst to increase the reduction rate. While a few procedures for determination of phosphate content in latex have been reported (Tunnicliffe, 1956; Van der Bie, 1947), those procedures require either cationic column for removal of interferences and are time consuming. Moreover, up to date, no report has been made to explain which form of phosphate affecting a quality of latex and which sample preparation is suitable for soluble and total phosphates. Hence, we attempted to develop a sample preparation of concentrated latex to determine phosphate in the forms of soluble and total phosphates, with spectrophotometric detection based on the ammonium molybdate method with ascorbic acid as a reducing agent.

2. Experimental

2.1. Reagents and Chemicals

Natural high (~0.7%) and low (~0.4%) ammonia preserved concentrated latexes were obtained from five different factories in the south of Thailand. All reagents used were of analytical grade. Deionized water was used for the preparation of standard and reagent solutions. Nitric acid-washed glasswares were used.

A stock phosphate solution (100 mg PO_4^{3-}/L) was prepared by dissolving 0.0143 g of anhydrous potassium dihydrogen phosphate with deionized water. Working standard solutions of phosphate were prepared by diluting a stock solution with deionized water. A combined reagent was 2.5 mol/L sulfuric acid, 0.01 mol/L potassium antimonyl tartrate, 4.0% ammonium molybdate and 0.1 mol/L ascorbic acid in the ratio of 10 : 1 : 3 : 6 (by volume). This solution remains stable for 2 days at room temperature.

2.2. Procedure for Soluble and Total Phosphates in Concentrated Latex

For soluble phosphate determination, 2.5 g of concentrated latex was added into an aluminium cup. Ethanol was added drop−wise onto the latex surface and the compound was mixed slowly. Serum was weighted and transferred to a 30–mL test tube. After adding 10 mL of acetone to precipitate rubber particles, the solution was left for 15-20 min and 5 mL of serum solution was filtered through Whatman no. 42 filter paper into a 25–mL volumetric flask. Four milliliters of a combined reagent was added, followed by diluting with deionized water. The reaction solution was left for 15 min prior to absorbance measurement at 890 nm with Spectronic 20 (Genesys^{TM}, USA).

For total phosphate determination, 0.25 g of concentrated latex was added into a 50–mL test tube and digested with 4 mL of nitric acid in a paraffin oil bath at 160°C until the mixture darkened and 1 mL of nitric acid was added. After cooling to room temperature, in order to digest the remained organic matters, 0.5 mL of 30% hydrogen peroxide and 2 drops of nitric acid was added into a solution, which was again digested at 160°C until the solution became pale yellow. One milliliter of acid digested solution was transferred into a 50–mL volumetric flask and diluted to volume with deionized water. One milliliter of the diluted solution was pipetted and one drop of 1% phenolphthalein was added. After the adjustment pH of the solution with 5 mol/L sodium hydroxide, 2 mL of a combined reagent was added into the solution. The reaction was allowed to settle at room temperature for 15 min and measured at 890 nm.

2.3. Tolerance Limit Study of Interferences by Ascorbic Acid Method

Interferences for the determination of phosphate were investigated by spiking 0.5 mg PO_4^{3-}/L solution with up to 1000 mg/L of interferences and analyzed by ascorbic acid method. Interferences were divided into two groups, anions and cations. According to other studies (AOAC, 2000; Phattanakul et al, 2009; Galli et al, 2000), interest anions were formic, propionic, glycollic, citric, malic, maleic and succinic acids, and nitrate, while cations were copper (II), iron (II), iron (III), magnesium, zinc, calcium and manganese (II). The spiked standard was reacted with 4 mL of a combined reagent for 15 min. After reaction, a blue complex solution was obtained and its absorbance was measured at 890 nm.

3. Results and Discussion

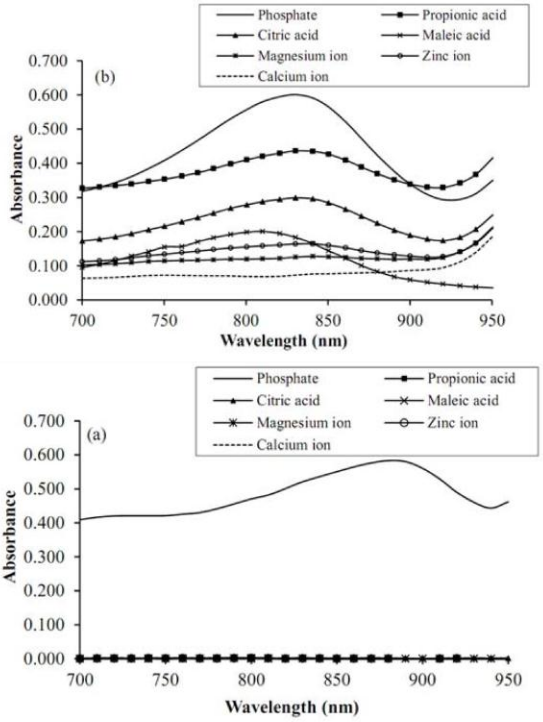
In order to develop a simple and rapid method for the determination of soluble and total phosphates in concentrated latex, the important parameters affecting the development, choice of spectrophotometric detection method and anion and cation interferences were investigated. The validation of the developed method, precision and accuracy, were also performed.

3.1. Comparison of Ascorbic Acid Method and Molybdenum Blue Method

In this study, two spectrophotometric detection methods were compared. Ammonium molybdate method using ascorbic acid as a reducing agent is known as ascorbic acid method, while that based on hydrazine sulfate is known as the molybdenum blue method which is described by Liberatore, (2009). Phosphovanadomolybdate method mentioned by a previous work (Muñoz et al, 1997) was not chosen since its yellow solution obtained at the end of the reaction is similar to the yellow-color of latex serum, resulting in the low sensitivity for the colorimetric detection of phosphate. Some interferences (Phattanakul et al, 2009; Galli et al, 2000; Scott et al, 2003), namely, propionic acid, citric acid, maleic

acid, magnesium ion, zinc ion and calcium ion, were studied for both ascorbic acid method and molybdenum blue method, as shown in Figure 1. The result showed that most studied interferences appeared to interfere with the blue–colored solution detected by molybdenum blue method. From our point of view the ascorbic acid method when compared to molybdenum blue method provides more advantages such as its non–complicate reaction method, low cost for the total analysis of phosphate due to a no heating step for reaction and blue–colored stability at room temperature. Therefore, ascorbic acid method was preferred and used throughout our experiment.

Figure 1: Effect of interferences (50 mg/L) on the absorbance of phosphate (0.5 mg PO_4^{3-}/L) measured by (a) ascorbic acid method and (b) molybdenum blue method



3.2. Optimization of Parameters for Ascorbic Acid Method

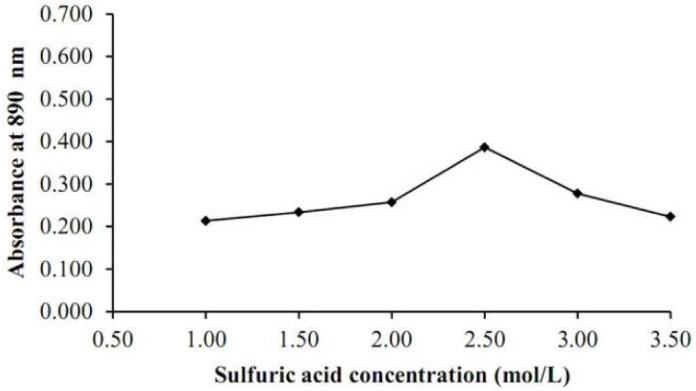
The developed spectrophotometric method was principally based on the reaction of phosphate, ammonium molybdate and potassium antimonyl tartrate to form phosphomolybdic acid followed by its reduction with ascorbic acid in sulfuric acid medium, resulting in the formation of an intense colored molybdenum blue. The parameters affecting the intensity and stability of colored solution were investigated and optimized in order to obtain the highest detection limit of phosphate concentration in concentrated latex. The optimization of parameters was performed by varying one parameter and keeping all others constant. One-way ANOVA at the 95% confidence limit was used for the data analysis.

3.2.1. Effect of Sulfuric Acid Concentration

The absorbance measurement was found to be greatly dependent on pH values of the medium solution. Figure 2 shows the effect of sulfuric acid at various concentrations (1.0-3.5 mol/L) for phosphate content determination. The results indicated that the absorbance of phosphate complex increased up to

2.5 mol/L sulfuric acid and decreased after the sulfuric acid concentration higher than that. Therefore, 2.5 mol/L sulfuric acid was chosen due to the highest absorbance of phosphate complex obtained.

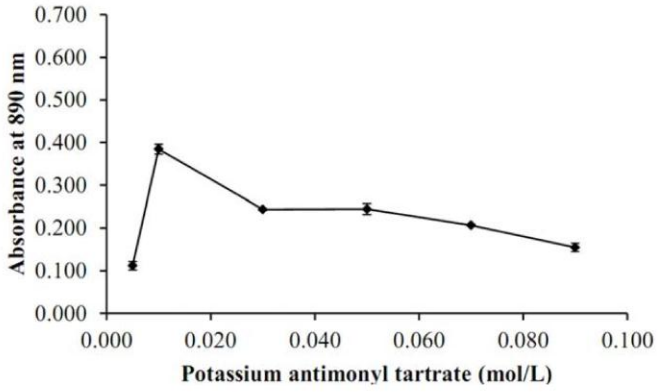
Figure 2: Effect of sulfuric acid concentration (0.5 mg PO_4^{3-}/L). Conditions: 5 mL of 0.01 mol/L potassium antimonyl tartrate; 15 mL of 4.0% ammonium molybdate; 30 mL of 0.1 mol/L ascorbic acid



3.2.2. Effect of Potassium Antimonyl Tartrate Concentration

Potassium antimonyl tartrate was used for the increasing rate of reduction with ascorbic acid (Drummond and Maher, 1995), therefore, the effect of its concentration on phosphate content determination in the range of 0.005 to 0.09 mol/L was studied (Figure 3). The maximum absorbance of phosphate complex was obtained when the solution contained 0.01 mol/L potassium antimonyl tartrate, hence, the concentration of potassium antimonyl tartrate at 0.01 mol/L was chosen and the reaction time was reduced to only 2 min.

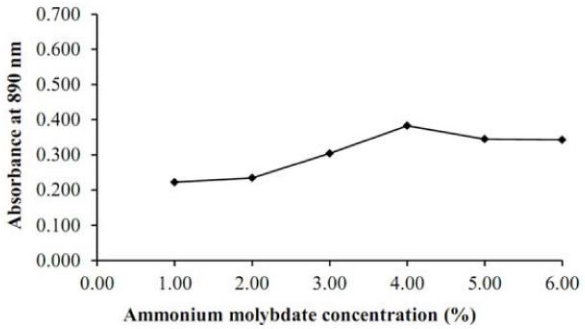
Figure 3: Effect of potassium antimonyl tartrate concentration (0.5 mg PO_4^{3-}/L). Conditions: 50 mL of 2.5 mol/L sulfuric acid; 15 mL of 4.0% ammonium molybdate; 30 mL of 0.1 mol/L ascorbic acid



3.2.3. Effect of Ammonium Molybdate Concentration

Ammonium molybdate affects on the color intensity of the complex. The concentration of ammonium molybdate was investigated from 1.0 to 6.0% (Figure 4). The absorbance of phosphate complex was increased with the addition of ammonium molybdate concentration up to 4.0% and decreasing gradually after that concentration. Therefore, 4.0% ammonium molybdate was chosen in order to obtain the highest sensitivity of phosphate determination in samples.

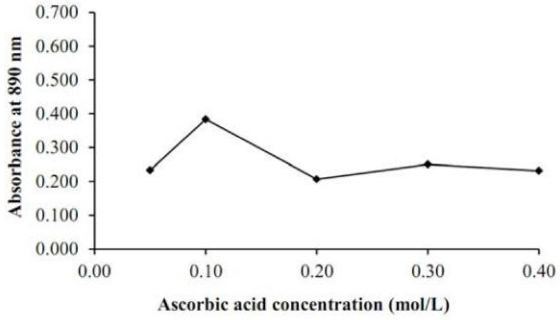
Figure 4: Effect of ammonium molybdate concentration (0.5 mg PO_4^{3-}/L). Conditions: 50 mL of 2.5 mol/L sulfuric acid; 5 mL of 0.01 mol/L potassium antimonyl tartrate; 30 mL of 0.1 mol/L ascorbic acid



3.2.4. Effect of Ascorbic Acid Concentration

Ascorbic acid is the most economical reducing reagent and has been found to reduce time of the reaction (Drummond and Maher, 1995). Figure 5 illustrates the effect of concentration of ascorbic acid in the range of 0.05-0.40 mol/L. The highest absorbance of phosphate complex was obtained at 0.10 mol/L ascorbic acid, with decreasing its concentration significantly and with the increase of ascorbic acid. Furthermore, our result showed that 0.10 mol/L ascorbic acid reduced the reaction time to 2 min, with a similar rate of the reaction time obtained when using concentration of ascorbic acid over 0.01 mol/L. Going and Eisenreich (1974) reported that it was necessary to use an ascorbic acid concentration of at least twenty times the maximum phosphate present in samples in order to obtain the full color development within 10-30 min. In our study, 0.10 mol/L ascorbic acid was chosen since it was excess enough to reach an equilibrium within 15 min.

Figure 5: Effect of ascorbic acid concentration (0.5 mg PO_4^{3-}/L). Conditions: 50 mL of 2.5 mol/L sulfuric acid; 5 mL of 0.01 mol/L potassium antimonyl tartrate; 15 mL of 4.0% ammonium molybdate



3.3. Tolerance Limits of Interference

Since concentrated latex sample is a complex compound containing some organic compounds, i.e., proteins, glycolipids, and metals, i.e., magnesium, iron (II) and manganese (II), it is necessary to evaluate the selectivity of coexistence ions for the analysis of phosphate by ascorbic acid method. Phattanakul et al (2009) showed that carboxylic groups, malate and succinate, were the highest contents of anions aparted from phosphate in latex whereas Galli et al (2000) purposed these anions as indicators for a quality control of latex. Furthermore, *Hevea brasiliensis* latex also contains cations or metal ions in the concentration of millimolar level, i.e., magnesium, calcium and potassium, and those in the concentration of micromolar level, i.e., zinc, copper (II) and manganese (II), could interfere the determination of phosphate (Scott et al, 2003). Therefore, both anions and cations were investigated in terms of the tolerance limit which is the maximum concentration causing an error of $\leq \pm 5\%$ in the

absorbance measurement (Khlyntseva et al, 2011). The results indicated that most interferences did not interfere the determination of phosphate (Table 1). Citrate, iron (II), iron (III) and calcium ion as calcium citrate were lesser tolerated limits than other interferences in the range between 470 and 750 mg/L for phosphate determination obtained by the developed method.

Table 1: Anion and cation interferences for the determination of phosphate (0.5 mg PO_4^{3-}/L)

Interference	Added as	Tolerance limits (mg/L)
Formate	Sodium formate	>1000
Propionate	Propionic acid	>1000
Glycolate	Glycollic acid	>1000
Citrate	Citric acid	500
Nitrate	Sodium nitrate	>1000
Succinate	Succinic acid	>1000
Malate	Malic acid	>1000
Maleate	Maleic acid	>1000
Copper (II) ion	Copper (II) sulfate	965
Magnesium ion	Magnesium sulfate	>1000
Magnesium ion	Magnesium citrate	950
Zinc ion	Zinc sulfate	>1000
Calcium ion	Calcium sulfate	>1000
Calcium ion	Calcium citrate	750
Manganese ion	Manganese sulfate	>1000
Iron (II) ion	Iron (II) sulfate	650
Iron (III) ion	Iron (III) citrate	470

3.4. Method Validation for Determination of Phosphate

The standard calibration curve performed using an optimum condition and spectrophotometric detection base on ascorbic acid method was found to be a linear response in the concentration range of 0.05 to 1.00 mg PO_4^{3-}/L, with a good correlation coefficient of 0.9970. The molar absorptivity (ε) of our result was 8.93×10^4 L/mol/cm which higher than of the value reported by Khlyntseva et al, (2011) ($\varepsilon = 3.7\times10^4$ L/mol/cm). Mathematical limit of detection and limit of quantification (Miller and Miller, 2005) were found to be 0.03 mg PO_4^{3-}/L at the signal to noise ratio of 3 and 0.05 mg PO_4^{3-}/L at the signal to noise ratio of 10, respectively. The inter-day reproducibility (n=6) was better than 5% for soluble phosphate and 2% for total phosphate, while the intra-day reproducibility (n=6) for soluble and total phosphates was less than 5% and 2%, respectively. The method employed to evaluate the analytical recoveries for soluble and total phosphates was based on spiking both low and high ammonia concentrated latex samples collected from five different factories with three final concentration levels of 30.0, 60.0 and 90.0 mg PO_4^{3-}/L. Mean percentage recoveries were found to be in range over 82.3-106.7% for soluble phosphate and 86.6-105.1% for total phosphate, with the RSD less than 8.0% (n=6). These results demonstrated that our developed sample preparation and spectrophotometric method could be applied effectively for the determination of soluble and total phosphates in concentrated latex.

3.5. Determination of Soluble and Total Phosphates in Concentrated Latex

Applications of the developed method were carried out by determining phosphates in low and high ammonia preserved concentrated latex samples collected from five different latex factories located in the south of Thailand. As shown in Table 2, the concentrations of soluble phosphates in concentrated latex samples were detected in the range of 2.7 to 3.9 mg PO_4^{3-}/L whereas those of total phosphates detected were between 25.7 and 31.7 mg PO_4^{3-}/L, with the standard deviation less than 5.0%. Karunanayake and Perera (2006) reported that the best quality of latex and dipped products should contain phosphate at a concentration of 30 mg PO_4^{3-}/L. The concentrated latex samples studied over a sampling period herein are likely to represent a good quality for industry use. It can be seen that soluble phosphate presented in concentrated latex at approximately 8.0-9.5% of that of total phosphate

content. Tunnicliffe (1956) reported that the free phosphate presented in rubber latex at approximately 16.7-17.9% of that total phosphorus content. It is still not known whether soluble or non-soluble phosphate has more effect on natural rubber product production. Hence, with this established method, the natural rubber latex industry has the possibility to understand more about the effect of phosphate compound on their products.

Table 2: Concentration of soluble and total phosphates in concentrated latex samples collected from five different factories (n=6)

Sample [*]	Mean soluble phosphate mg/kg (RSD) [**]		Mean total phosphate mg/kg (RSD)	
	LA [***]	HA [****]	LA	HA
Hb 1	3.9 (1.2)	3.9 (1.6)	27.5 (0.6)	26.6 (0.7)
Hb 2	3.2 (1.9)	3.8 (1.1)	31.7 (0.5)	29.9 (0.5)
Hb 3	3.6 (0.9)	3.8 (1.4)	28.9 (0.5)	29.0 (0.7)
Hb 4	2.7 (1.7)	2.9 (1.3)	25.7 (0.7)	29.7 (0.9)
Hb 5	3.2 (1.9)	2.9 (1.1)	29.0 (0.3)	28.8 (0.4)

[*] *Hevea brasiliensis* latex.
[**] Relative standard deviation (%).
[***] Low ammonia preserved concentrated latex.
[****] High ammonia preserved concentrated latex.

4. Conclusions

In conclusion, the developed sample preparation and spectrophotometric method for the determination of phosphates in concentrated latex was found to be an effective method. This developed method provides a procedure that can distinguish between soluble and non-soluble phosphate compounds. Moreover, this developed method also offers a wide linear range, good precision and accuracy with a high tolerance of coexistence anions and cations appeared in concentrated latex. It has been successfully applied to determine soluble and total phosphates in real samples.

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