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,
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™, 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, 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
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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 PO₄³⁻/L) measured by (a) ascorbic acid method and (b) molybdenum blue method

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**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.
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.

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 ≤ ±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)

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

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 ($\varepsilon$) of our result was $8.93 \times 10^{4}$ L/mol/cm which higher than of the value reported by Khlyntseva et al, (2011) ($\varepsilon = 3.7 \times 10^{4}$ 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)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean soluble phosphate mg/kg (RSD)</th>
<th>Mean total phosphate mg/kg (RSD)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>LA (*** )</td>
<td>HA (****)</td>
</tr>
<tr>
<td></td>
<td>LA (*** )</td>
<td>HA (****)</td>
</tr>
<tr>
<td>Hb 1</td>
<td>3.9 (1.2)</td>
<td>3.9 (1.6)</td>
</tr>
<tr>
<td>Hb 2</td>
<td>3.2 (1.9)</td>
<td>3.8 (1.1)</td>
</tr>
<tr>
<td>Hb 3</td>
<td>3.6 (0.9)</td>
<td>3.8 (1.4)</td>
</tr>
<tr>
<td>Hb 4</td>
<td>2.7 (1.7)</td>
<td>2.9 (1.3)</td>
</tr>
<tr>
<td>Hb 5</td>
<td>3.2 (1.9)</td>
<td>2.9 (1.1)</td>
</tr>
</tbody>
</table>

* * 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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