

## **EXAMINATION OF THE FACTORS AFFECTING PHOSPHOROUS MEASUREMENT BY THE UV-VIS**

*Đến tòa soạn: 30 - 5 - 2016*

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### **TÓM TẮT**

#### **KHẢO SÁT CÁC YẾU TỐ ẢNH HƯỞNG ĐẾN VIỆC ĐO HÀM LƯỢNG PHOTPHO BẰNG PHƯƠNG PHÁP UV-VIS**

*Quy trình đo photpho (P) từ dịch chiết đất bằng máy quang phổ UV-Vis đã được sử dụng từ lâu, tuy nhiên vẫn còn đó nhiều yếu tố có tiềm năng gây sai số cho kết quả đo mà vẫn chưa báo cáo. Nghiên cứu này được thực hiện nhằm khảo sát ảnh hưởng của 6 yếu tố đến mật độ quang P, bao gồm hàm lượng P cao trong dung dịch, thời gian lưu giữ dung dịch, độ bền màu xanh phức phosphomolybdenum, and sự có mặt của silicate, nitrite và chromium. Hàm lượng P cao hơn 2 ppm trong dung dịch đo làm giảm mối tương quan giữa nồng độ P và mật độ quang, với hệ số tương quan thay đổi từ 0.999 xuống 0.988. Trong lúc thời gian lưu trữ dung dịch đo nhỏ hơn 5 ngày không làm thay đổi đáng kể mật độ quang, thời gian kể từ khi thêm các hợp chất tạo màu có ảnh hưởng đến mật độ quang, đặc biệt sau 30 phút. Điều này cho thấy rằng màu xanh phức phosphomolybdenum có độ ổn định tốt trong vòng 30 sau khi thêm vào dung dịch đo. Sự có mặt của ion silicate làm tăng tuyến tính sự hấp thụ tia UV của P, ngược lại sự có mặt của các ion nitrite và chromium làm giảm mật độ quang P. Sự hấp thụ cực đại tia UV tại các bước sóng gần nhau của silicate và P có thể giải thích cho mối tương quan cộng hưởng giữa silicate và mật độ quang P. Trong khi đó ảnh hưởng của nitrite và chromium có thể được giải thích theo các cơ chế khác là sự oxi hóa phức xanh và thay đổi chỉ số pH của dung dịch. Các kết quả này cho thấy rằng kết quả đo P từ dịch chiết đất bằng máy quang phổ UV-Vis có thể bị ảnh hưởng bởi các yếu tố được khảo sát trong nghiên cứu này. Do đó, điều cần thiết có thêm nhiều nghiên cứu khác để cải tiến quy trình đo P bằng máy quang phổ UV-Vis.*

*Keyword: Storage duration, Stability, Color-developing reagent, Silicate, Nitrite, Chromium.*

### **1. INTRODUCTION**

Phosphorous is an important parameter of soil and water as it significantly affects agriculture production and environment quality. There have been a lot of methods used to quantify soil P contents (<sup>1</sup>). The basis different among the methods is the soil-P extracting method to get wanted P that can be available (<sup>2</sup>), microbial (<sup>3</sup>), or total (<sup>4</sup>) in

soils. On common step from those methods is the use of UV-Vis spectrophotometer to measure UV absorption of the extracts at 882-nm wavelength for P estimation (<sup>5-7</sup>).

One principle of the UV-Vis method is that the intensity of the blue color derived from phosphomolybdenum complex, determined by UV-Vis at 882-nm wavelength, is proportionally related to P concentration. Rodriguez et al. (<sup>8</sup>) identified that the blue color intensity as well as its stability could greatly depend on the properties of measured solutions. The authors further reported that stability of the blue color strongly depended on the measured-solution pH and that the color-developing reagent was also time dependent. There are many factors may influence the stability and intensity of the blue color, such as presences of unwanted ions (silicate, nitrite and chromium) in the measured solutions, storage duration and P concentration of the measured solution.

Actually, the UV-Vis has been used to measure silicate content at 815 nm (<sup>9</sup>), nitrite content at 210 nm (<sup>10</sup>), and chromium content at 540 nm (<sup>11</sup>). Different compounds have peak absorption of the UV at different wavelengths. Because the wavelength, 882 nm, at which peak UV absorption by P was identified, is close to that of silicate at 815 nm, the presence of the silicate may increase the UV absorbance assigned due to P, resulting in an overestimation of P content. Meanwhile, the influence of nitrite and chromium may be different from that of silicate due to their much lower UV adsorption wavelength, compared to that of P. The two ions may influence the blue-color intensity and stability by oxidation of the phosphomolybdenum complex and thus reducing P absorbance, if co-present. However, there had been no report on these issues, and thus it is necessary to examine these effects on P measurement.

In addition, UV absorption by P could be influenced by high P contents itself, meaning that the measured solution having quite high P content may reduce the correlation coefficient between absorbance and P concentration. Stability of the phosphomolybdenum blue color, determining the success of the UV-Vis method, is also a problem and assumingly much depending on time duration since addition of the color-developing reagent. Therefore, the current study was conducted in a laboratory with four independent tests. The aims of the study were to examine (1) effect of high P contents on the correlation coefficient between absorbance and P concentration, (2) effects of time duration including storage time and duration since addition of color-developing reagent, and (3) effects of the presences of silicate, nitrite, and chromium on P absorbance of the UV-Vis at 882-nm wavelength.

## 2. METHODS

### 2.1. P measurements by UV-Vis

The method used to quantify aqueous solution P was ascorbic acid-molybdenum blue procedure reported by Rodriguez et al. (<sup>8</sup>). Briefly the method included (1) preparation of measured solutions (see below for more details), (2) addition of mixed

color-developing reagents including 1.2M H<sub>2</sub>SO<sub>4</sub>, 0.023 M ammonium molybdate, 0.0019 M antimony potassium tartrate and 0.136 M ascorbic acid to the measured solutions and (3) absorbance measurements using the UV-Vis spectrophotometer (G10S UV-Vis spectrophotometer, Thermo Scientific Waltham, MA, USA) at 882-nm wavelength.

## 2.2. Solution preparation and measurements

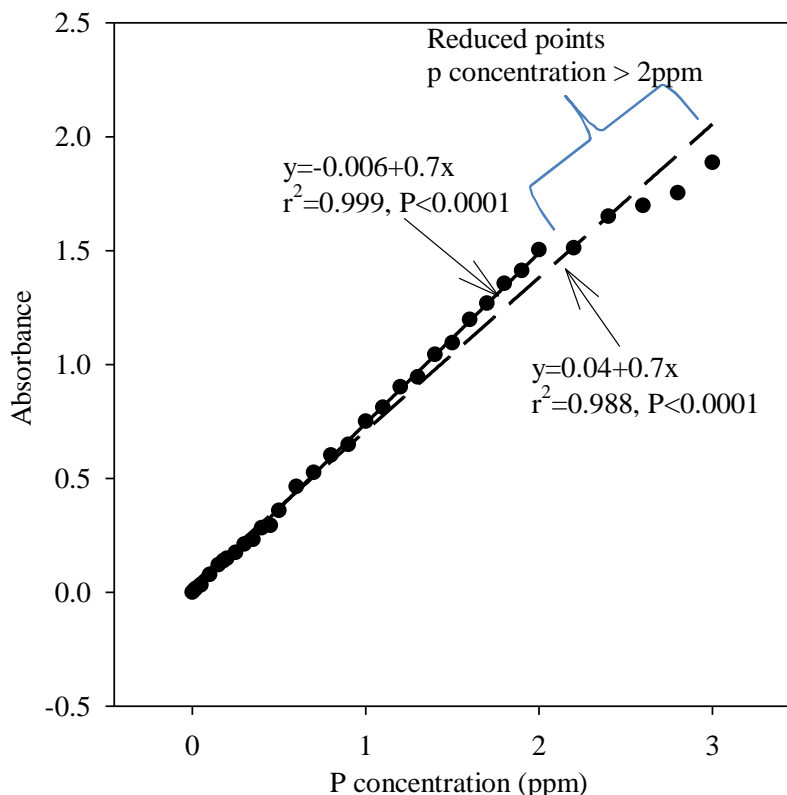
There were four independent tests to examine six factors potentially affecting P absorbance, reported in the current study. The first test was to examine the effect of high P concentration on the absorbance. Thirty four solutions with varying P concentrations in ppm were prepared, including 0.00, 0.01, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.2, 2.4, 2.6, 2.8 and 3.0. The second test was to examine the effect of storage time on absorbance. Two solutions with two P concentrations at 0.1 ppm and 1.0 ppm were prepared in 15 replicates each. The total 30 solution tubes were prepared at the same day and each day was measured with 6 solution tubes (2 concentrations and 3 replicates). The measurements were lasted for 5 consecutive days and the unmeasured solutions were stored at room temperature in a laboratory. The third test was to examine the stability of the blue color derived from phosphomolybdenum complex. Two solutions at 0.1 and 1.0-ppm P concentrations were prepared into 15 tubes each. The measurements were started after 5, 15, 30, 45 and 60 minutes since addition of the color-developing reagents. The forth test was to examine the effects of silicate, nitrite and chromium on absorbance. Two solutions at 0.1 and 1.0-ppm P concentrations were prepared into 105 tubes each. Thirty tubes at 0.1-ppm P and thirty tubes at 1.0-ppm P were added with SiO<sub>3</sub><sup>2-</sup> solution to make the measured solutions containing 0.0, 0.1, 1.0, 3.0, 5.0, 10, 15, 20, 25 and 30-ppm silicate (each concentration having 3 replicates). Another 60 tubes were also added with NO<sub>2</sub><sup>-</sup> solution in a similar way to make the same nitrite concentrations (each concentration having 3 replicates). The rest 90 tubes (45 at 0.1-ppm P and 45 at 1.0-ppm P) were added with Cr<sup>6+</sup> solution to make the measured solutions containing 0.0, 0.1, 0.3, 0.4, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5-ppm Cr (each having 3 replicates). All the tubes above were added with color-developing reagents and measured with the UV-Vis after 20-30 minutes.

## 2.3. Statistical analyses

The absorbances from three replicates were averaged and percentage changes were calculated and used to make Figures. For the test of P concentration, all absorbances were fitted with a simple linear regression model and correlation coefficients were calculated to examine proportional relationships between absorbance and P concentration.

### 3. RESULTS AND DISCUSSION

#### 3.1. Effect of high P concentrations



*Figure 1. Effect of high P concentration of measured solutions on absorbance.*

As the P concentration increased from 0 to 3 ppm, absorbance increased significantly with  $r^2 = 0.988$  (Figure 1). Meanwhile, with the P concentration changing from 0 to 2 ppm, the absorbance proportionally increased with  $r^2 = 0.999$ . The high P concentration of the measured solutions created some impact to the UV-Vis readings, forming a weaker relationship between P content and absorbance. Some reasons accounting for the impact could be that the intensity of the blue color was not well proportionally related to the high P concentrations possibly due to lack of the color-developing reagent. Therefore, it is recommended to measure solution with P less than 2 ppm.

### 3.2. Effect of time duration

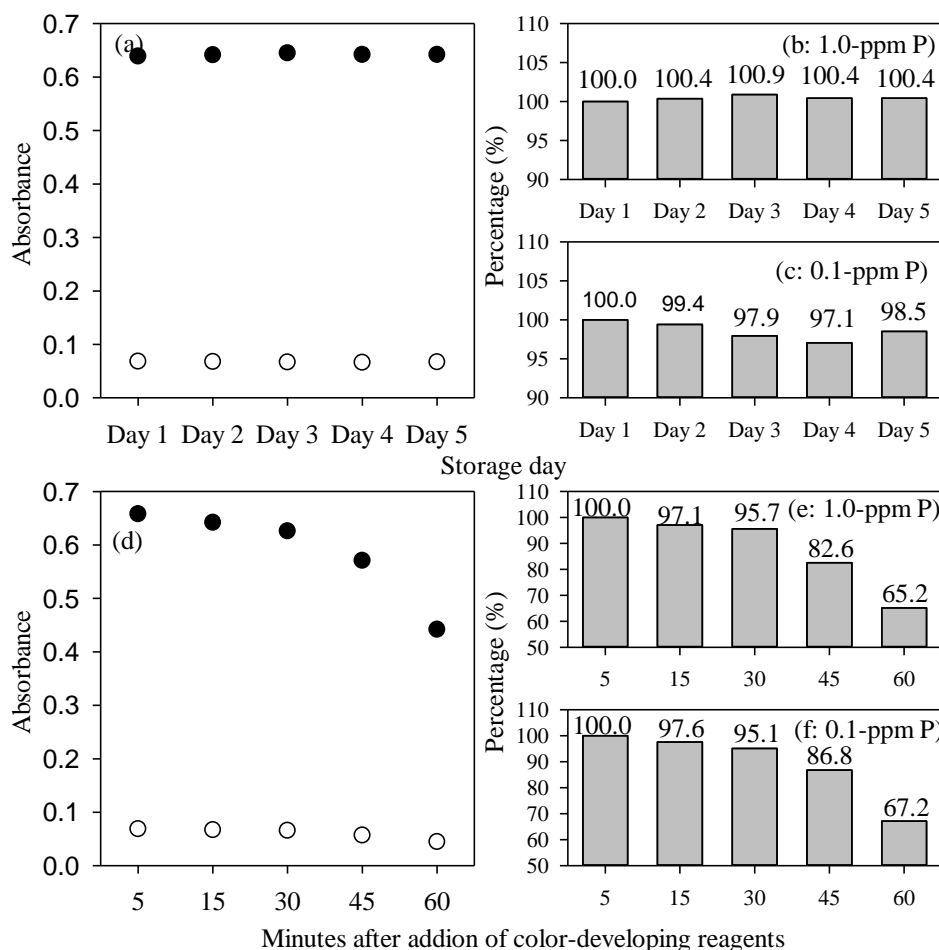


Figure 2. Effects of storage day (a) and time duration since addition of color-developing reagents (d) on absorbance. Figures b, c, e and f were the percentages compared to day 1 and/or minute 5. The filled circles were absorbance from 1.0-ppm-P solution and the unfilled circles were those from 0.1-ppm-P solution.

For one or other reasons, it is not always convenient to measure the extracts for P content right after filtering the digested solution. Therefore, the extracts may be stored in some places such as bridge for a few days before measurement. Figure 2a showed that the absorbance of day 1 was similar to those from other days, 2, 3, 4, 5 for each of the two solutions. Absorbance from the 0.1-ppm-P solution varied from 0.064 to 0.068, and that from the 1.0-ppm-P solution varied from 0.639 to 0.645. For the 1.0-ppm-P solution, the variations of absorbance were less than 1 % (Figure 2b), and for the 0.1-ppm-P solution, the variations were less than 3 % (Figure 2c), compared to the day 1. These indicated that the extracts can be stored for about five days before UV-Vis reading without significantly changing the P content.

In contrast, Figures 2b, 2e, 2f showed that within 30 minutes after adding the color-developing reagent the solutions should be read with the UV-Vis, otherwise the P content may drop down significantly. Forty five minutes after addition of the color-developing reagent, the absorbance changed significantly from 0.069 to 0.057, equal 13%, for the 0.1-ppm-P solution and from 0.66 to 0.57, equal 17%, for the 1.0-ppm-P solution. These indicated that the blue color derived from phosphomolybdenum complex was stable for about 30 minutes. Measurement of the P solution after 30 minutes may underestimate the P content.

### 3.3. Effect of silicate ion

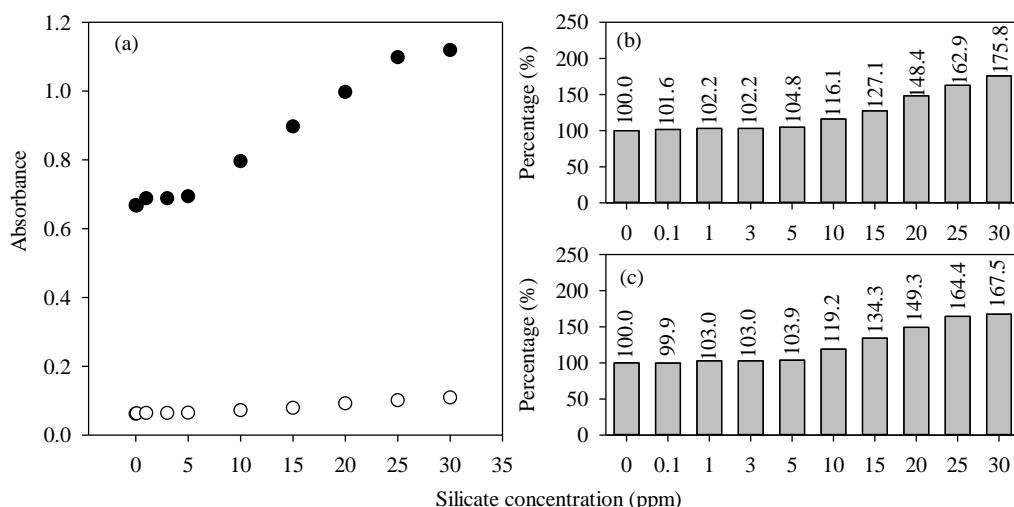


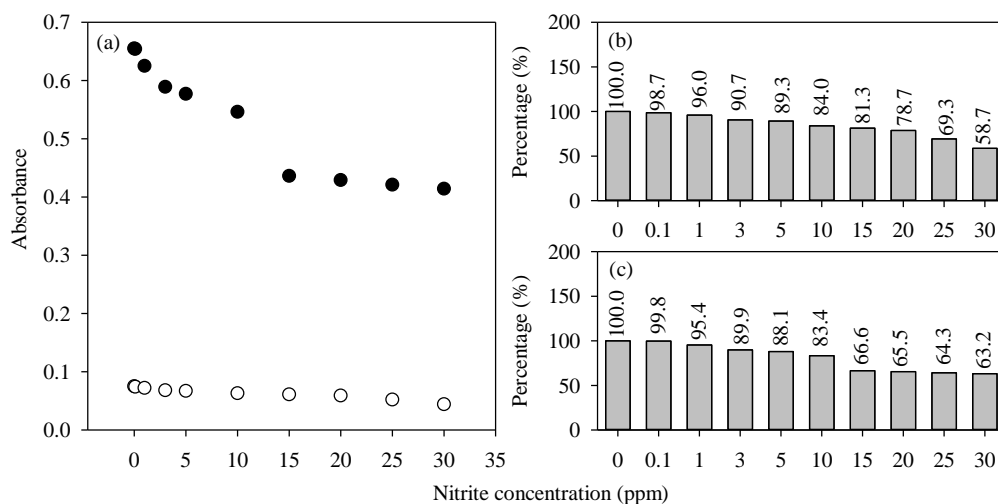
Figure 3. Effect of silicate ion concentration on absorbance. (a) was absorbance and (b) and (c) were percentages of 1.0-ppm-P solution, and of 0.1-ppm-P solution, respectively. The filled circles were absorbance from 1-pmm-P solution and the unfilled circles were those from 0.1-ppm-P solution.

With an increase in silicate ion concentration in the measured solution, the absorbance increased significantly, especially when the silicate ion concentration was higher than 5 ppm for both P concentrations (Figures 3a, 3b, and 3c). While silicate concentration in the measured solution increased from 0.0 to 5ppm, the absorbance increased by <5% for both solutions. An increase in silicate content to 10 ppm or above resulted in a great rise of absorbance, more than 16%.

The use of UV-Vis following molybdenum blue method to measure silicate content at 815 nm was reported by Samadi-Maybodi & Atashbozorg <sup>(9)</sup>. Because the peak wavelength of silicate ion is 815 nm, quite close to the applied wavelength to measure P content in the current study (882 nm), silicate ion may also absorb UV light and thus increased the absorbance, as seen in the Figure 3a. In fact, silicate is abundant in most soils. Silicate was also reported to compete with phosphorous for absorption sites on soil clay and the competition was controlled by soil pH <sup>(12)</sup>. The authors also found that at soil pH 7 or above, silicate reduced soil P adsorption, suggesting that the

presence of silicate may reduce P content in alkaline soils due to leaching. Therefore, it should take much attention on the presence of silicate when measuring P in those soils.

### 3.4. Effect of nitrite ion



*Figure 4. Effect of nitrite ion concentration on absorbance. (a) was absorbance and (b) and (c) were percentages of 1.0-ppm-P solution, and of 0.1-ppm-P solution, respectively. The filled circles were absorbance from 1-pmm-P solution and the unfilled circles were those from 0.1-ppm-P solution.*

As the nitrite concentration changed from 0 to 30 ppm, absorbance reduced from 0.075 to 0.044, equal 41%, for the 0.1-ppm-P solution, and from 0.66 to 0.41, equal 37%, for the 1.0-ppm-P solution (Figure 4a, 4b, 4c). This indicates that nitrite ion had some negative impact, reducing the UV absorption, which was in contrast to silicate's effect. Actually, solution containing nitrite ion can absorb UV at low wavelength, maximum at 210 nm, and thus UV-Vis was used to measure nitrite concentration at the same wavelength (<sup>10</sup>). With a peak absorption at relatively low wavelength, 210 nm, the nitric ion may absorb a very small amount of UV light at high wavelength, 882 nm which was applied to quantify P in the current study. If the UV absorption by the nitrite were significant when measuring P content, then the absorbance would increase with an increase of the nitrite concentration. The opposite result shown in Figure 4a indicated that nitrite ion may have other mechanisms to affect the absorbance. There are two possibilities that nitrite may introduce impacts to the absorbance, including (1) nitrite may oxidize the phosphomolybdenum blue complex, and thus reducing the blue-color intensity (<sup>13</sup>), and (2) nitrite addition may change the solution pH.

### 3.5. Effect of Chromium

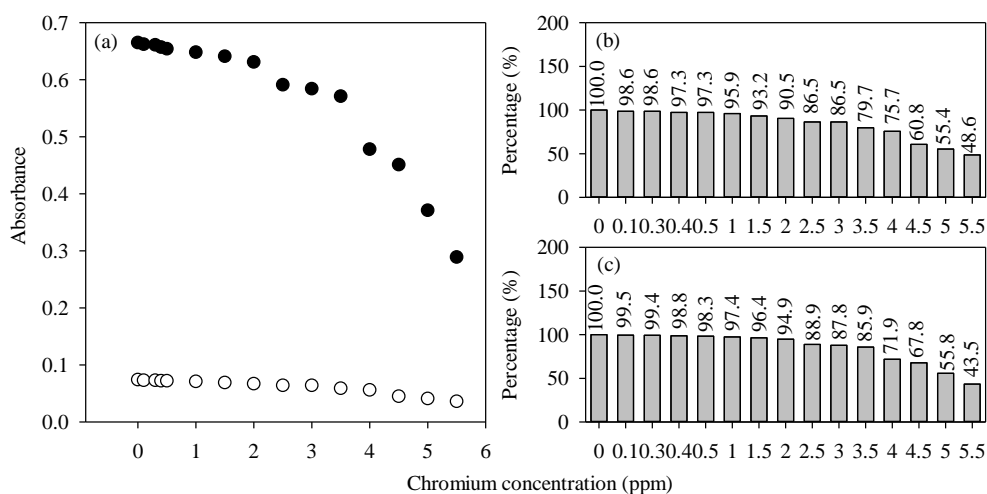


Figure 5. Effect of chromium ion concentration on absorbance. (a) was absorbance and (b) and (c) were percentages of 1.0-ppm-P solution, and of 0.1-ppm-P solution, respectively. The filled circles were absorbance from 1-pmm-P solution and the unfilled circles were those from 0.1-ppm-P solution.

Similar to the nitrite's effect, chromium with concentration increasing from 0 to 5.5 ppm also reduced UV absorption from 0.074 to 0.036, equal 51%, and from 0.67 to 0.29, equal 57%, for 0.1-ppm-P and 1.0-ppm-P solutions, respectively, (Figure 5a, 5b and 5c). In fact, the UV-Vis spectrophotometer was used to measure chromium concentration at 540 nm (<sup>14</sup>). The reducing mechanisms by chromium may be similar to nitrite because the wavelength at which maximum UV absorption by chromium was found was much lower than that applied to quantify P in the current study.

### 4. CONCLUSIONS

The UV-Vis spectrophotometer is commonly used to measure P content of soil extracts using molybdenum blue color methods. The soil extracts more likely contain other ions such as silicate, nitrite and chromium. The presences of these ions were found to change P absorbance significantly. High P contents in the measured solutions also reduced correlation coefficients between P absorbance and P concentration. The P absorbance was additionally influenced by the time duration since addition of color developing-reagent. These findings suggest that the blue color method for P measurement should be improved with consideration of the examined factors for better results.

### 5. ACKNOWLEDGMENTS

The current study was conducted at the Industrial University of Ho Chi Minh city, the Institute of Environmental Science, Engineering and Management. The current study was funded by the Institute of Environmental Science, Engineering and Management. The authors are grateful to research group for their helps.



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