Determination of available phosphorus in alkaline soil by molybdenum blue spectrophotometry

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Abstract. A rapid and accurate method for the determination of available phosphorus in alkaline soil by molybdenum blue spectrophotometry was established. The alkaline soil samples were extracted by Olsen method. The effects of absorption wavelength, amount of chromogenic agent, amount of reducing agent and colour reaction time on the determination results were investigated. The results showed that the optimal conditions were as follows: the maximum absorption wavelength was 700 nm, the amount of chromogenic agent was 1 mL, the amount of reducing agent was 1 mL, and the colour reaction time was 15 min. The linear range of phosphorus concentration was 0.3 μg/mL ~ 1.8 μg/mL, and the recoveries ranged from 88.67 % to 92.17 %. The method is simple, sensitive and accurate.

1. Introduction
Phosphorus is an indispensable element in the process of plant growth [1]. Phosphorus can promote the formation of new roots and germination of flower buds, make flowers bright and fruits early-maturing, and even improve the cold resistance and drought resistance of plants. If the phosphorus intake is insufficient, the nutritional level of the plant will be reduced, resulting in its slow growth. Although phosphorus is good, when the phosphorus in soil is too much, it will strengthen the respiration of plants, make the consumption of sugars too much, reduce the accumulation of energy, affect the synthesis of starch, and lead to the decline of yield. At the same time, the accumulation and loss of excessive phosphate fertilizer will cause serious environmental problems. Therefore, the determination of available phosphorus content in soil is helpful to understand the soil phosphorus supply capacity, and has great significance to guide farmers to apply fertilizer reasonably and effectively. Spectrophotometry has the characteristics of low cost, high sensitivity and simple operation. It is widely used in food [2-3], drug [4-6], environment [7-8] and other fields. In this paper, the content of available phosphorus in vegetable garden soil was determined by molybdenum blue spectrophotometry with satisfactory results.

2. Materials and methods

2.1. Apparatus and reagents
AL104 analytical balance, 721G visible spectrophotometer, pH-3C acidity meter, ZWF-111 constant temperature reciprocating oscillator, DK-S24 electric constant temperature water bath, dihydrogen phosphate potassium (GR), ascorbic acid (AR), 98 % sulfuric acid, sodium bicarbonate extractant (42
g/L, pH=8.5), 2,4-dinitrophenol indicator (2 g/L), molybdate solution (0.175 g of potassium antimony tartrate was dissolved in 50 mL of distilled water for solution A, 6.180 g of ammonium molybdate was dissolved in 50 mL of distilled water, and mixed with 150 mL of 50 % sulfuric acid after cooling for solution B. Solution A was mixed with solution B and stored in brown reagent bottles).

2.2. Solution preparation
Preparation of phosphate standard stock solution (100 μg/mL): 0.2196 g of potassium dihydrogen phosphate was weighed accurately and dissolved in proper amount of water. Then 5 mL of 50 % sulfuric acid was added, and the volume was fixed with distilled water to 500mL.

Preparation of phosphorus standard solution (15 μg/mL): 15 mL of phosphate standard stock solution was transferred into a 100 mL volumetric flask, and the volume was fixed with distilled water to the scale line,

2.3. Soil collection and treatment

2.3.1. Soil collection. The soil used in the experiment was from the vegetable garden. Soil samples were collected from 10 different locations in the root layer of vegetables (0 ~ 20 cm) by the "S" route, and about 150 g of each sample was collected and mixed. The soil was separated by quartering method to ensure that the mass of mixed sample was about 0.5 kg.

2.3.2. Soil treatment. The soil was naturally dried and then grinded to powdery. The 2 mm sieve was used to remove excess impurities. A 10 g sample (accurate to 0.001) was weighed and placed in a dry 150 mL stopper conical flask. About 1 g of phosphorus-free activated carbon was added, and 50 mL of sodium bicarbonate extract was added. The cap was sealed and placed on an oscillator, oscillated at 25 ℃ for 30 min at a frequency of 180 r/min. Then the solution was obtained by filtration with nonphosphorus filter paper.

2.4. Determination method of soil available phosphorus
20.00 mL of the solution treated according to the method in 2.3.2 was transferred to a 50 mL volumetric flask at room temperature. Then add a drop of 2, 4-dinitrophenol indicator and add 2 mol/L H2SO4 solution drop by drop until the solution becomes colorless. After that, 1 mL10 % ascorbic acid solution and 1 mL molybdate solution were successively added to the volumetric flask. The volume was fixed with distilled water to the scale line, and the absorbance value was measured at 700 nm wavelength after 15 min.

3. Results and discussion

3.1. Selection of absorption wavelength
At room temperature, 2 mL of phosphorus standard solution was transferred into a 50 mL volumetric flask, then 1 mL of 10 % ascorbic acid and 1 mL molybdate solution were successively added, and the volume was fixed to the scale line. After 15 min, the reagent blank without phosphate was taken as the reference solution, and the absorbance values were determined at different wavelengths. The results are shown in Figure 1.

Figure 1 shows that the absorbance first increases and then decreases with the increase of wavelength. The absorbance reaches the peak at 700 nm wavelength, so 700 nm is selected as the best absorption wavelength.
3.2. Selection of color reaction time

At room temperature, 2 mL of phosphorus standard solution was transferred into a 50 mL volumetric flask, then 1 mL of 10 % ascorbic acid and 1 mL molybdate solution were successively added, and the volume was fixed to the scale line. The reagent blank without phosphate was taken as the reference solution, and the absorbance values were determined every few minutes at the wavelength of 700 nm. The results are shown in Figure 2.

Figure 2 shows that the curve of color reaction time rises sharply in the range of 0-15 min, indicating that the color reaction is taking place at this time. The absorbance value remained unchanged within 15 ~ 40 min, indicating that the reaction was basically completed within 15 min. Therefore, 15 min is the best time.

3.3. Selection of the amount of reducing agent (ascorbic acid)

At room temperature, 2 mL of phosphorus standard solution, 0.2 ~ 1.4 mL of 10 % ascorbic acid, 1 mL molybdate solution were added to a 50 mL volumetric flask respectively, and the volume was fixed to
the scale line. The absorbance was determined at 700 nm wavelength with the solution without reducing agent as the reference solution after 15 min. The results are shown in Figure 3.

![Figure 3](image)

**Figure 3.** Effect of the amount of reducing agent on absorbance.

As shown in Figure 3, the absorbance increases with the increase of the amount of the reducing agent, and then gradually stabilizes. When the amount of reducing agent is too low, the reaction is incomplete and the result is too low. When the amount of reducing agent is not less than 1mL, the absorbance value tends to be stable, so 1mL is the best amount of reducing agent.

### 3.4. Selection of the amount of chromogenic agent (molybdate)

At room temperature, 2 mL of phosphorus standard solution, 1 mL of 10 % ascorbic acid, 0.5 ~ 5.0 mL molybdate solution were added to a 50 mL volumetric flask respectively, and the volume was fixed to the scale line. The absorbance was determined at 700 nm wavelength with the solution without molybdate as reference solution after 15 min. The results are shown in Figure 4.

![Figure 4](image)

**Figure 4.** Effect of the amount of chromogenic agent on absorbance.

Theoretically, the more molybdate is used, the more favorable it is to the formation of blue compounds. However, if the amount of molybdate is too much, other reactions will occur, which will affect the determination of the experiment. As shown in Figure 4, the absorbance value is the largest when the molybdate dosage is 1 mL, so 1 mL is the best amount of chromogenic agent.
3.5. Linear range and detection limit

1.00 mL, 2.00 mL, 3.00 mL, 4.00 mL, 5.00 mL, 6.00 mL phosphorus standard solution were taken respectively for analysis and determination according to the selected experimental conditions (the concentration was equivalent to 0.3 μg/mL, 0.6 μg/mL, 0.9 μg/mL, 1.2 μg/mL, 1.5 μg/mL, 1.8 μg/mL). Taking absorbance as ordinate and concentration as abscissa, the standard curve was drawn. It was found that the concentration of phosphorus in the range of 0.3~1.8 μg/mL had a good linear relationship with absorbance. The linear equation was $y = 0.3911x + 0.0768$, and the correlation coefficient was 0.9998. The detection limit was 0.053 μg/mL when the signal-to-noise ratio was 3 times.

3.6. Determination of available phosphorus in soil

According to the method described in 2.4, the available phosphorus content of three soil samples (soil A, soil B, soil C) was determined, and their phosphorus supply capacity was evaluated. The results are shown in Table 1.

| Soil sample | Available phosphorus content / (mg/kg) | Phosphorus supply capacity | Effect of phosphorus application |
|-------------|----------------------------------------|-----------------------------|---------------------------------|
| A           | 8.99                                   | low (5~10)                  | effective                       |
| B           | 7.59                                   | low (5~10)                  | effective                       |
| C           | 12.28                                  | medium (10~20)              | partial effective               |

3.7. Experiment on recovery of standard addition

To examine the recovery, a known amount of the phosphorus standard solution was added to the soil sample A. The samples were treated according to the method in 2.3.2 and determined according to the experimental method in 2.4. The results are shown in Table 2.

| Background value / μg | Amount added / μg | Determined / μg | Recovery / % | RSD / % (n=5) |
|-----------------------|-------------------|-----------------|--------------|---------------|
| 89.9                  | 30                | 116.5           | 88.67        | 3.91%         |
|                       | 45                | 131.0           | 91.33        | 3.05%         |
|                       | 60                | 145.2           | 92.17        | 2.68%         |

4. Conclusion

A molybdenum blue spectrophotometric method for the determination of available phosphorus in alkaline soil was established. The method is low cost, easy to operate, accurate and reliable. It has been applied to the actual determination of vegetable soil samples with satisfactory results.

References

[1] Tian L, Guo Q, Zhu Y, et al. Research and application of method of oxygen isotope of inorganic phosphate in Beijing agricultural soils [J]. Environmental Science & Pollution Research, 2016, 23(23):1-9.

[2] Stachniuk A, Szmagara A, EA Stefaniak. Spectrophotometric assessment of the differences between total nitrate/nitrite contents in peel and flesh of cucumbers[J]. Food Analytical Methods, 2018, 11(10): 2969-2977

[3] ZEHRA GÜLER. Determination of synthetic colorants in confectionery and instant drink powders consumed in turkey using uv/vis spectrophotometry[J]. Journal of Food Quality, 2005, 28(1): 98-108

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[4] Afkhami A, Khatami H A. Determination of some catecholamines based on their reaction with periodate [J]. Journal of Analytical Chemistry, 2003, 58(2):135-138.

[5] Shamsa F, Amani L. Determination of sulfamethoxazole and trimethoprim in pharmaceuticals by visible and UV spectrophotometry [J]. Iranian Journal of Pharmaceutical Research, 2006, 5(1):31

[6] L. Chierentin, H. R. N. Salgado. Performance characteristics of UV and visible spectrophotometry methods for quantitative determination of norfloxacin in tablets [J]. Journal of Scientific Research, 2014, 6(3): 531-541

[7] R. Anitha, G. R. Rajarajeswari. Selective Detection of sub-hundred picomolar mercuric ion in aqueous systems by visible spectrophotometry using gripe water functionalized gold nanoparticles [J]. Journal of Cluster Science, 2019, 30(4): 907-917

[8] R Gürkan, Hİ Ulusoy, M Akçay. Simultaneous determination of dissolved inorganic chromium species in wastewater/natural waters by surfactant sensitized catalytic kinetic spectrophotometry [J]. Arabian Journal of Chemistry, 2017, 10(1): 450-460.