

Spectrophotometric determination of phosphate in presence of arsenate

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Abstract: A simple and sensitive spectrophotometric method has been optimized for the determination of phosphate in the presence of arsenate. This method is based on the formation of phosphomolybdate complex from phosphate and added molybdate ion in an acidic medium, followed by the reduction of the complex with hydrazine hydrate. The system obeys Lambert-Beer's law in the concentration range 60-1080 ppb. The optimized volume of different reagents was 3.5 mL of 0.25% ammonium molybdate, 1.5 mL of 0.25 N sulfuric acids, and 1 mL of 0.5 M of hydrazine hydrate. The time required for the full-color development was 15 minutes at 30°C. The arsenate interference was successfully removed by using sodium thiosulphate and sodium metabisulfite below the arsenic concentration of 100 ppb. 0.2 mL of 1 M sodium metabisulfite and 1 mL of 0.1 M sodium thiosulphate were found to be the optimum volume for inhibiting arsenate interference below 100 ppb arsenate concentration.

Keywords: Molybdenum blue; Phosphate; Soil; Water.

Introduction

Phosphorus is one of the most abundant elements in the earth's crust. It can be found in the terrestrial, aquatic, and atmospheric environments, as well as in aquatic biota¹. The elemental form of phosphorus is toxic and is found in nature in the form of phosphate; orthophosphate, polyphosphate, and organic phosphate²⁻⁶. Phosphorus has great biological importance as it is involved in many physiological activities such as cell division, seed germination, photosynthesis, and respiration⁷. It also stimulates the growth of plankton and aquatic plants, provides food for aquatic animals.

Phosphate has numerous industrial applications as well. Its usage as calcium phosphate for the ceramic industry, sodium phosphate, and polyphosphate in baking powder, food additives, etc. signifies its industrial importance. However, it is primarily used in fertilizer production, deter-

gent manufacture, sugarcane juice clarification^{8,9}. Phosphorus in natural waters consists of inorganic ortho, condensed, and organic phosphate in both dissolved and particulate form. The most bioavailable form of phosphorus, orthophosphate, is the most frequently measured in natural waste and processed waters as a means of assessing phosphorus impact on the trophic status of receiving waters¹⁰. Although phosphate is a vital nutrient for plants and aquatic animals, the elevated level of phosphorus can disrupt the balance of nutrients and lead to the proliferation of algae and aquatic weeds. Such unrestrained growth often results in lower dissolved oxygen content that causes fish kills¹¹. For this reason, the determination of phosphate is detrimental.

There are numerous analytical methods developed for the determination of phosphate at different concentration

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ranges. Titrimetry and complex- gravimetry have been used to determine phosphate at higher concentrations, whereas analytical methods such as colorimetry, atomic absorption spectroscopy, HPLC, flow injection analysis, ion chromatography, and spectrophotometry, among others, have been developed to determine phosphate at ppm-level. However, due to the simple procedure and requiring low-cost equipment, the spectrophotometric method involving the formation of molybdenum blue is the most used method¹². The molybdenum blue method involves the formation of a heteropoly acid, from tetrahedral orthophosphate and molybdate under acidic conditions, which is then reduced to form an intensely colored phosphomolybdenum blue (PMB) species. Various reducing agents have been used for the reduction of heteropoly acid to form molybdenum blue such as ascorbic acid, stannous chloride, hydrazine sulfate etc¹³.

However, one major drawback of the molybdenum blue method is that it suffers from the interference from arsenate, silicate, and germanate, out of which the interference from arsenate is the most pronounced. The tetrahedral AsO_4^{3-} ion reacts with acidic molybdate in a manner very similar to that of the PO_4^{3-} . The molar absorptivities, chemical properties, and formation conditions of arsenomolybdenum blue and phosphomolybdenum blue are so similar that these two ions in the same solution cannot be quantified simultaneously¹³. Furthermore, because phosphate and arsenate have chemical and structural similarities in aqueous solution, phosphate has a significant interference in the presence of arsenate¹⁴. Therefore, phosphate from the sample containing arsenate can only be determined after reducing arsenate to arsenite.

Many modifications have been developed to the molybdenum blue method for the quantification of both ions simultaneously. Johnson presented a method for simultaneous determination of arsenate and phosphate in seawater in which the simultaneous determination was carried out by reducing arsenate to arsenite by adding a mixture of sodium thiosulphate and sodium metabisulfite while phosphate remains unaffected by the added reagents.

After reducing arsenate, only the phosphomolybdenum blue was formed and the absorbance was solely due to the formation of phosphomolybdenum only. However, the time required for full-color development was found to be 90 minutes¹⁵. Blomqvist et al. attempted to reduce the arsenate interference by timing the photometric recording as the formation rate of arsenomolybdenum blue (AsMB) was slower than that of the PMB. They also pointed out that the interference can also be reduced by diluting the sample solution¹⁶. Carvalho et al. presented a method for simultaneous determination of phosphate and arsenate based on the reduction of arsenate to arsenite by L-cysteine. This method was quite rapid as compared to that of Johnson as the reaction time for color development was lowered to less than 15 min by the addition of acetone¹⁷.

Linge and Oldham also modified the spectrophotometric method for simultaneous determination of both anions by eliminating the interference by reducing arsenate with sodium sulfite. Although it improved the measurement, the procedure was quite lengthy, requiring a long reaction time of one and a half hour¹⁸. Tsang et al. used sodium dithionite to reduce arsenate to arsenite keeping the reaction temperature at 50–80 °C. The dithionite reduction was found to be effective even at sample pH and reduction time depended on the concentration of dithionite¹⁹. Besides these methods involving the reduction of arsenate to arsenite, Borgnino et al. proposed a continuous flow method based on different kinetic characteristics of arsenate and phosphate. Because arsenate takes longer than phosphate to form molybdenum blue complex, they took advantage of the differences in behavior of phosphate and arsenate towards acidic molybdate²⁰.

In this study, it has been attempted to optimize the spectrophotometric molybdenum method for the determination of phosphate by studying the effects of the concentration of various reagents, viz., the concentration of sulfuric acid, ammonium molybdate, and reducing agent hydrazine hydrate, and to develop a method for the simultaneous determination of phosphate and arsenate.

Materials and methods

Instruments

For the spectrophotometric measurements, the UV-Visible spectrophotometer (Model WPS-104, Japan) with 1 cm matched quartz cells was used. An electronic weighing balance (Model PH3203NE, India) was used.

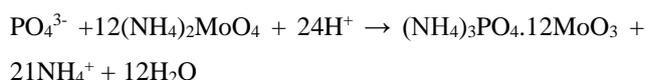
Chemicals and reagents

All the chemicals and reagents were of analytical grade, and they were employed without additional purification. All the solutions were prepared in deionized water. Standard stock solutions of phosphate, arsenate (1000 ppm) was prepared by dissolving the exact amount of potassium dihydrogen phosphate (KH_2PO_4) and disodium hydrogen arsenate, respectively in deionized water and stored in the refrigerator for further use. The working solutions were made by diluting the corresponding stock solutions. 5 mL of 5% stock ammonium molybdate reagent was taken in a 100 mL volumetric flask and was diluted up to the mark.

A stock solution of 10 N H_2SO_4 was prepared by diluting conc. H_2SO_4 (36 N) and a working solution of 0.25 N sulfuric acid was prepared by diluting 10 N sulfuric acids. Exact quantities of corresponding reagents were used to make 0.5 M hydrazine hydrate, 1 M sodium meta bisulfite, and 0.1 M sodium thiosulfate solutions.

Determination of λ_{max}

The inorganic phosphate determination by molybdenum blue method is a well-established and commonly used method. When the acidified solution of phosphate was treated with ammonium molybdate it gave a hetero poly acid which was then reduced by hydrazine hydrate to give phosphomolybdenum blue i.e., a blue color complex. The reaction involved in the formation of molybdenum blue can be given as ²¹:



Thus, formed phosphomolybdenum blue was subjected to measure absorbance from wavelength 800 nm to 900 nm spectrophotometrically. The absorbance values were

plotted against wavelength and the λ_{max} was determined. The wavelength corresponding to maximum absorbance was observed and was considered as the λ_{max} ²¹.

Optimization of concentration of ammonium molybdate

The effect of the concentration of ammonium molybdate on the intensity of the blue color formed was studied by taking varying volumes (0.5-5.0) mL of 0.25% ammonium molybdate. To this, 2 mL of 5 ppm phosphate solution, 0.5 mL of 0.25 N sulfuric acids, and 1 mL of 0.5 M hydrazine hydrate were added, and the final volume was made 25 mL by diluting with distilled water. Then the solutions were kept aside for color development, and the absorbance was measured after 35 minutes at 830 nm.

Optimization of concentration of sulphuric acid

The optimization was done by taking different volumes (0.5 -5.0) mL of 0.25 N sulphuric acid, 2 mL of 5 ppm phosphate, 3.5 mL of 0.25% ammonium molybdate, and 1 mL of 0.5M hydrazine hydrate in a 25 mL volumetric flask and the solutions were made up to the mark by diluting with distilled water. The absorbance was measured after keeping them aside for the development of full color.

Optimization of concentration of hydrazine hydrate

The concentration of the reducing agent is also one of the factors that influence the sensitivity of molybdenum blue method. The concentration of hydrazine hydrate was optimized by taking 0.2-3.0 mL of 0.5 M hydrazine hydrate, 2 mL of 5 ppm phosphate, 3.5 mL of 0.25% ammonium molybdate, and 1.5 mL of sulphuric acid in 25 mL volumetric flask. The final volume was made 25 mL and absorbance was measured after full-color development.

Effect of arsenate on the absorbance of phosphomolybdenum blue

The effect of the presence of arsenate on the time to reach the maximum absorbance and on the color stability of the complex formed was determined. For this, absorbance changes as a function of time for the solution containing a mixture of phosphate and arsenate at a concentration of 400 ppb of each were measured.

Optimization of concentration of sodium metabisulfite

The present work was aimed at finding a suitable method for the removal of arsenate interference during the determination of phosphate so that these two ions can be determined simultaneously. Many methods have been developed for such purpose as mentioned in the literature review but in this paper, the method developed by Johnson was revisited and was applied with slight modification¹⁵. In his work, he had used a mixture of sodium metabisulfite and sodium thiosulfate for the inhibition of arsenate interference. In our work, hydrazine hydrate was also added to the reaction mixture after the addition of the thiosulphate-metabisulfite mixture.

Results and discussion

Determination of λ_{\max}

On plotting absorbance against wavelength, the λ_{\max} was found at 830 nm for the given phosphate solution. Pradhan and Pokhrel also observed an analogous λ_{\max} value²¹.

The maximum absorbance was obtained from the solution containing 3.5 mL of ammonium molybdate solution, which corresponds to the concentration of 0.035% of ammonium molybdate. Above this concentration, absorbance was constant (Figure 1).

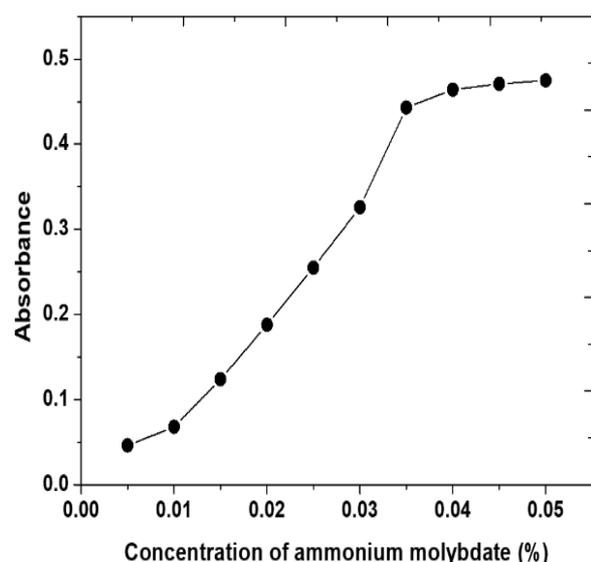


Figure 1: Optimization of concentration of ammonium molybdate

Optimization of concentration of sulphuric Acid

The absorbance data showed that maximum absorbance was obtained by the solution corresponding to 1.5 mL of sulphuric acid. This volume was used for the whole experiment. At higher acid volume, the absorbance was constant for a few extra volumes but decreased then after (Figure 2).

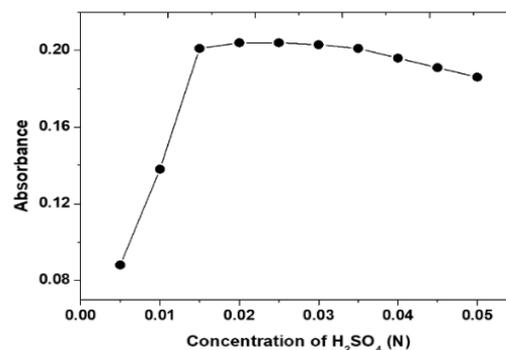


Figure 2: Optimization of concentration of sulfuric acid

Shyla and Nagendrappa, Pradhan and Pokhrel, and Khongpet et al. also observed a similar pattern of absorbance with the concentration of acid^{9, 21, 22}. The high concentration of acid may lead to a decrease in the sensitivity of the reaction to phosphate due to the decomposition of 12-MPA as well as inhibition of its formation. Self-reduction of molybdate may occur even in absence of phosphate at low acid concentrations²².

Optimization of concentration of hydrazine hydrate

The absorbance data showed that 1 mL hydrazine hydrate was sufficient to give maximum absorbance (Figure 3). The optimized volume i.e., 1 mL of hydrazine hydrate, was used throughout the experiment.

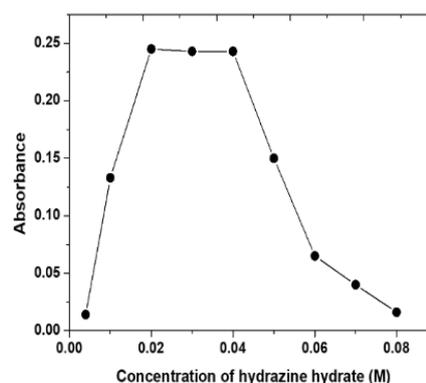


Figure 3: Optimization of concentration of hydrazine hydrate

Comparable results were obtained by Shyla and Nagendrappa and Pradhan and Pokhrel^{9, 21}. The reason for the decreased color formation at a higher concentration of reducing agents may be attributed to the early decomposition of 12-MPA formed²¹.

Effect of time in the formation of phosphomolybdenum blue

The formation of PMB is a time-dependent process that is slow at the beginning and increases gradually with time until it remains constant for a brief period. A further increase in time resulted the in a decrease in absorbance value. The plot of absorbance versus time is given in Figure 4, which shows that the time required for full-color development was 15 minutes at 30°C and the color remained stable for the next 25 minutes. The decrease in the absorbance on further increase in time may be due to the dissociation of the phosphomolybdenum blue (PMB) complex²¹.

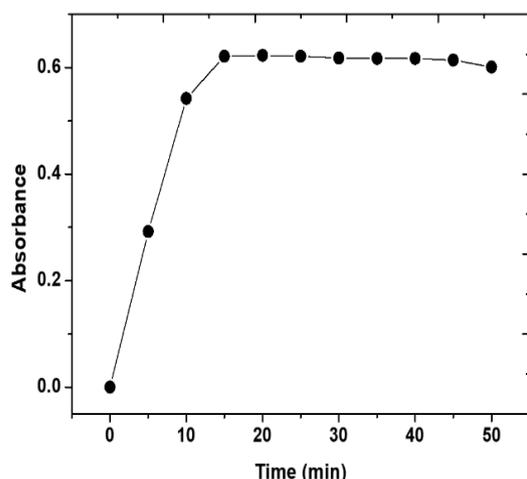


Figure 4: Effect of time in the color stability of PMB complex

Figure 4 shows that the reaction for the formation of PMB is completed in 15 minutes. Analogous results were obtained by Pradhan and Pokhrel although the time to reach the maximum absorbance in their study was 35 minutes²¹. The lowered reaction time in the present study might be attributed to the use of a low concentration of acid since the reaction time is found to increase invariably in the case when a high concentration of acid is used¹³.

Effect of arsenate on the absorbance of phosphomolybdenum blue

As seen from Figure 5, the presence of arsenate on the sample didn't alter the time for full-color development. The time required for full-color development for the sample containing either of the ions is 15 minutes whereas that for the sample containing both the anions is 20 minutes. However, arsenate enhances the absorbance of PMB complex which is due to the formation of arsenomolybdenum blue (AsMB) along with PMB.

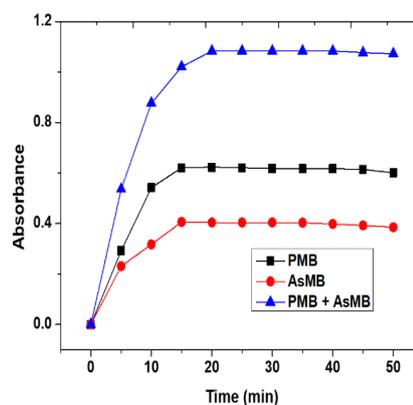


Figure 5: Effect of the presence of arsenate on the absorbance of phosphomolybdenum blue

Optimization of concentration of sodium metabisulfite

The concentration of sodium metabisulfite required for the reduction of As (V) to As (III) was optimized using 200 ppb arsenate solution, 1 mL of 0.1 M sodium thiosulphate, and varying the volume (0.2-1.0 mL) of 1M sodium metabisulfite. The concentration of sodium metabisulfite corresponding to 1mL was enough to suppress the formation of AsMB completely (Figure 6).

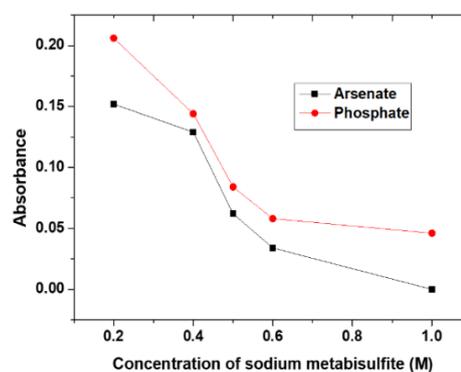


Figure 6: Optimization of concentration of sodium metabisulfite

The inhibitory effect of sodium thiosulphate and metabisulfite on the AsMB is due to the reduction of arsenate to arsenite, which, due to lack of tetrahedral structure, cannot form the heteropolymolybdenum blue. The sodium thiosulphate acts as the reducing agent whereas sodium metabisulfite acts as the source of SO₂ gas which is required for the thiosulphate to function as a reducing effect¹³. The need for metabisulfite in the reduction of arsenate was tested by adding only the thiosulfate reagent to the solution containing arsenate, where it did not affect the color development, whereas the solution containing both reagents inhibited the formation of AsMB. But the optimized concentration of sodium metabisulfite had the problem of inhibiting the formation of PMB also, which is shown in Figure 6.

The concentration of sodium metabisulfite, which didn't affect the formation of PMB (i.e., 0.2 mL) was taken and tested on synthetic samples of arsenate to see whether it could inhibit the AsMB formation or not. It was found that this concentration of sodium metabisulfite inhibits the AsMB formation below the arsenate concentration of 100 ppb.

The reduction of arsenate (As (V)) to arsenite (As (III)) using sulfur-containing reductant is a very slow process¹³. Also, it has been known from the literature that the reducing agents used to reduce arsenate affect the rate of formation of PMB. So, in our study also, the time required for full-color development for sodium metabisulfite treated phosphate was also determined (Figure 7).

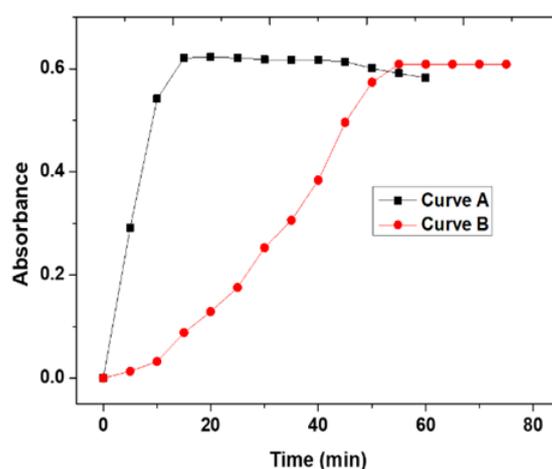


Figure 7: Time of formation of PMB (a) curve A for the phosphate sample without added metabisulfite and thiosulphate, and (b) curve B for phosphate sample with the same concentration with metabisulfite and thiosulphate solution.

Table 1: Concentration of phosphate before and after arsenate interference removal.

Conc ⁿ of phosphate (ppb)	Conc ⁿ of phosphate with 100 ppb arsenate (ppb)	Conc ⁿ after adding sodium thiosulphate and sodium metabisulfite (ppb) ^a	S.D.*	R.S.D.** (%)
200	219	202	2.081	1.053
300	330	301	2.160	0.717
400	422	402	2.943	0.733
500	525	501	3.109	0.619

*S.D.: Standard Deviation, **R.S.D.: Relative Standard Deviation, ^amean value of the triplicate reading

Time for color development after addition of reducing agent.

According to Johnson, the time of formation of PMB for phosphate sample treated with sodium metabisulfite and sodium thiosulphate is 90 minutes¹⁵. In this study, it took 55

minutes. This might be because the reaction temperature in this experiment was kept a little above room temperature. According to the arsenate interference removal literature, the time of color development in the study conducted by

Carvalho et al., was 5 minutes whereas it was 15 minutes in the experiment conducted by Tsang et al.^{17, 19}.

Conclusions

The concentration of reagents in the molybdenum blue technique for determining phosphate has been optimized. The optimized approach produces superior results with lower reagent concentrations and reduces the reaction time required for complete color development. Similarly, a technique for determining phosphate and arsenate simultaneously has been devised, allowing phosphate measurement when arsenate content in the sample is less than 100 ppb. The procedure is straightforward, requiring only a few low-cost chemicals and a low reagent concentration. As a result, the toxicity, as well as any contamination caused by these reagents, is reduced.

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