

# Rapid Determination of Trace Amounts of Phosphate and Arsenate in Water by Spectrophotometric Detection of their Heteropoly Acid - Malachite Green Aggregates Following Pre-concentration by Membrane Filtration

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A simple and rapid method for the determination of trace amounts of phosphate and arsenate in water is proposed. Molybdophosphate - and molybdoarsenate - Malachite Green aggregates, formed by reaction of a reagent consisting of a mixed solution of ammonium molybdate and Malachite Green, were selectively collected on a nitrocellulose membrane filter (pore size 3  $\mu\text{m}$ ) and dissolved in methylcellosolve together with the membrane filter. The absorbance ( $\lambda = 627 \text{ nm}$ ), denoted  $A(P + \text{As})$ , was proportional to the sum of the concentrations of phosphate and arsenate with a molar absorptivity of  $2.7 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$ . Thiosulphate, a reducing agent for arsenate, was added to water samples, and the absorbance of the solution,  $A(P)$ , was measured as described above. The absorbance  $A(P)$  corresponds to the concentration of phosphate alone. The difference,  $A(P + \text{As}) - A(P)$ , then corresponds to the arsenate concentration. The proposed method makes it possible to determine phosphate and arsenate at levels ranging from 0.3 to 150 p.p.b.

**Keywords:** Spectrophotometry; arsenate; phosphate; heteropoly acid - Malachite Green; water

The toxicity and possible carcinogenicity of arsenate and the biological pollution of environmental waters enriched with phosphate have made necessary their careful measurement in the environment.

There are several methods available for the determination of phosphate in water.<sup>1-3</sup> Most procedures for the spectrophotometric determination of phosphate are based on the formation of 12-molybdophosphate followed by its reduction to heteropoly blue species. However, it is difficult to determine phosphate at levels less than  $1 \mu\text{g l}^{-1}$  (1 p.p.b.) in water samples. Therefore, a pre-concentration step, such as solvent extraction, which requires a considerable time and reduces the accuracy of analysis, is usually carried out.<sup>4,5</sup> Pre-concentration by the collection of the heteropoly blue of phosphate on a membrane filter is a method that has already been developed.<sup>6,7</sup> However, as the molar absorptivity of heteropoly blue is  $1 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ , this sensitivity is not sufficient for the detection of nanogram levels of  $\text{P}^{\text{V}}$ , even with pre-concentration.

Itaya and Ui<sup>8</sup> reported that molybdophosphate, formed in acidic media, reacts with Malachite Green to produce a coloured compound that can be used for the spectrophotometric determination of phosphate in serum. Altmann *et al.*<sup>9</sup> studied the mechanism of the colorimetric reaction between molybdophosphate and Malachite Green and concluded that the ion-association complex  $(\text{Malachite Green})_3(\text{PMo}_{12}\text{O}_{40})$  forms in acidic media containing sulphuric acid.

Motomizu *et al.*<sup>10,11</sup> reported a spectrophotometric method in which a molybdophosphate aggregate is formed with Malachite Green, and the sensitivity of which is enhanced by pre-extraction of the aggregate with toluene - isobutyl methyl ketone. However, the procedure for concentrating the aggregate is complicated and the maximum concentration factor is about 20.

Arsenic species can be determined at low levels in water samples using electrothermal atomisation atomic absorption spectrometry coupled with analytical separations.<sup>12,13</sup> The procedure, however, is time consuming and requires expensive apparatus. The simultaneous presence of arsenate and phosphate in natural waters has given rise to the development of methods for determining both species.<sup>12-14</sup>

To determine trace amounts of these elements in water samples, a simple, precise, rapid and economical procedure is desirable. The present studies were carried out in an attempt

to meet this need. Using the proposed method, it is possible to rapidly determine both phosphate and arsenate in amounts ranging from 0.3 to 150 p.p.b. as phosphorus and arsenic.

## Experimental

### Reagents

All chemicals were of analytical-reagent grade and were used without further purification.

**Malachite Green solution**,  $2 \times 10^{-3} \text{ M}$ . Prepared by dissolving 0.976 g of Malachite Green (oxalate) in 1 l of water.

**Ammonium molybdate solution**, 0.0971 M. Prepared by dissolving 120 g of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  in 1 l of water.

**Molybdate - Malachite Green (Mo - MG) reagent**. Prepared by mixing 300 ml of ammonium molybdate, 250 ml of Malachite Green and 47 ml of concentrated sulphuric acid. After allowing the mixture to stand for about 30 min, it was filtered through a 0.45  $\mu\text{m}$  pore size membrane filter. The filtrate was used as the Mo - MG reagent.

**Phosphate standard solution**,  $2.00 \times 10^{-3} \text{ M}$ . Prepared by dissolving 0.2722 g of potassium dihydrogen orthophosphate in 1 l of water.

**Arsenate standard solution**,  $1.00 \times 10^{-2} \text{ M}$ . Prepared by dissolving 0.3120 g of disodium hydrogen orthoarsenate in 100 ml of water.

### Apparatus

Absorbance was measured with a Hitachi Model 210 double-beam spectrophotometer and a JASCO Uvidec 660 double-beam spectrophotometer, using 1.0-cm cuvettes.

### Procedure

A 1-ml volume of 7.5 M sulphuric acid and 3 ml of Mo - MG reagent were added to 20 ml of a water sample containing phosphate and arsenate at total concentrations ranging from  $2 \times 10^{-9}$  to  $2 \times 10^{-5} \text{ M}$ .

For a sample containing little or no phosphate, standard phosphate solution was added to the sample solution at a concentration of  $4 \times 10^{-7} \text{ M}$  so as to accelerate the formation of the arsenate aggregate. In any event, pre-addition of phosphate solution in this manner is preferred when the

phosphate content of a sample is unknown. The solution was then filtered through a 3  $\mu\text{m}$  pore size membrane filter (made of nitrocellulose - acetylcellulose or nitrocellulose) to collect the aggregates of 12-molybdophosphate and 12-molybdoarsenate with Malachite Green. The membrane filter and aggregates were dissolved in 4 ml of methylcellosolve. The absorbance,  $A(P + As)$ , was measured at 627 nm using a reagent blank as reference. The absorbance obtained corresponded to the sum of the concentrations of phosphate and arsenate in the sample.

A 1-ml volume of 7.5 M sulphuric acid and 0.3 ml of  $2 \times 10^{-2}$  M sodium thiosulphate, used to reduce the arsenate to arsenite, were added to 20 ml of the sample and the solution was allowed to stand for 5 min at room temperature. The absorbance,  $A(P)$ , of the solution was measured as described above. The absorbance obtained corresponded to that of the phosphate alone, as arsenite does not form an aggregate with Malachite Green. The difference,  $A(P + As) - A(P)$ , therefore corresponds to the arsenate concentration in the sample. When the total concentration of phosphorus and arsenic in sample water was less than 3 p.p.b., 200 ml of sample water were required.

## Results and Discussion

### Optimum Conditions

Absorption spectra of the molybdophosphate - Malachite Green aggregate in methylcellosolve and aqueous solutions are shown in Fig. 1. The molar absorptivities of the aggregate in methylcellosolve and aqueous solutions were found to be  $2.7 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$  at  $\lambda_{\text{max}}$ , 627 nm and  $7.8 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$  at  $\lambda_{\text{max}}$ , 650 nm, respectively. The absorption spectrum of the arsenate aggregate in methylcellosolve was obtained by subtracting the spectrum of the phosphate aggregate solution from that of the solution containing both phosphate and arsenate aggregates. The spectrum obtained was similar to that of the phosphate aggregate shown in Fig. 1, indicating that arsenate, in addition to phosphate, reacts with the Mo - MG reagent and forms coloured species of similar composition. Consequently, both phosphate and arsenate can be determined by measurement of the absorbance of the methylcellosolve solution at 627 nm.

To establish the conditions that would be appropriate for the formation of aggregates with Malachite Green, the effects of the aqueous solution acidity, Mo - MG reagent constituents, membrane filter material and pore size and the organic solvents used to dissolve the aggregates were examined.

The effect of the sulphuric acid concentration on absorbance was examined first. A constant response was obtained

with sulphuric acid concentrations in the range 0.25–0.75 M, and the final concentration was therefore adjusted to about 0.45 M. To obtain a constant and maximum absorbance, the final concentrations of molybdate and Malachite Green in solution before filtration were adjusted to  $4 \times 10^{-2}$  and  $1 \times 10^{-4}$  M, respectively, that is, their concentrations in the Mo - MG reagent were fixed at 0.34 and  $8.4 \times 10^{-4}$  M, respectively. These values ensured that the optimum conditions recommended by Motomizu *et al.*<sup>10</sup> for determining phosphate in aqueous solution would be met.

An examination was made of the applicability of various types of membrane filters, such as those made of nitrocellulose, nitrocellulose - acetylcellulose, regenerated cellulose, polycarbonate and PTFE. Nitrocellulose and nitrocellulose - acetylcellulose membrane filters were found to give the most effective aggregate collection and both were used. The effect of pore size on aggregate collection is shown in Fig. 2. Constant and maximum absorbance was obtained over a wide range of pore size, from 0.3 to 8  $\mu\text{m}$ , and a 3  $\mu\text{m}$  pore size was subsequently used. In this experiment, collection of the aggregate on membrane filters was found to depend not on molecular size but on the adsorptivity of the aggregate. This point should be studied in greater detail.

Methylcellosolve, dimethyl sulphoxide, dimethylformamide and acetone were tested as solvents for dissolving the aggregates. Methylcellosolve was the best owing to its lower viscosity and the high absorbances obtained for the aggregates. Maximum absorbance was achieved within 1 min and was stable for at least 3 h.

The presence of small amounts of phosphate markedly accelerated the formation of the molybdoarsenate aggregate. The effect of phosphate concentration on the rate of colour development of the arsenate aggregate in sulphuric acid (0.45 M) was examined using the standard phosphate and arsenate solutions. The results are shown in Fig. 3. In the absence of phosphate, arsenate gave less than 50% of its final response even after standing for 2 h, whereas in the presence of  $4 \times 10^{-7}$  M phosphate it could be detected to the extent of more than 95% within 5 min. Hence the formation of the molybdoarsenate aggregate in the sample solution should be accelerated by the addition of adequate amounts of standard phosphate solution. The  $\text{PO}_4^{3-}$  to  $\text{AsO}_4^{3-}$  ratio in natural waters is usually very high, so the addition of standard phosphate solution is not always necessary. Murphy and Riley,<sup>14</sup> Stauffer<sup>15</sup> and Johnson and Pilson<sup>16</sup> have also reported the acceleration of the development of 12-molybdoarsenate by phosphate at low concentrations. This finding was applied in the present work to increase considerably the rate of aggregate. As both phosphate and arsenate react

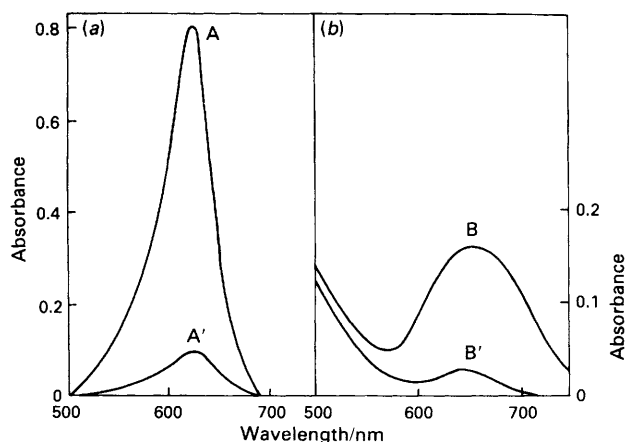


Fig. 1. Absorption spectra of the molybdophosphate - Malachite Green aggregate in (a) methylcellosolve and (b) aqueous solutions. Concentrations of phosphate: (A)  $2.4 \times 10^{-6}$  M; (B)  $2 \times 10^{-6}$  M; (A' and B') 0 M

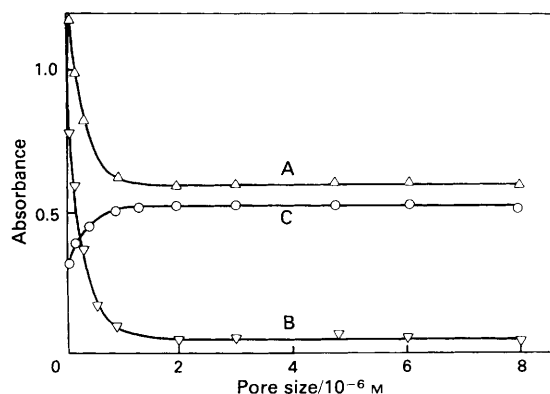


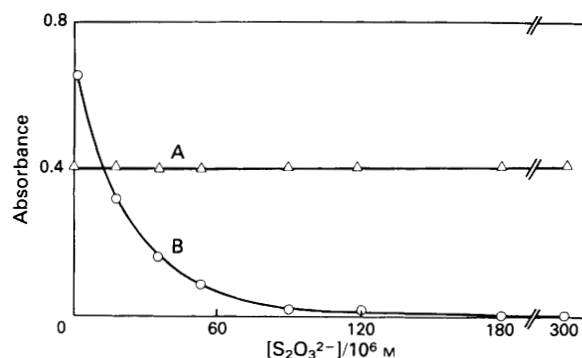
Fig. 2. Effect of nitrocellulose membrane filter pore size on the absorbances at 627 nm of (A) molybdophosphate - Malachite Green aggregate and (B) reagent blank. (C) was obtained by subtracting (B) from (A). Concentration of phosphate,  $4 \times 10^{-7}$  M

**Table 1.** Effects of foreign substances on the determination of phosphate and arsenate. Concentrations of phosphate and arsenate present:  $2 \times 10^{-7}$  M

| Cation added             | Concentration/<br>M | PO <sub>4</sub> <sup>3-</sup> and AsO <sub>4</sub> <sup>3-</sup><br>found, % | Anion added                            | Concentration/<br>M | PO <sub>4</sub> <sup>3-</sup> and AsO <sub>4</sub> <sup>3-</sup><br>found, % |
|--------------------------|---------------------|--|--|---------------------|--|
| Ca <sup>2+</sup> .. .. . | $1 \times 10^{-3}$  | 101.1  | AsO <sub>3</sub> <sup>2-</sup> .. .. . | $4 \times 10^{-6}$  | 100.5  |
| Fe <sup>3+</sup> .. .. . | $4 \times 10^{-7}$  | 120.0  | Cl <sup>-</sup> .. .. .                | $2 \times 10^{-1}$  | 100.2  |
|                          | $1 \times 10^{-7}$  | 105.0  | SiO <sub>3</sub> <sup>2-</sup> .. .. . | $2 \times 10^{-5}$  | 105.0  |
|                          | $4 \times 10^{-8}$  | 100.5  |  | $4 \times 10^{-6}$  | 101.0  |
| K <sup>+</sup> .. .. .   | $1 \times 10^{-1}$  | 100.2  | SO <sub>4</sub> <sup>2-</sup> .. .. .  | 1                   | 100.0  |
| Na <sup>+</sup> .. .. .  | $1 \times 10^{-1}$  | 100.0  |  |                     |  |

**Table 2.** Determination of phosphate and arsenate in real water samples

| Sample                  | PV,<br>p.p.b. | AsV,<br>p.p.b. |
|-------------------------|---------------|----------------|
| <i>Mineral waters:</i>  |               |                |
| A .. .. .               | 7.2           | 10.7           |
| B .. .. .               | 18.1          | 7.6            |
| C .. .. .               | 81.1          | 1.5            |
| D .. .. .               | 44.3          | 9.1            |
| E .. .. .               | 19.3          | 9.3            |
| <i>Tap waters:</i>      |               |                |
| F .. .. .               | 11.8          | 2.0            |
| G .. .. .               | 6.5           | 3.4            |
| H .. .. .               | 1.8           | 1.0            |
| <i>Hot well waters:</i> |               |                |
| I .. .. .               | 52.1          | 74.0           |
| J .. .. .               | 40.9          | 36.1           |
| <i>River waters:</i>    |               |                |
| Hayato River ..         | 9.7           | 6.2            |
| Mizusawa River ..       | 13.2          | 3.6            |

**Fig. 4.** Effect of concentration of thiosulphate on the absorbance of the aggregates at 627 nm. (A)  $4 \times 10^{-7}$  M PO<sub>4</sub><sup>3-</sup>; (B)  $6 \times 10^{-7}$  M AsO<sub>4</sub><sup>3-</sup>

sample solution was kept at room temperature for 5 min following the addition of thiosulphate to a concentration of  $1.2 \times 10^{-4}$  M prior to colour development by the Mo - MG reagent.

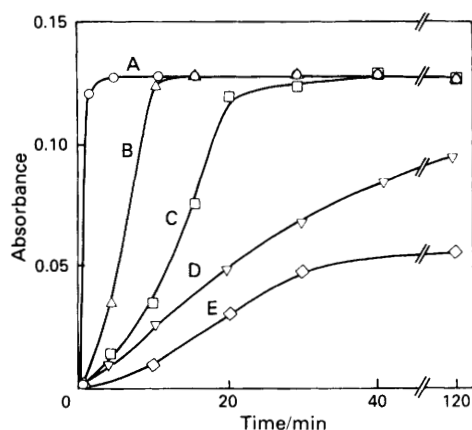
The conditions for the determination of phosphate and arsenate as described above were chosen on the basis of the experimental results. The plots of absorbance obtained with the standard phosphate or arsenate solution against concentration gave a linear relationship,  $y = 1.03 \times 10^6 x - 0.005$  ( $y$  and  $x$  refer to the absorbance and molar concentration of phosphate or arsenate in the sample), correlation coefficient  $r = 0.998$ , over the concentration range  $2 \times 10^{-9}$ – $2 \times 10^{-5}$  M. The coefficients of variation for phosphate and arsenate were 2.0 and 3.2%, respectively, ( $n = 10$ ) at  $1 \times 10^{-7}$  M. The coefficient of variation for  $1 \times 10^{-8}$  M phosphate (0.3 p.p.b. phosphorus) was 7.0% ( $n = 12$ ).

### Interferences

The effects of foreign substances on the absorbance corresponding to the phosphate and arsenate ions are given in Table 1. Almost all of the substances, except iron(III) and silicate, caused not more than a 5% error even in the presence of as much as  $10^6$  times the concentration of phosphate or arsenate. The presence of iron(III) in an amount equal to that of phosphate caused an increase in absorbance. The interference of iron(III) was avoided by passing the water sample through a column packed with a cation-exchange resin (Dowex 50WX-8). The presence of silicate in an amount 100 times that of phosphate caused a minor error in the absorbance. Heating the sample water acidified with sulphuric acid eliminated the interference of silicate. Arsenite had no effect on the absorbance of phosphate or arsenate.

### Determination of Phosphate and Arsenate in Real Water Samples

The method was applied to the determination of phosphate and arsenate in real water samples and the results are given in Table 2. Phosphate and arsenate at parts per billion levels

**Fig. 3.** Absorbance at 627 nm of the molybdoarsenate aggregate with Malachite Green vs. standing time for the standard arsenate in 0.45 M sulphuric acid. Concentration of arsenate,  $2 \times 10^{-6}$  M. Concentration of phosphate: (A)  $2.0 \times 10^{-6}$  M; (B)  $4.0 \times 10^{-7}$  M; (C)  $2.0 \times 10^{-7}$  M; (D)  $8.0 \times 10^{-8}$  M; and (E) 0.0 M

with the Mo - MG reagent, the signal obtained corresponds to the sum of the concentrations of the two ions in the sample.

The determination of phosphate in a sample containing both ions therefore requires use of an agent that prevents arsenate from forming an aggregate. Thiosulphate was used to reduce arsenate to arsenite, which is incapable of forming an aggregate.<sup>16-18</sup> Fig. 4 shows the effect of thiosulphate on the absorbance of the standard arsenate and phosphate solutions. By allowing the solution containing above  $1 \times 10^{-5}$  M thiosulphate to stand for 5 min at room temperature, the absorbance corresponding to the arsenate disappeared, but the signal corresponding to the phosphate remained unchanged. Therefore, in the determination of phosphate, the

Table 3. Recovery of arsenate and phosphate

| Sample            | Original content, p.p.b. |                 | Added, p.p.b.  |                 | Recovery of amount added, * % |                 |
|-------------------|--------------------------|-----------------|----------------|-----------------|-------------------------------|-----------------|
|                   | P <sup>v</sup>           | As <sup>v</sup> | P <sup>v</sup> | As <sup>v</sup> | P <sup>v</sup>                | As <sup>v</sup> |
| Tap water . . .   | 6.6                      | 2.0             | 5.0            | 7.5             | 100.2                         | 101.3           |
| River water A . . | 44.3                     | 6.6             | 5.0            | 7.5             | 100.0                         | 96.7            |
| River water B . . | 18.1                     | 7.7             | 5.0            | 7.5             | 98.0                          | 92.7            |

\* Average of five determinations.

were determined in a short time, 20 samples being analysed within 60 min.

Table 3 gives the results of recovery tests. The analytical recoveries of phosphate and arsenate in tap water and river water were between 92.7 and 101%.

Aggregates with Malachite Green give a high molar absorptivity of  $2.7 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$ . The collection of aggregates on a membrane filter and their dissolution in a small volume of methylcellosolve thus provides a sensitive, simple, rapid and economical method for the determination of trace amounts of phosphate and arsenate in environmental waters.

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