# Spectrophotometric method for the determination of phosphorus in natural waters using the bismuth-phosphomolybdate complex

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## Abstract

A very simple, easy and sensitive spectrophotometric manual determination method of phosphorus for the P-PO$_4^{3-}$ ion in natural waters, based on formation of the blue bismuth-phosphomolybdate complex (BiPMo), was developed. Ascorbic acid is used for the reduction of bismuth-phosphomolybdic acid to bismuth-phosphomolybdate complex. Beer's law is obeyed for the concentration range to 0.6 mg·$\ell^{-1}$ (aqueous solution) and to 1.2 mg·$\ell^{-1}$ P (IBMK). The effect of foreign ions on absorbance was examined. Ions do not affect the spectrophotometric determination of phosphorus using the BiPMo complex at concentrations usually found in natural waters.

The limit of detection (blank + 3 S.D.) was 0.0059 mg·$\ell^{-1}$ (aqueous solution) and 0.0050 mg·$\ell^{-1}$ (IBMK) using the bismuth-phosphomolybdate complex (BiPMo). The P-PO$_4^{3-}$ in river water, mineral water and spring water was determined by this method. The results obtained by the proposed method are compared with those of the phosphoantimonylmolybdenum blue method. The method of standard addition was also applied to the determination of phosphorus in natural waters.

**Keywords**: spectrophotometry, phosphorus determination, natural waters, bismuth-phosphomolybdate complex

## Introduction

Phosphorus (P) is an essential element for all life including plant growth and photosynthesis in algae (Daniel, 1998; Haugarth and Jarvis, 1999; McDowell, 2001).

A multiplicity of phosphorus forms, including ortho-, pyro-, poly-, meta-, organic, colloidal, and suspended phosphorus, can be present in natural waters. Each of these forms can be measured either partially or fully (depending on the reaction conditions) as orthophosphate via hydrolysis. Phosphate stimulates the growth of plankton and aquatic plants which provide food for fish. This may cause an increase in the fish population and improve the overall water quality. However, if an excess of phosphate enters a water-way, algae and aquatic plants grow excessively, choking up the waterway and using up large amounts of oxygen. This condition is known as eutrophication or over-ferti-lisation of the receiving waters. This process, in turn, causes the death of aquatic life because of the lowering of dissolved oxygen levels. Extensive monitoring is needed to provide data on this nutrient for effective water quality management. An indispensable key to the accomplishment of this objective are methods for analysing phosphates that are accurate, specific, and reliable.

In recent years, many rapid and simple methods are used for the determination of phosphorus as phosphate in water samples. Spectrophotometric methods are based on the formation of yellow molybdophosphoric acid and its reduction to a blue het-eropoly compound, phosphomolybdenum blue (*Standard Methods*, 1998; Lyddy-Meaney et al., 2002; Zhang and Chi, 2002). There have been many reports on various modifications of these methods in order to make the sensitivity and stability high, such as the malachite green method (Susanto et al., 1995), rhodamine B method (Zenhai et al., 2005), thiamin method (Perez-Ruiz et al., 2001) and luminol method (Yaqoob et al., 2004).

However, it is known that the silicate ion has a significant effect on determination of phosphorus in all methods based on phosphomolybdenum blue. Silicate interference can be reduced or eliminated using lower temperatures (silicomolybdenum blue complex is favoured at higher temperatures) or by reducing the pH (Zhang and Chi, 1999). Masking agents such as tartaric acid can also be effective provided that they are added before the molybdophosphate or molybdosilicate species are formed (Yaqoob et al., 2004).

In order to decrease silicate interference and increase the sensitivity of the phosphomolybdenum blue method Murphy and Riley (1962) carried out the reduction of molybdophosphoric acid with tin(II)-chloride or ascorbic acid in the presence of potassium-antimonyl tartarate and obtained a blue solution, the absorption maximum of which was at 882 nm. These authors stated that antimony ions (III) have the role of catalyst. Somewhat later, Going and Eisenreich (1974) surmised the stoichiometry of the reduced heteropoly acid to be PSb$_2$Mo$_{10}$O$_{40}$. The reduction is not instantaneous, nor is the blue product stable; full colour develops in 6 to 10 min and fades gradually thereafter (Minear and Keith, 1984). Subsequently, the Murphy and Riley technique has become one of most frequently used procedures, achieving a tentative status in the *Standard Methods* (1998).

Ueda et al. (2001) used a phosphoantimonylmolybdenum blue complex in a ratio of H$_2$O:CH$_3$CN (1:1) for the spectropho-

tometric determination of phosphorus in river and sea waters.

Boltz and Mellon (1948) observed that the addition of bismuth to the molybdophosphoric acid solution, with subsequent reduction, resulted in an increase in the blue hue. Campbell and Mellon (1960) used this catalytic effect for the determination of bismuth in an alloy for bearing metals and Goldman and Hargis (1969) determined the structure of the formed complex (the ratio P, Bi and Mo is 1:1:18 ).

The fact that the bismuth-phosphomolybdate complex (BiPMo) is more stable than antimonyl-phosphomolybdate over a longer period of time was used for the spectrophotometric determination of phosphorus in coal and coal ashes (Mihajlovic et al., 2003).

In this study, we developed a procedure for the direct spectrophotometric determination of  P-PO$_4^{3-}$ using the bismuth-phosphomolybdate complex in natural waters and aqueous solution, even after the extraction of this complex by isobutyl methyl ketone.

## Experimental

### Apparatus and reagents

All the experiments were carried out with a Carl Zeiss 11 spectrophotometer, Jena.

A stock standard solution of bismuth (1 g·$\ell^{-1}$) was prepared by dissolving 1.0000 g of bismuth metal in the minimum volume of 1:1 HNO$_3$ and diluting the solution to 1 $\ell$ with 2% (v/v) HNO$_3$. The solution was further diluted to obtain a 0.001 mol·$\ell^{-1}$ solution.

A  standard phosphorus solution (0.1 g·$\ell^{-1}$) was obtained by dissolving 0.2197 g of potassium dihydrogen phosphate, (previously dried) in distilled water to make 500 m$\ell$ of solution. The working solution of phosphorus (2 mg·$\ell^{-1}$) was prepared by dilution of the standard solution.

Ascorbic acid (2 g) was dissolved in 100 m$\ell$ of water. Since ascorbic acid solutions are not stable for long periods of time at room temperature, they were freshly prepared immediately prior to use.

Ammonium heptamolybdate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, (2.2287 g) was dissolved in distilled water to 100 m$\ell$, giving a 0.12 mol·$\ell^{-1}$ solution.

Concentrated (d=1.54; 60% (t/t)) HClO$_4$ (271.8 m$\ell$) was diluted to 500 m$\ell$ with distilled water. This solution was 5 mol·$\ell^{-1}$.

## Procedures

### Procedure for obtaining the calibration curves

**Procedure A**. In order to construct a calibration curve, 2.5 m$\ell$ of 5 mol·$\ell^{-1}$ solution of HClO$_4$, 1 m$\ell$ of 0.12 mol·$\ell^{-1}$ ammonium molybdate, 2 m$\ell$ of 0.001 mol·$\ell^{-1}$ bismuth solution and various volumes (1-15 m$\ell$) of the working phosphorus solution were placed in 50 m$\ell$ flasks. Then 2.5 m$\ell$ of ascorbic acid solution was added and the mixtures diluted with distilled water up to the mark. The solution was allowed to stand for 10 min. The absorbance of the coloured solution was measured at 720 nm in a 1 cm cell against a reagent blank.

**Procedure B**. A solution of the blue complex formed by Procedure A was transferred into a 150 m$\ell$ separating funnel. It was then extracted with 25 m$\ell$ of isobutyl methyl ketone (IBMK) by shaking for 1 min. The absorbance was measured at 670 nm against the blank solution.
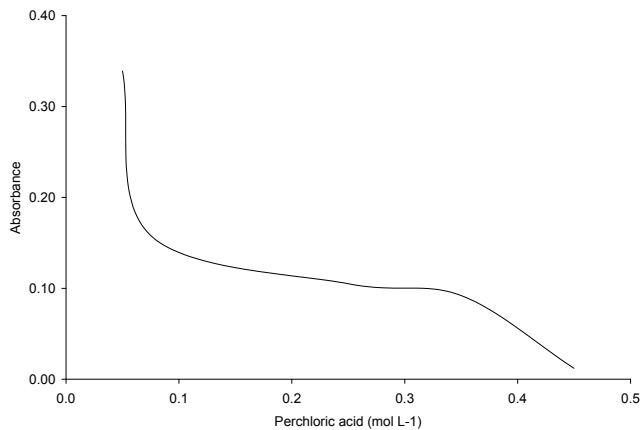


**Figure 1**
*Absorbance at 720 nm as a function of the perchloric acid concentration for 0.2 mg·$\ell^{-1}$ P, ammonium molybdate 2.40 mmol $\ell^{-1}$, bismuth 0.04 mmol·$\ell^{-1}$ and ascorbic acid 5.68 mmol·$\ell^{-1}$. The absorbance was measured after standing at room temperature for 10 min.*

### The procedure for the determination of phosphorus in natural water samples

An aliquot of a natural water sample (containing not more than 30 µg of phosphorus) was measured into a 50 m$\ell$ volumetric flask and the same procedure as used for the establishment of the calibration curve was followed.

## Results and discussion

### Effect of concentration of perchloric acid

Since the BiPMo complex is the most stable in perchloric acid solutions (Goldman and Hargis, 1969), all investigations were performed in solutions of this acid.

In order to determine the concentration of perchloric acid in which the reduced form of the BiPMo complex has maximum absorption, the absorbances of aqueous solutions of this complex in which the concentration P was 0.2 mg·$\ell^{-1}$ and the concentration of perchloric acid was in the range 0.05 to 0.45 mol·$\ell^{-1}$were measured.

The results of the measurements show (Fig.1) that the absorbance of the aqueous solutions of the reduced form of the BiPMo complex is the highest at the lowest concentration of perchloric acid (0.05 mol·$\ell^{-1}$), which leads to a wrong conclusion that all determinations of phosphorus via the BiPMo complex should be performed at this concentration of the acid.

However, the absorbance of this complex changes rapidly with time at this concentration of perchloric acid (Fig.2) and, after a short period of standing, there is no linear dependence between the measured absorbance and the concentration of phosphorus in the solution. This increase of the absorbance of aqueous solutions of the BiPMo complex in solutions of lower acidity is due to the reduction of the surplus molybdate into a blue product. Spectrophotometric determinations of phosphorus in aqueous solutions of the BiPMo complex should, therefore, be performed at such an acidity in which the absorbance of a solution of a certain concentration is sufficiently high, the change of absorbance is minimal with respect to time and the dependence of the absorbance on the concentration of phosphorus is linear. All those demands are satisfied with a perchloric acid concentra-

tion of 0.25 mol·ℓ$^{-1}$. At this concentration of acid, the absorbance of an aqueous solution BiPMo is stable for at least 89 h. For this reason a perchloric acid concentration of 0.25 molℓ·ℓ$^{-1}$ was used in all further investigations.

If the BiPMo complex is extracted with IBMK, the concentration of perchloric acid should also be 0.25 mol·ℓ$^{-1}$. At lower acid concentrations the surplus molybdate is reduced into a product which dissolves in IBMK, thus disturbing the determination.

## Effect of ascorbic acid concentration

The effect of ascorbic acid concentration (0.11 to 22.72 mmol·ℓ$^{-1}$) on the rate of BiPMo-complex formation was investigated by monitoring the changes of absorbance of a series of aqueous solutions of this complex (0.2 mg·ℓ$^{-1}$ P, 2.40 mmol·ℓ$^{-1}$ ammonium molybdate, 0.04 mmol·ℓ$^{-1}$ bismuth, 0.25 mol·ℓ$^{-1}$perchloric acid) with time. The value of the absorbance changes very slowly with time at an ascorbic acid concentration of 0.11 mmol·ℓ$^{-1}$, and only after 20 minutes is the maximum value of the absorbance of the BiPMo complex achieved (Fig. 3). If the concentration of ascorbic acid is higher then the time of achieving the maximum absorbance is shorter. At concentrations higher than 5.68 mmol·ℓ$^{-1}$, there is no significant effect of the concentration of ascorbic acid on the rate of attainment of the maximum absorbance. Therefore, this concentration was used in all further investigations.

## Selection of solvent

Since the BiPMo complex is a product of the reduction of a phosphomolybdate polyacid, such as phosphomolybdate and antimonyl phosphomolybdate, which are 100% extracted in IBMK, this solvent was employed in all our investigations of the extraction of the BiPMo complex.

## Absorption spectra

The absorption spectrum of an aqueous solution of BiPMo complex (containing 0.2 mg·ℓ$^{-1}$ P) is shown in Fig. 4, curve a. The wavelength of the absorption maximum is 720 nm. This complex has an absorption maximum in IBMK at a wavelength of 670 nm (curve b).

## Calibration graph and sensitivity

The working conditions described in the procedure (0.25 mol·ℓ$^{-1}$ perchloric acid, 2.40 mmol·ℓ$^{-1}$ ammonium molybdate, 0.04 mmol·ℓ$^{-1}$ bismuth and 5.68 mmol·ℓ$^{-1}$ ascorbic acid) provide a linear analytical range of the BiPMo complex formed from 0 to 0.6 mg·ℓ$^{-1}$ phosphorus in an aqueous solution and that formed from 0 to 1.2 mg·ℓ$^{-1}$ phosphorus in IBMK as well.

If the concentrations of the reagents are increased (bismuth from 0.04 to 0.08 mmol·ℓ$^{-1}$, ammonium molybdate from 2.40 to 4.80 mmol·ℓ$^{-1}$) during the determination of phosphorus using the proposed method, the range of the linear dependence of the absorption in aqueous solution is increased up to 1.2 mg·ℓ$^{-1}$ of phosphorus (Fig. 5).

The molar absorption coefficient was 1.66 x 10$^4$ ℓ·mol$^{-1}$·cm$^{-1}$ at 720 nm in water and 2.1 x 10$^4$ ℓ· mol$^{-1}$·cm$^{-1}$ at 670 nm in IBMK.

The limit of detection of the proposed method (blank + 3 S.D.) was 0.0059 mg·ℓ$^{-1}$ (aqueous solution) and 0.0050 mg·ℓ$^{-1}$ (IBMK).
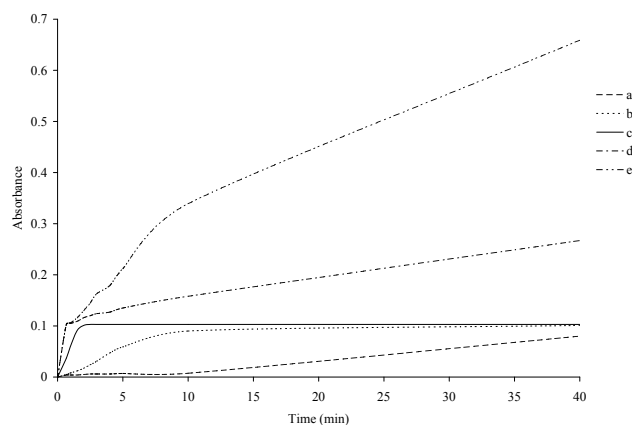


**Figure 2**
*Changes in the absorbance at 720 nm as a function of the reaction time for different concentrations of perchloric acid (**a** – 0.45; **b** – 0.35; **c** – 0.25; **d** – 0.075; **e** – 0.05 mmol·ℓ$^{-1}$). Experimental conditions: 0.2 mg·ℓ$^{-1}$ P, ammonium molybdate 2.40 mmol·ℓ$^{-1}$, bismuth 0.04 mmol·ℓ$^{-1}$and ascorbic acid 5.68 mmol·ℓ$^{-1}$.*
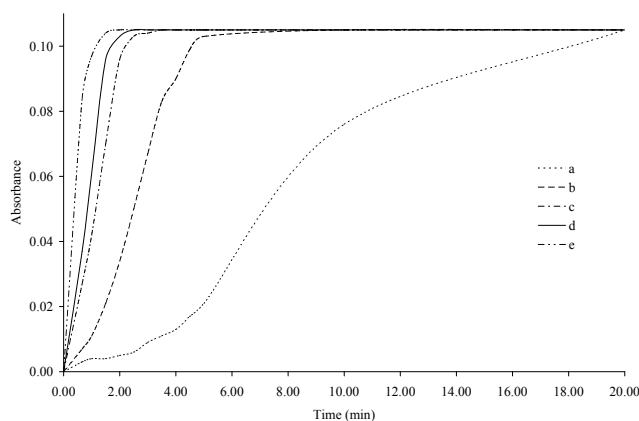


**Figure 3**
*Changes in the absorbance at 720 nm as a function of the reaction time for different concentrations of ascorbic acid (**a** – 0.11; **b** – 0.28; **c** – 2.27; **d** – 5.68; **e** – 22.72 mmol·ℓ). Experimental conditions: 0.2 mg·ℓ$^{-1}$ P, ammonium molybdate 2.40 mmol·ℓ$^{-1}$, bismuth 0.04 mmol·ℓ$^{-1}$and perchloric acid 0.25 mol·ℓ$^{-1}$.*



**Figure 4**
*Absorption curves for bismuth-phosphomolybdate complex:*
**a** – *aqueous solution,* **b** – *IBMK.*

**Figure 5**
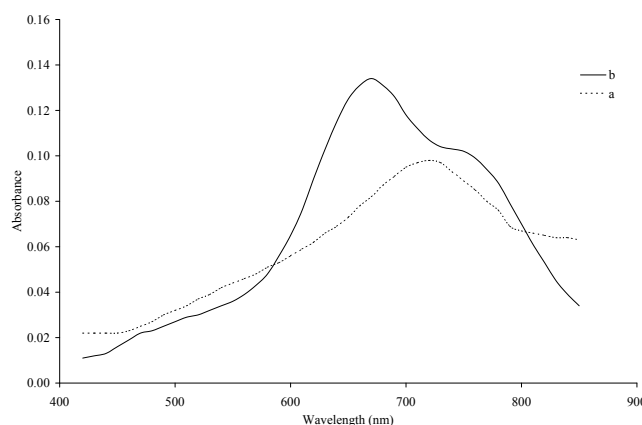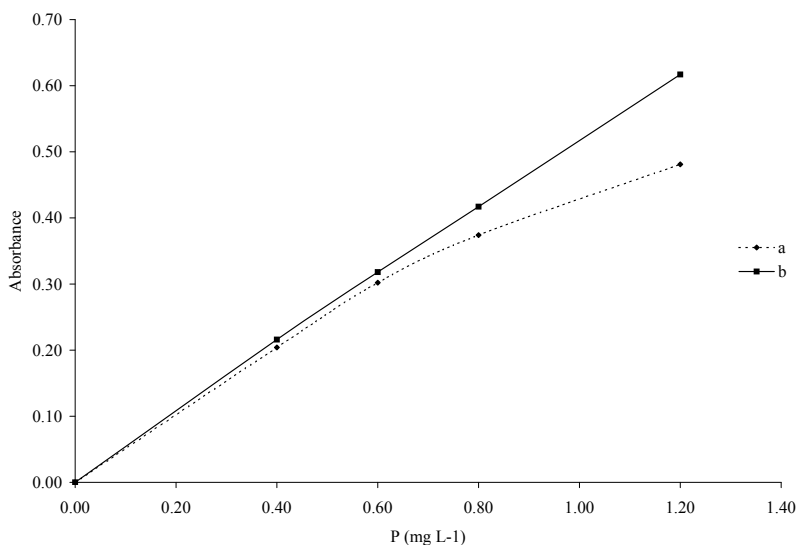*Absorbance at 720 nm as a function of the phosphorus concentration. Experimental conditions: **a.** Ammonium molybdate 2.40 mmol·ℓ⁻¹, bismuth 0.04 mmol·ℓ⁻¹, perchloric acid 0.25 mol·ℓ⁻¹ and ascorbic acid 5.68 mmol·ℓ⁻¹ and **b.** Ammonium molybdate 4.80 mmol·ℓ⁻¹, bismuth 0.08 mmol·ℓ⁻¹; perchloric acid 0.25 mol·ℓ⁻¹ and ascorbic acid 5.68 mmol·ℓ⁻¹.*

| TABLE 1 Tolerance limits (within $\pm$ 2% error) of diverse ions in the determination of 0.4 mg·ℓ⁻¹ phosphorus | |
| --- | --- |
| **Tolerance limit (mg·ℓ⁻¹)** | **Ion** |
| 8 000 | $NO_3^-$ |
| 3 000 | $Sr^{2+}, Ba^{2+}$ |
| 2 500 | $K^+, Mg^{2+}$ |
| 2 130 | $Cl^-$ |
| 1 370 | $Na^+$ |
| 1 040 | $Cr^{3+}$ |
| 1 000 | $Cs^+$ |
| 800 | $BO_3^{3-}, SO_4^{2-}, Ce^{4+}$ |
| 700 | $I^-$ |
| 500 | $Ca^{2+}, Si^{4+}$ |
| 400 | $Co^{2+}, Zn^{2+}$ |
| 300 | $Li^+, HCO_3^-$ |
| 200 | $Al^{3+}, Fe^{3+}$ |
| 100 | $Cu^{2+}, Hg^{2+}, Mn^{2+}, Cr^{6+}$ |
| 80 | $F^-$ |
| 50 | $Ni^{2+}, V^{3+}$ |
| 35 | $Pb^{2+}, Ag^+$ |
| 20 | $Se^{4+}, As^{3+}$ |
| 10 | $Sb^{3+}, Ti^{3+}$ |
| 8 | $NO_2^-$ |

## Effect of foreign ions

Since the goal of this study was to propose a novel spectrophotometric method for the determination of phosphorus in natural waters, it was decided to investigate the interference of only those ions whose presence in the analysed samples was to be expected, as well as of those which are known to interfere with molybdenum blue methods, such as As, Si, Ge, Ti, Th, Zr, V, Nb, Ce, Sb (react with molybdenum) (Going and Eisenreich 1974) and Cr(VI), Fe(III), $NO_2^-$, $WO_4^-$, $VO_2^-$ etc. (Nasu and Kan, 1988). The investigation of the effects of foreign ions on the spectrophotometric determination of phosphorus by the proposed method was performed by measuring the absorption (at 720 nm) of the solutions containing 0.4 mg·ℓ⁻¹ P, 0.25 mol·ℓ⁻¹ perchloric acid, 2.40 mmol·ℓ⁻¹ ammonium molybdate, 0.04 mmol·ℓ⁻¹ bismuth and 5.68 mmol·ℓ⁻¹ ascorbic acid, as well as different ion concentrations of the foreign ion.

The interference of the ions within the tolerance limits ($\pm$ 2%) is presented in Table 1.

Positive errors higher than 2% were caused by $V^{3+}$ (50 mg·ℓ⁻¹), $Se^{4+}$ and $As^{3+}$ (>20 mg·ℓ⁻¹) and $Sb^{3+}$ (10 mg·ℓ⁻¹). Negative errors higher than 2% were caused by $NO_2^-$, $Ni^{2+}$, $F^-$, $I^-$, $Hg^{2+}$, $Cr^{6+}$, $Fe^{3+}$, $Cl^-$ at concentrations higher than those listed in Table 1.

$K^+$ ions at a concentration of 2.5 g·ℓ⁻¹, $Rb^+$ ions at a concentration of 2 g·ℓ⁻¹ and $Cs^+$ ions at a concentration 1 g·ℓ⁻¹ did not interfere because hard solvent perchlorates of these elements are precipitated. When the concentration of $Ag^+$ ions is higher than 35 mg·ℓ⁻¹, silver is reduced by the ascorbic acid and so disturbs the determination.

The determination of phosphorus using the proposed method is performed in acid medium, therefore, $CO_2$ bubbles are formed in the presence of high concentrations of bicarbonates (higher than 300 mg·ℓ⁻¹) which disturb the determination. The interference of $HCO_3^-$ (in mineral waters) is eliminated by prior acidification and heating of the samples.

According to the results of the investigations, it can be concluded that no ions effect the spectrophotometric determination

| TABLE 2 Results on the determination of phosphorus in samples of natural water | | | |
| --- | --- | --- | --- |
| | **Concentration P (µg·ℓ⁻¹)[a]** | | |
| | **A method** | **B method** | **C method** |
| River water | 56.86 ± 1.63 | 57.46 ± 0.75 | 58.49 ± 1.09 |
| River water | 37.95 ± 0.84 | 38.06 ± 1.01 | 39.26 ± 1.32 |
| Mineral water | 35.56 ± 1.09 | 35.07 ± 2.92 | 36.18 ± 1.30 |
| Spring water | 195.72 ± 1.28 | 197.79 ± 2.21 | 194.63 ± 1.82 |

*[a] Results are based on six measurements*
*A. Bismuth-phosphomolybdate complex method (aqueous solution)*
*B. Bismuth-phosphomolybdate complex method (IBMK)*
*C. Standard method (antimonyl-phosphomolybdate complex)*

of phosphorus using the BiPMo complex at concentrations usually found in natural waters.

## Analysis of natural water samples

The proposed procedure was applied to natural water samples. The results obtained by the proposed and a standard spectrophotometric method are presented in Table 2. The standard procedure described in the literature, which is based on the formation of the antimonyl-phosphomolybdate complex, was taken as the reference (*Standard Methods,* 1998).

When the results obtained by the proposed method are compared with those obtained by the standard method (*Standard Methods,* 1998), it can be concluded that these two methods are in agreement.

The method of standard addition was also applied to the determination of phosphorus in natural waters. According to the obtained results (Table 3), it can be concluded that there is no interference of the elements present in the analysed samples dur-

**TABLE 3**
**Results of the determination of phosphorus in natural waters samples via the BiPMo complex (aqueous solution)**

| Simple | Added P $\mu g \cdot \ell^{-1}$ | Found P $\mu g \cdot \ell^{-1}$ | Found P% |
|--------|--------|--------|--------|
| 1 | 0 | 7.18 | |
|   | 119 | 125.45 | 99.42 |
|   | 238 | 248.89 | 101.52 |
| 2 | 0 | 26.32 | |
|   | 119 | 148.06 | 101.89 |
|   | 238 | 267.04 | 101.03 |
| 3 | 0 | 59.82 | |
|   | 119 | 179.43 | 100.34 |
|   | 238 | 302.52 | 101.58 |
| 4 | 0 | 9.57 | |
|   | 119 | 131.64 | 102.39 |
|   | 238 | 252.07 | 101.82 |
| 5 | 0 | 38.29 | |
|   | 119 | 159.49 | 101.40 |
|   | 238 | 280.79 | 101.63 |

ing the determination of phosphorus by the proposed method.

The concentration of orthophosphate changed with time of storage in polyethylene bottles for up to 48 h (Boyd and Tucker, 1980) at 25°C, samples should ideally be analysed within 1h of collection, although, storage for 2 to 8 h does not greatly effect the concentration of orthophosphate.

## Conclusions

A modified spectrophotometric method for the determination of phosphorus in natural waters is proposed. The sensitivity of the method is close to the sensitivity of the spectrophotometric method via the SbPMo complex, but the BiPMo complex is more stable. The absorbance of the solution does not change during more than 89 h. If the BiPMo complex is extracted with IBMK, the sensitivity of the suggested method is increased.

The proposed method allows the sensitive, quick and simple determination of trace amounts of phosphorus as orthophosphate in natural waters even in laboratories equipped with simple instruments.

On the basis of our recent investigation, this method shows potential for application to the determination of phosphorus in sediments, soil, plants and ores, as well as in biological materials (urine, blood serum). Studies aimed at investigating this hypothesis are in progress.

## Acknowledgements

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