An atomic absorption spectrometric method for the determination of phosphorus in foodstuffs using the bismuth phosphomolybdate complex[*]

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A new indirect AAS method using the bismuth phosphomolybdate complex for the determination of phosphorus in foodstuffs is suggested. The bismuth phosphomolybdate complex in acid medium was extacted with isobutyl methylketone and the phosphorus was determined through bismuth in an air/acetylene flame by utilising the 223.06 nm resonance line of bismuth. The interference caused by antimony and titanium can be neglected in the presence of excess of bismuth. The detection limit of the method is 0.008 μg/mL of phosphorus.

Keywords: Atomic absorption spectrometry, phosphorus determination, foodstuffs, bismuth phosphomolybdate complex.

INTRODUCTION

Phosphorus is an important constituent of many inorganic and organic materials. The determination of traces of phosphorus is important for industry, geochemistry, biochemistry and agrochemistry, as well as for other fields of science. Because of the great importance of phosphorus, numerous methods for its determination have been described in the literature. Most of these methods are based on the absorption of yellow phosphomolybdic and vanadomolybdic acid or their reduction products in aqueous and organic solvents.[1–6]

Atomic absorption spectrometry (AAS) is often preferred to spectrophotometric methods for the determination of numerous of elements because of its sensitivity and freedom from interference other ions present. However, the direct determination of phosphorus by the AAS method is very difficult because the resonance lines of phosphorus (177.5, 178.3 and 178.8 nm) are in far ultraviolet region where the absorption of these lines by atmospheric gases is very pronouced. For this reason, measurements in this region of the spectrum demand that the optical system of the spectrophotometer be under vacuum or in some inert gas (nitrogen, argon).

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Phosphorus can be determined directly by flame[7–14] and electrothermal AAS[15–21] by using the less sensitive nonresonant lines in the UV and visible region of the spectrum. In flame AAS the absorption or emission of P atoms,[7–9] PO molecules[7–10,14] or HPO molecules[11–13] are measured. In all direct methods for the determination of phosphorus the detection limits are very high (5–540 μg/mL) except for the electrothermal method (≥0.1 μg/mL). The contents of phosphorus in food[9] (at 178.3 and 178.6 nm), detergent,[22] biological materials,[21] steel,[23] fats and oil[19] have been determined by the application of these methods.

Indirect methods of for the determination of phosphorus are based on the formation of binary complex of phosphorus and molybdenum[24] and ternary complex of phosphorus and molybdenum with antimony and phosphorus and molybdenum with vanadium. After extraction with some organic solvent, the complex is aspirated into the flame or an aliquot of the solution is placed into a graphite cuvette and the content of phosphorus is determined through the signal for molybdenum, antimony or vanadium.

The content of phosphorus has been determined indirectly through molybdenum in biological materials,[26] milk and blood serum,[27] steel,[28] aluminium alloys,[29] synthetic mixtures[30] and rocks[24] in air-acetylene and nitrous oxide/acetylene flames. The interference of many ions, especially of arsene and silicon were noticed in the application of the air/acetylene flame. The ternary complex P, Sb an Mo was applied for the determination of phosphorus through antimony by using the 217.6 nm[31] resonant line in an air-acetylene flame, as well as for the determination of phosphorus in a phosphorosilicate glass film deposited on a silicon wafer by thermal AAS.[32] The ternary complex of phosphorus and molybdenum with vanadium was applied for the determination of phosphorus through V in steel and iron[33] in an N_2O-C_2H_2 flame.

High performance liquid chromatography has been used to determine orthophosphate, pyrophosphate and tripolyphosphate.[38] Quantitative analysis of orthophosphate, pyrophosphate and tripolyphsphate are performed using high performace liquid chromatography with inductively coupled argon plasma emission spectrometric detection.

Molybdenum and phosphorus also form ternary complex with Ti, Th, Zr, Nb and Bi in which the ratio of phosphorus and these elements is 1:1.[34]

As early as in 1948, Boltz and Mellon[35] noticed that the addition of bismuth to molybdophosphoric acid solution, with subsequent reduction, resulted in an increase in the blue hue. Campbell and Mellon used this catalytic effect for the determination of bismuth in an alloy for bearing metals,[36] and Goldman and Hargis[37] determined the structure of the formed complex (the ratio P, Bi and Mo is 1:1:18).

Proceeding from the fact that bismuth forms a stable complex with phosphorus and molybdenum in precise stoichiometric ration, the aim of this work was to develop a procedure for the indirect determination of phosphorus in foodstuffs utilizing bismuth by the AAS method with an air-acetylene flame.

EXPERIMENTAL

Apparatus

All the experiments were performed with a Perkin Elmer 3300 double-beam atomic absorption spectrometer.

The experimental parameters selected were: wavelength: 223.1 nm; slit width: 0.20 nm; flame: air/acetylene; oxidant flow-rate: 10 L/min; acetylene flow-rate: 2 L/min; lamp: electrodeless discharge (EDLs) watts 8.

Reagent

1. Stock standard solution of bismuth: Bismuth metal (1.0000 g) was dissolved in the minimum volume of 1:1 HNO_3. This solution was diluted to 1 L with 2%(v/v) HNO_3. The solution was further diluted to obtain a 0.001 M solution in 0.5 M perchloric acid; a stock solution corresponding to 209 μg/mL of bismuth was thus obtained.

2. Standard phosphorus solution: Potassium dihydrogen phosphate, KH_2PO_4, (0.2197 g) was dissolved in distilled water to 500 mL. 1 mL of this solution contained 100 μg of phosphorus. The working solution of phosphorus (2μg/mL) was prepare by dilution of the standard solution (100 μg/mL).

3. Ascorbic acid: Ascorbic acid (1 g) was dissolved in 100 mL of water. As ascorbic acid solutions are not stable for long periods of time at room temperature, they were freshly prepared immediately prior to use.

4. Ammonium molybdate solution (0.12 M): Amomonium heptamolibdate, $(NH_4)_6Mo_7O_{24}$ ·$4H_2O$, (2.2287 g) was dissolved in distilled water to 100 mL.

5. Perchloric acid (5M): Concentrated $HClO_4$ (271.8 mL) was diluted to 500 mL with distilled water.

Sample preparation

The foodstuff samples (0.2–0.5 g) were wet-ashed with 10 mL of concentrated nitric acid and 2 mL hydrogen peroxide solution. After the decomposition the solution was diluted to 50 mL.

Establishment of the calibration curve.

Various volumes (1, 2, 4, 8 and 15 mL) of the working solution were transferred to a 50 mL volumetric flask containing 2.5 mL 5 M $HClO_4$, 1 mL of the 0.12 M ammonium molybdate and 2 mL of 0.001 M bismuth solution (8.36 μg/mL). Then 5 mL of the 1% ascorbic acid solution was added and the volume made up to 50 mL. The solution was allowed to stand for 10–15 min.

A blue complex formed and the solution was transferred into a 150 mL separating funnel. It was then extracted with 25 mL of isobutyl methyl ketone by shaking for *ca.* 1 min. After aspiration of the blank solution, the extracted volume was aspirated into the flame atomic absorption spectrometer and the absorbance due to bismuth was read from the computer monitor for each solution (Table I).

The procedure of the determination of phosphorus

A certain volume of the sample (depending on the content of phosphorus) was measured into a 50 mL volumetric flask, and the same procedure as used for the establishment of the callibration curve was performed. The concentration of phosphorus in organic layer was read directly from the computer monitor and the content of phosporus in the sample was calculated from this value and the callibration curve (Table III).

RESULTS AND DISCUSSION

Electrothermal AAS, a very sensitive but expensive method, can be applied in the direct determination of phosphorus, as can be seen from the literature. The sensitivity of other direct methods is very low and some condition (vacuum, nitrogen, argon) are needed for some of them.

For the indirect determination of phosphorus through molybdenum and vanadium, warmer flames must be used, such as N_2O/C_2H_2, which is less applied in analytical practice because of its toxicity and explosive nature.

Therefore, in the inidirect determination of phosphorus by the AAS method, a metal should be chose which can be determined in an air-acetylene flame under the condition that the sensitivity of the determination is high and that the other ions present in the sample do not interfere. Bismuth fulfills all the above mentioned conditions. It form a very stable ternery complex with phosphorus and molybdenum which may be quantitively extrated with MIBK after reduction with ascorbic acid. The dependence between the absorbance and the concentration of phosphorus in the solution is linear in the range 0–1.2 µg/mL (Table I).

TABLE I. Relationship betwen the phosphorus concentration and the absorbance

Phosphorus concentration µg/mL	Absorbance	
	(Bi)	(Sb)
0.02	0.009	
0.04	0.017	
0.10	0.037	0.016
0.20	0.075	0.036
0.40	0.151	0.066
0.80	0.303	
1.00	0.374	
1.20	0.440	
1.50	0.482	

Effect of acidity

In order to determine the perchloric acid concentration resulting in the maximal absorption signal for bismuth, the absorbance change of a solution containing 0.4 µg/mL P in dependence on the acidity of the solution was monitored (Fig. 1). The results of this investigation show that the absorbance begins to decline when the concentration of acid is higher than 0.30 M. If the concentration of the
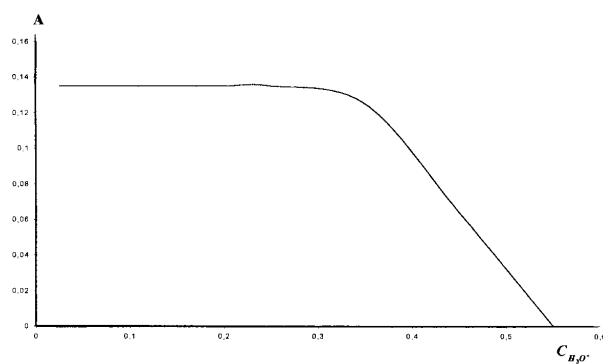

Fig. 1. Effects of $c_{H_3O^+}$ on the absorbance

acid is less than 0.1 mol/L molybdate is reduced into a blue product which dissolves in MIBK, thus disturbing determination.

Interference studies

   It is well known that not only P but also As, Ge, Ti, Th, Zr, V. Nb, Ce and Sb react with molbdenum. All these ion are expected to eject bismuth out of ternery complex with phosphorus and molybdenum and so interfere with the phosphorus determination. Beside, data in the literature indicate that even other ions, such as Cr(VI), nitrites *etc*, interfere with the formation of the phosphomolybdate complex. Since, in this paper, a new method for the determination of phosphorus in foodstuffs is being proposed, we decided to investigate the interference of only those ions whose presence in the analysed samples, is to be expected, as well as of those for which ambiguous interference date exist in the literature. In these investigation the concentrations of interferents and phosphorus were varied while the concentration of bismuth was kept constant (8.2 μg/mL).

TABLE II. Interference by diverse ions

Interferent ions	μg/mL	Taken P μg/mL	Found P %
Ca^{2+}	8	0.1	100.0
	8	0.5	99.7
K^+	8	0.1	98.5
	8	0.5	99.1
Al^{3+}	20	0.1	100.0
	20	0.5	99.4
Fe^{3+}	20	0.1	98.5
	20	0.5	99.7
Cr^{3+}	8	0.1	98.5
	8	0.5	99.7
Ti^{4+}	5	0.1	100.0
	10	0.1	95.6
Si^{4+}	60	0.1	100.0
	60	0.5	99.1
Sb^{3+}	10	0.1	0.00
	5	0.1	33.3
		0.5	30.4
	1	0.1	70.5
	1	0.5	70.8
	0.5	0.1	77.5
	0.5	0.5	83.5
	0.05*	0.1	98.8
	0.05	0.5	100.0
NO_2^-	8	0.1	0.00
	8	0.5	0.00

*Tolerance limit

According to the obtained result (Table II), it can be seen that there is no signal for bismuth when the concentration of Sb was 10 μg/mL and that of phosphorus 0.1 μg/mL, which means that antimony disturbs the determination of phosphorus through bismuth. If the concentration of bismuth is increased threefold, the signal for bismuth increases seven times. This means that an increase in the ratio of Bi an Sb reduces the interference of antimony. Since foodstuffs contains about 5×10^{-4} % of antimony, the interference of antimony can be neglected when the concentration of bismuth is 8.2 μg/mL (this amount is added in the application of this method).

At a concentration ratio of Ti and Bi of 10:8, the bismuth signal decreases by 5 % and when this ratio is decreased (5:8) no interference is caused by titanium. Since the contents of titanium in foodstuffs is very low these interferences may be neglected as well.

Nitrites disturb the determination of phosphorus, therefore they should be destroyed in the aqueous solution before extraction of the ternery complex with MIBK.

TABLE III. Determination of phosphorus in some foodstuffs

Sample	Proposed method (AAS) %	Molybdenum blue method ($PSb_2Mo_{10}O_{40}$) %
Coconut	0.121	0.123
Banana	0.071	0.071
Camomile	0.307	0.310
Bilberry	0.081	0.082
Celery	0.315	0.315
Wheat (1)*	0.122	0.124
Wheat (2)	0.152	0.151
Wheat (3)	0.191	0.187
Wheat (4)	0.158	0.158
Wheat (5)	0.136	0.136
Wheat (6)	0.163	0.162

*1 (sort "Europa"), 2 (sort "Kg 56 S"), 3 (sort "Balkan", 4 (sort "Lepa"), 5 (sort "Takovčanka"), 6 (sort "Krajinka").
Sensitivity = (Conc. of std × 0.0044)/Measured abs = (0.4 × 0.0044)/0.151 = 0.013 μg/mL
Detection limit = (Standard conc × 2 std. dev)/Mean = (0.4 × 2 × 0.0014)/0.138 = 0.008 μg/mL
Standard deviation (sample Wheat (1), 9 determinations) = 0.0014 %.

The interferences caused by chromium and other cations are with the tolerance limits (±2 %).

The results of the determination of phosphorus in some foodstuffs are shown in Table III. The results presented in Table III are the average values of three measurements. The three values were read one after another following the aspiration of the organic extract into the flame. The phosphorus contents were determined in the same solutions by a standard spectrophotometric method (The molybdenum blue method). The analytical results obtained by the proposed method were in good agreement with those obtained by spectrophotometry. The detection limit of the proposed method is 0.008 μg/mL.

CONCLUSIONS

A new indirect AAS method for the determination of phosphorus in foodstuffs is proposed. It offers the following advantages over other indirect methods:

1. An air/acetylene flame is used instead of N_2O/C_2H_2 one.

2. Extraction of the ternary complex by MIBK is quantitative.

3. The sensitivity is two times higher in comparison with the indirect AAS method through antimony.

4. The ternary complex (BiPMo) is stable for more than 3 h.

И З В О Д

АТОМСКА АПСОРПЦИОНА СПЕКТРОФОТОМЕТРИЈСКА МЕТОДА ЗА ОДРЕЂИВАЊЕ ФОСФОРА У ПРЕХРАМБЕНИМ ПРОИЗВОДИМА ПРИМЕНОМ БИЗМУТ-ФОСФОР-МОЛИБДАТНОГ КОМПЛЕКСА

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Предложена је нова индиректна атомска апсорпциона спектрофотометријска метода за одређивање фосфора у прехрамбеним производима применом бизмут-фос-фор-молбдатног комплекса. Бизмут-фосфор-молибдатни комплекс у киселој средини екстрахован је метилизобутил кетоном и фосфор одређиван преко бизмута у пламену ваздух-ацетилен коришћењем резонантне линије 223,06 nm за бизмут. Интерференције од антимона и титана могу се занемарити у присуству вишка бизмута. Границе детекције предложене методе износи 0,008 μg/mL.

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REFERENCES

1. F. D. Boltz, G. M. Mellon, *Anal Chem.* **20** (1948) 577
2. F. J. Kennedy, A. D. Weetman, *Anal. Chim. Acta* **55** (1971) 448
3. A. R. Chalmer, A. D. Thomson, *Anal. Chim. Acta* **18** (1958) 575
4. S. N. Ging, *Anal. Chem.* **28** (1956) 1330
5. Q. E. Laws, J. D. Webley, *Analyst* **84** (1959) 28
6. S. P. Chen, Y. T. Taribara, H. Warner, *Anal. Chem.* **23** (1951) 192
7. R. K. Skogarboe, A. S. Gravatt, G. H. Morrison, *Anal. Chem.* **39** (1967) 1602
8. D. C. Manning, S. Slavin, *At. Absorp. Newsl.* **8** (1969) 132
9. G. F. Kirkbright, M. Marshall, *Anal. Chem.* **45** (1973) 1610
10. A. Davis, F. J. Dinan, E. J. Labbett, J. D. Chazin, L. E. Tufts, *Anal. Chem.* **36** (1964) 1066
11. R. M. Dagnall, K. C. Thompson, T. S. West, *Analyst* **93** (1968) 72
12. K. M. Aldous, R. M. Dagnall, T. S. West, *Analyst (London)* **95** (1970) 417
13. A. Syty, *Anal. Lett.* **4** (1971) 531
14. H. Haraguchi, K. Fu Wa, *Anal. Chem.* **48** (1976) 784
15. V. B. L'vov, A. D. Khartsyzov, *Zh. Prikl. Spektrosk.* **11** (1969) 413
16. R. D. Ediger, A. R. Knott, G. E. Peterson, R. D. Beaty, *At. Absorp. Newsl.* **17** (1978) 28
17. R. D. Ediger, *At. Absorp. Newsl.* **15** (1976) 145

18. D. J. Driscoll, A. D. Clay, H. C. Rogers, R. H. Jungers, E. F. Butler, *Anal. Chem.* **50** (1978) 767
19. A. Prevot, M. Gente-Jauniaux, *At. Absorp. Newsl.* **17** (1978) 1
20. V. B. L'vov, A. L. Pelieva, *Zh. Anal. Khim.* **33** (1978) 1572
21. J. F. Langmyhr, M. J. Dahl, *Anal. Chim. Acta* **131** (1981) 303
22. C. G. Taralballa, J. G. Spielholtz, J. R. Steinberg, *Mikrochim. Acta* **4** (1972) 484
23. J. P. Whiteside, J. W. Price, *Analyst* **102** (1977) 618
24. C. Riddle, A. Turek, *Anal. Chim. Acta* **92** (1977) 49
25. S. W. Zangg, J. R. Knox, *Anal. Biochem.* **20** (1967) 282
26. S. W. Zangg, J. R. Knox, *Anal. Chem.* **38** (1966) 1759
27. G. Linden, S. Turk, T. B. de la Fuente, *Chim. Anal. (Paris)* **53** (1971) 244
28. T. Suzuki, H. Morinaga, A. Sasaki, *Tetsu To Hagane* **63** (1975) 1063
29. L. J. Bernal, J. M. del Nozal, L. Deban, J. A. Aller, *Talanta* **28** (1981) 469
30. R. T. Hurford, F. D. Boltz, *Anal. Chem.* **40** (1968) 379
31. K. P. Gupta, K. A. Sarkar, *Indian J. Chem.* **17A** (1979) 312
32. K. Tsujii, K. Kuga, E. Kitazume, *Anal. Chim. Acta* **115** (1980) 143
33. J. Janousek, *Chem. Anal. (Warsaw)* **24** (1979) 59
34. E. J. Going, J. S. Eisenreich, *Anal. Chim. Acta* **70** (1974) 95
35. F. D. Boltz, G. M. Mellon, *Anal. Chem.* **19** (1947) 837
36. R. H. Campbell, G. M. Mellon, *Anal. Chem.* **32** (1960) 54
37. D. H. Goldman, G. L. Hargis, *Anal. Chem.* **41** (1969) 491
38. A. Morita, *Anal. Chem.* **53** (1981) 1997.