The Spectrophotometric Determination of Trace Amounts of Arsenate and Arsenite in Natural Waters with Special Reference to Phosphate Determination

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(Received April 6, 1964)

The current methods for the determination of arsenic,[1-5] in which molybdoarsenate or its reduction product, "*molybdenum blue*", is determined spectrophotometrically, are only applicable to microgram quantities of the element and are inconvenient for usual natural waters, the arsenic content of which is from several tenths to several micrograms per liter. It is a time-consuming and laborious work to concentrate the necessary liters of a sample. Furthermore, when the available amount of the sample is limited or the sample is extremely

1) N. W. Rakestraw and F. B. Lutz, *Biol. Bull.*, **65** 397 (1933).
2) S. Gorgy, N. W. Rakestraw and D. L. Fox, *J. Mar. Research*, **7**, 22 (1948).
3) M. Ishibashi, T. Shigematsu, Y. Nakagawa and Y. Ishibashi, *Bull. Chem. Research, Kyoto Univ.*, **24**, 68 (1951).
4) K. Sugawara and S. Kanamori, This Bulletin, **29**, 670 (1956).
5) W. Dozanska and H. Czarnodolowa, *Roczniki Panstwowego Zakladu Hig.*, **10**, 217 (1959).

diluted, the methods are useless.

The present authors have previously reported a spectrophotometric method for determining submicrogram quantities of orthophosphate in natural waters.[6] The principle was to isolate molybdophosphate by solvent extraction, to liberate molybdate by decomposition from the extracted molybdophosphate in which the molybdenum is coordinated to phosphate in the ratio of 1 : 12, and finally to determine the liberated molybdenum spectrophotometrically.

In the present work the authors have extended this principle so that it is possible to separately determine 0.01～1.00 μg. of phosphate-P, 0.02～2.00 μg. of arsenate-As, and 0.02～2.00 μg. of arsenite-As from a sample smaller than 250 ml.

The method consists of the following five steps: 1) Phosphate and arsenate are extracted as molybdophosphate and molybdoarsenate successively with the proper solvents. 2) The arsenite in the remaining solution is oxidized into arsenate. Then the arsenate is extracted according to step 1. 3) The three fractions, phosphate, arsenate and arsenite, are washed. 4) The respective heteropolymolibdate are stripped and subjected to decomposition, with the liberation of free molybdate. 5) The liberated molybdate is spectrophotometrically determined as thiocyanate for the respective fractions.

Experimental

Apparatus. — The absorbance is measured by the use of a Beckman spectrophotometer, type DU, with 10 mm. cells of Pyrex glass.

All glass vessels must be free from any contamination of arsenic. Especially, it is recommended that the 500 ml. separatory funnel for the extraction of molybdoarsenate be washed with hot mineral acid for several days.

Reagents. — All reagents should be of the analyzed reagent grade, and water distilled with copper still must be used.

Ascorbic Acid: a 0.05% aqueous solution.

Hydrochloric Acid: concentrated. One milliliter of methanol is added per liter of the acid to reduce the trace of chlorine.

n-Butanol, Chloroform and Acetone: distilled.

Solvent A: *n*-butanol and chloroform are mixed in a 4 : 6 proportion by volume.

Solvent B: *n*-butanol, chloroform and acetone are mixed in a 3 : 7 : 3 proportion by volume.

Ammonium Molybdate: a 10% aqueous solution.

Washing Solution A: 60 ml. of *n*-butanol and 40 ml. of concentrated nitric acid are dissolved to 1 l. with distilled water.

Washing Solution B: 60 ml. of *n*-butanol, 140 ml. of acetone, and 10 ml. of concentrated nitric acid are dissolved to 1 l. with distilled water.

Potassium Iodate: a saturated aqueous solution.

Sodium Hydroxide: a 1 N aqueous solution.

Ferrous Ammonium Sulfate: 1 g. of the hexahydrate is dissolved in 100 ml. of 0.2 N sulfuric acid.

Potassium Thiocyanate: a 10% aqueous solution.

Stannous Chloride: 10 g. of the dihydrate is dissolved in 100 ml. of 1 N hydrochloric acid.

Phosphate Standard Solution. — A stock solution containing 1 mg. of phosphate-P per ml. is prepared by dissolving 0.439 g. of potassium dihydrogen phosphate to 100 ml. with distilled water. The addition of a few drops of chloroform keeps the solution from a mold growth. The working standard solutions are prepared by properly diluting the stock solution.

Arsenite Standard Solution. — A stock solution containing 1 mg. of arsenite-As per ml. is prepared as follows, 0.132 g. of arsenic trioxide is dissolved in 10 ml. of a 1 N sodium hydroxide solution, slightly acidified with diluted hydrochloric acid and made up to 100 ml. This stock solution is properly diluted for the preparation of working standard solutions.

Arsenate Standard Solution. — One milliliter of an arsenite stock standard solution is evaporated up with 20 ml. of concentrated nitric acid and 1 ml. of concentrated hydrochloric acid on a water bath, and then the residue is dissolved in hot water and properly diluted.

Procedure. — *The Separation of Phosphate, Arsenate and Arsenite.* — Into a 500 ml. separatory funnel, 250 ml. of a sample water, 0.3 ml. of an ascorbic acid solution, 4 ml. of hydrochloric acid, 15 ml. of *n*-butanol, and 10 ml. of solvent A are taken; the funnel is then shaken for 3 min. to saturate the aqueous phase with solvent A. The addition of the ascorbic acid protects the arsenite from any possible oxidation, leaving the coexisting phosphate and arsenate untouched. The organic layer is replaced by a new 5 ml. of solvent A. After 2.5 ml. of the ammonium molybdate solution is added, the funnel is immediately shaken for 3 min. The extract is transferred to a 100 ml. separatory funnel. The extraction is repeated with another 5 ml. of solvent A. For the determination of phosphate, these two extracts of the phosphate-fraction are combined in a 100 ml. separatory funnel.

The remaining aqueous phase is again subjected to extraction by two or more 5 ml.-portions of the solvent A to remove the last trace of phosphate. Then 7.5 ml. of the ammonium molybdate solution and 35 ml. of acetone are added to the funnel and the arsenate is extracted by shaking the mixture for 3 min. with two 5 ml.-portions of solvent B. For the determination of arsenate, these two extracts of the arsenate fraction are combined in a 100 ml. separatory funnel.

The remaining aqueous phase is extracted with two or more 5 ml.-portions of solvent B to ensure the complete separation of the arsenate. Then 5 drops of a saturated potassium iodate solution are added to oxidize the arsenite to arsenate. The arsenate thus formed is extracted according to the step for the separation of arsenate described above,

6) K. Sugawara and S. Kanamori, This Bulletin, **34**, 258 (1961).

and the extracts of the arsenite fraction are combined in a 100 ml. separatory funnel.

*The Determination of Phosphate**. — The phosphate fraction separated above is shaken for 3 min. with 50 ml. of washing solution A to wash out any uncombined molybdate and any contaminative heteropolymolybdate other than molybdophosphate. The remaining organic layer is then transferred to a 50 ml. separatory funnel. After the addition of 10 ml. of the 1 N sodium hydroxide solution the funnel is shaken for 1 min. to strip the molybdophosphate completely from the oily phase, and then the organic layer is drawn off. Next, 3 ml. of concentrated hydrochloric acid, 1 ml. of the ferrous ammonium sulfate solution, 3 ml. of the potassium thiocyanate solution and 3 ml. of the stannous chloride solution are successively added. The molybdenum thiocyanate with an amber color is extracted with two small portions of solvent A. The extract is made up to 5 ml. in order to subject it to spectrophotometry at 475 mμ. Here, to protect the ferrous iron from oxidation, the addition of a few small crystals of stannous chloride to the joint extract is recommended.

The Determination of Arsenate. — The arsenate fraction is shaken with two 50 ml.-portions of washing solution B for 3 min. Through the washing process, only molybdoarsenate remains in the organic phase; the other molybdenum compounds contaminating the molybdoarsenate are washed out.

The organic layer is transferred to a 50 ml. separatory funnel in order to subject it to the stripping of molybdoarsenate with an alkali solution and to the final step of spectrophotometry, as has been described in the determination of phosphate.

The Determination of Arsenite. — The extract of the arsenite fraction is processed as described in the determination of arsenate.

Discussion

The Separation of Phosphate, Arsenate and Arsenite.—*The Extraction of Molybdophosphate.* —Figures 1 and 2 show the relation of the percent extraction of phosphate, arsenate, germanate and silicate as respective heteropolymolybdates to the acidity in hydrochloric acid and to the concentration of ammonium molybdate in the sample solution before extraction. When the concentration of ammonium molybdate is fixed at 0.08%, the extraction of molybdophosphate is practically complete at the acidity of higher more than 0.19 N and when the acidity is fixed at 0.19 N, it is complete at the concentration of ammonium molybdate of higher more than 0.08%. Moreover the extraction of the other ions is less than 0.3% in the whole range examined and shows a tendency to increase with the acidity and the concentration of molybdate. Accordingly, the acidity of 0.19 N and the concentration of ammonium molybdate of 0.1% are chosen as the optimum conditions for the extraction of phosphate, under which conditions the other ions are extracted in percentages of less than 0.01%.

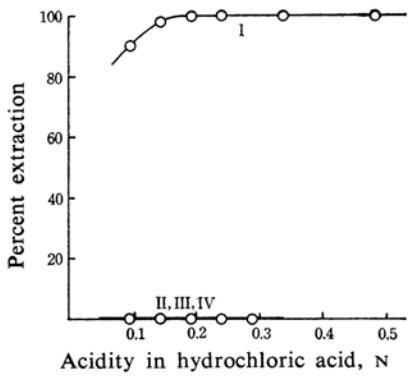

Fig. 1. Percent extraction of phosphate, arsenate, germanate and silicate and the acidity of aqueous phase before extraction.
I: Phosphate II: Arsenate
III: Germanate IV: Silicate
The concentration of ammonium molybdate: 0.08%

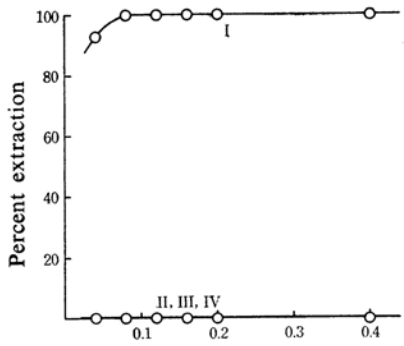

Fig. 2. Percent extraction of phosphate, arsenate, germanate and silicate and concentration of ammonium molybdate before extraction.
I: Phosphate II: Arsenate
III: Germanate IV: Silicate
The acidity in hydrochloric acid: 0.19 N

The Extraction of Molybdoarsenate.—Figures 3 and 4 show the relation of the percent extraction of arsenate, germanate and silicate as respective heteropolymolybdates to the acidity in hydrochloric acid and to the concentration of ammonium molybdate. When the concentration of ammonium molybdate is fixed

* When more than 2 μg. of phosphate-P is treated with, 2 ml. of the stannous chloride solution and 10 ml. of chloroform are added to the extract, which is then swirled to reduce the extracted molybdophosphate to the "molybdenum blue" completely. Then, 10 ml. of 1 N hydrochloric acid is added and the funnel is shaken for 1 min. to back-extract the "*molybdenum blue*" into the aqueous phase, which is subjected to spectrophotometry at 740 mμ.

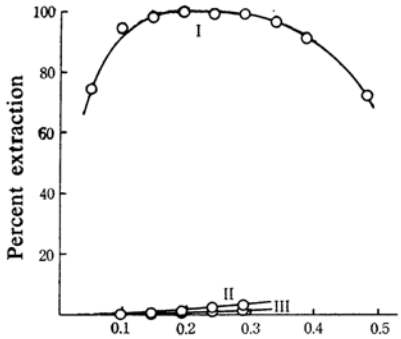

Fig. 3. Percent extraction of arsenate, germanate and silicate and acidity of aqueous phase before extraction.
I: Arsenate II: Germanate III: Silicate
The concentration of ammonium molybdate: 0.1%

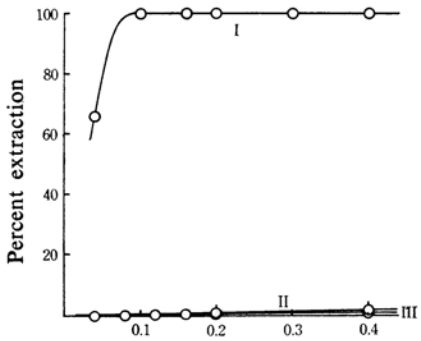

Fig. 4. Percent extraction of arsenate, germanate and silicate and concentration of ammonium molybdate in aqueous phase before extraction.
I: Arsenate II: Germanate III: Silicate
The acidity in hydrochloric acid: 0.19 N

at 0.1%, the extraction of arsenate is complete in the acidity range from 0.19 N to 0.24 N, and when the acidity is fixed at 0.19 N, it is complete at the concentration of ammonium molybdate of higher more than 0.1%. Moreover, the extraction of the other ions increases with the acidity and the concentration of molybdate up to 4 per cent. However, as will be shown later, a large amount of silicate interefers with the extraction of arsenate, because a considerable amount of molybdate is consumed by the silicate. Therefore, the acidity of 0.19 N and the concentration of ammonium molybdate of 0.4% are chosen as an appropriate conditions under which germanate and silicate are extracted in percentages as small as 1.5% and 0.5% respectively.

The Washing of the Extracts.—*The Washing of the Phosphate Fraction.*—Figure 5 shows the

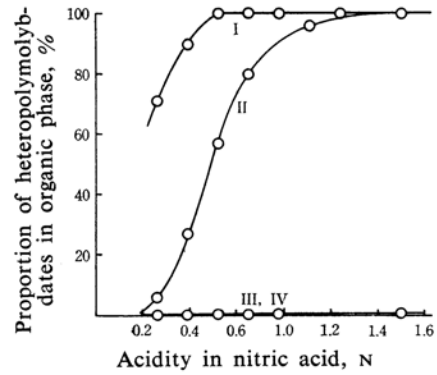

Fig. 5. Distribution of heteropolymolybdates of phosphate, arsenate, germanate and silicate and acidity of aqueous phase.
I: Molybdophosphate
II: Molybdoarsenate
III: Molybdogermanate
IV: Molybdosilicate

distribution of four heteropolymolybdates between 10 ml. of solvent A and 50 ml. of the 6% *n*-butanol aqueous solution at various acidities in nitric acid. The molybdophosphate remains completely in the organic phase at an acidity of higher more than 0.52 N, while almost all of the molybdogermanate and molybdosilicate is in the aqueous phase, and the proportion of molybdoarsenate in the organic phase increases with the increase in acidity. When the phosphate fraction of the extracts is washed with washing solution A (0.52 N in acidity), 57% of molybdoarsenate and less than 0.5% of the other heteropolymolybdates remain in the organic phase.

The Washing of the Arsenate Fraction. — The distribution of molybdoarsenate, molybdogermanate and molybdosilicate between 10 ml.

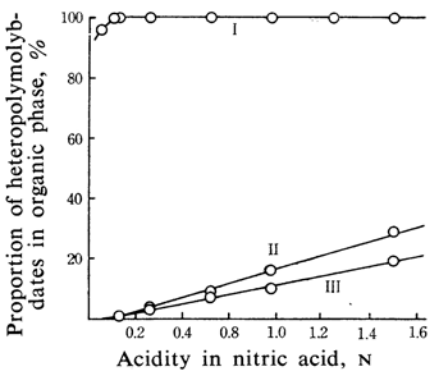

Fig. 6. Distribution of heteropolymolybdates of arsenate, germanate and silicate and acidity of aqueous phase.
I: Molybdoarsenate
II: Molybdogermanate
III: Molybdosilicate

TABLE I. THE DETERMINATION OF PHOSPHATE, ARSENATE AND ARSENITE IN THE PRESENCE OF GERMANATE AND SILICATE*

Added					Found		
Phosphate -P μg.	Arsenate -As μg.	Arsenite -As μg.	Germanate -Ge μg.	Silicate -Si μg.	Phosphate -P μg.	Arsenate -As μg.	Arsenite -As μg.
0.50	10	10	0	0	0.51		
0.50	0	0	100	0	0.50		
0.50	0	0	0	1000	0.51		
100	0.50	0.50	0	0		0.50	0.49
0	0.50	0.50	100	0		0.51	0.49
0	0.50	0.50	0	200		0.50	0.50
0	0.50	0.50	0	1000		0.06	0.05
0	0.50	0.50	0	1000		0.51**	0.50**

* The experiment was carried out with 50 ml. of the aqueous phase.
** The extraction of molybdoarsenate was carried out at the ammonium molybdate concentration of 1.2%.

TABLE II. APPLICATION OF THE PRESENT METHOD TO NATURAL WATERS

Sample	Sample taken ml.	Added			Found		
		Phosphate -P μg.	Arsenate -As μg.	Arsenite -As μg.	Phosphate -P μg.	Arsenate -As μg.	Arsenite -As μg.
Ground water from a well on the campus of Nagoya University	250	0	0	0	11.3	0.08	0.03
	250	3.0	0.05	0.05	14.2	0.14	0.07
Sea water from the north-western Pacific Ocean (33°12'N; 139°53'E)	250	0	0	0	13.6	0.25	0.13
	250	2.0	0.50	0.50	15.8	0.76	0.63

of solvent B and 50 ml. of a washing solution containing 6% *n*-butanol and 14% acetone at various acidities in nitric acid is shown in Fig. 6. The molybdoarsenate remains completely in the organic phase at an acidity of higher more than 0.10 N, and a small proportion of the other ions remains in the organic phase, although this proportion increases with the acidity. At the acidity of 0.13 N in washing solution B, less than 1% of molybdogermanate and molybdosilicate remain in the organic phase. Usual natural waters contain several milligrams of dissolved silica per liter, and, therefore, duplicated washing is needed to get exact results for arsenate determination.

Standard Curve. — The standard curves for phosphate-P and arsenate-As are shown in Fig. 7.

The molecular extinction coefficients were measured as 15.5×10^3 for molybdenum thiocyanate and as 185×10^3 and 184×10^3 for phosphate- and arsenate-equivalents respectively. Therefore, the P/Mo and As/Mo ratios in the extracted chemical species are 12.0 and 11.9 respectively; this shows that both extracted molybdophosphate and molybdoarsenate are 12-acid.

As is shown in Fig. 7, the sensitivity of the present method is high enough to be applicable to natural waters with no need to concentrate samples previously.

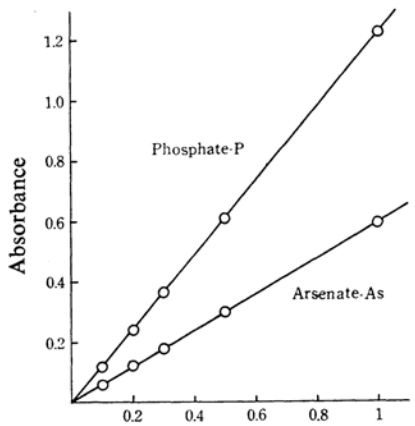

Fig. 7. Standard curves.

Interference

The detailed discussion of the successive isolation of phosphate, arsenate and arsenite

in the previous section suggests an applicability of the present method to the analysis of trace amounts of these ions in the presence of considerable amounts of germanate and silicate. This is also proved to be satisfactory by the results listed in Table I. Thus, 200 μg./l. each of arsenate-As and arsenite-As, 2 mg./l. of germanate-Ge, and 20 mg./l. of silicate-Si do not interfere with the determination of phosphate. One hundred micrograms of phosphate-P, 2 mg./l. of germanate-Ge, and 4 mg./l. of silicate-Si do not interfere with the determination of arsenate or arsenite, but 20 mg./l. of silicate-Si shows a marked negative interference; this is avoided by the extraction of molybdoarsenate at an increased concentration of ammonium molybdate (1.2%).

Large amounts of titanium(IV), vanadium(V) and tungsten(VI) interfere, but they are tolerated up to the concentrations of 20 μg./l., 20 μg./l. and 200 μg./l. respectively.

Sodium, potassium, calcium, magnesium, chloride, sulfate and nitrate cause no interference up to the concentrations found in sea water.

When a sample contains some oxidizing agent, there is danger that a part of the arsenite will be converted to arsenate during the process of analysis. The addition of a small amount of ascorbic acid will protect the arsenite from this oxidation.

Examples of the application of the present method to natural waters are listed in Table II.

The authors wish to thank Dr. Motoharu Tanaka for his many helpful discussions and suggestions during this work.

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