Spectrophotometric Determination of Submicrogram Quantities of Orthophosphate in Natural Waters

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(Received August 13, 1960)

In advanced researches of natural waters, need is felt for a method of determining phosphate more sensitive than the current Dénigè's colorimetric method¹⁾ especially when the quantity of the sample is limited. Such a need is partly filled with the radioactivation method$^{2,3)}$. However, a nuclear reactor is not easily accessible. Furthermore, irradiation and cooling are time-consuming. This situation initiated the present work.

It is true that there have been made various trials to increase the sensitivity of the determination of phosphate by the use of solvent extraction of phosphomolybdate$^{4-7)}$ where the yellow complex is extracted with an organic solvent to be directly or after reduction to molybdenum blue subjected to spectrophotometry. These methods, however, are not sufficiently sensitive to determine submicrogram quantities of the compound. The authors' idea was to step up this increased sensitivity. Thus the phosphomolybdate which was once extracted in an *n*-butanol-chloroform mixture is decomposed into the components. Then, the

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3) J. A. James and D. H. Richards, *Nature*, **176**, 1062 (1955); **177**, 1230 (1956).
4) T. Kiba and M. Ura, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **76**, 520 (1955).
5) Coe Wadeline with M. G. Mellon, *Anal. Chem.*, **25**, 1668 (1953).
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liberated molybdenum is separated to be spectrophotometrically determined as thiocyanate complex whereby 0.02$\sim$1.00 μg. of phosphorus as phosphate in a water sample less than 250 ml. can be determined with an accuracy of an error less than 5%.

Experimental

Apparatus.—Absorbance was measured with a Beckman spectrophotometer model DU by using 10 mm. cells of Pyrex glass.

Reagents.—Hydrochloric acid, concentrated.

n-Butanol and chloroform, distilled.

n-Butanol-chloroform mixture: *n*-Butanol and chloroform are mixed in the proportion of 3 : 7 by volume.

Ammonium molybdate solution, 10%.

Washing solution. Thirty mlliliter of *n*-butanol is dissolved in 500 ml. of 0.5 N nitric acid.

Sodium hydroxide solution, 1N.

Ferrous ammonium sulfate solution: In 100 ml. of 0.2 N sulfuric acid 1 g. of the hexahydrate is dissolved.

Potassium thiocyanate solution, 10%.

Stannous chloride solution: In 100 ml. of 1 N hydrochloric acid 10 g. of the dihydrate is dissolved.

Phosphate standard solution: The stock solution which contains 1 mg. of phosphorus as phosphate per ml. is prepared by dissolving 0.439 g. of dihydrogen potassium phosphate in distilled water and making it up to 100 ml. The addition of a few drops of chloroform keeps the solution from mold growth. The working standard solutions are prepared by properly diluting the stock solution.

Procedure. — A sample is properly diluted or evaporated so that 250 ml. contains 0.02$\sim$1.0 μg. of phosphorus. After having been filtered, if necessary, this 250 ml. is placed in a 500 ml. separatory funnel. Then, 10 ml. of hydrochloric acid, 15 ml. of *n*-butanol and 10 ml. of the *n*-butanol-chloroform mixture are added. The funnel is shaken for 1 min. to saturate the aqueous phase with the mixed solvent. When two layers clearly separate, the organic layer is drawn out to be replaced by a new addition of 5 ml. of the ammonium molybdate solution and 5 ml. of the mixed solvent. Two minutes shaking follows whereby the phosphomolybdate is formed and extracted into the solvent. The extract is transferred into a 100 ml. funnel. The extraction is repeated by using another 5 ml. of the mixed solvent. The two extracts are combined and shaken for 1 min. with 50 ml. of the washing solution, whereby the uncombined molybdate is completely washed out. The remaining organic layer which contains exclusively phosphomolybdate is transferred into a 50 ml. separatory funnel. After the addition of 10 ml. of 1 N sodium hydroxide solution and 5 ml. of distilled water, the funnel is shaken for 1 min. to strip the phosphomolybdate completely from the oily phase by the aqueous alkali solution. After 15 min. standstill during which the decomposition of the phosphomolybdate into molybdate and phosphate is complete, the organic layer is drawn off. With swirling, 3 ml. of concentrated hydrochloric acid, 1 ml. of ferrous ammonium sulfate solution, 3 ml. of thiocyanate solution and 3 ml. of stannous chloride solution are added successively. An amber color develops immediately. This is extracted with two small portions of the mixed solvent and made up to 5 ml. To the joint extract a few small crystals of stannous chloride are recommended to be added to protect the ferrous iron from oxidation. Otherwise ferric thiocyanate is formed to cause a high blank value because of its red coloration. The quantity of phosphate is determined from the absorbance reading at 475 mμ in reference to the standard curve. The curve has previously been constructed by following the same procedure with the standard solutions at different dilutions.

Discussion

Extraction of Phosphomolybdate. — The extraction must proceed on a condition where following two requirements are satisfied: 1) The extraction must be complete. 2) The possible contaminations of arseno-, germano- and silicomolybdate must be avoided. To this

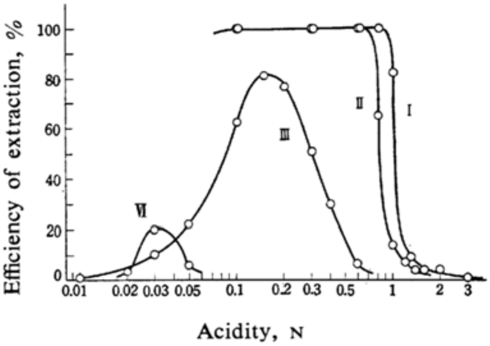

Fig. 1. Effect of the acidity on the extraction of heteromolybdates with *n*-butanol.

I Phosphomolybdate
II Arsenomolybdate
III Germanomolybdate
IV Silicomolybdate

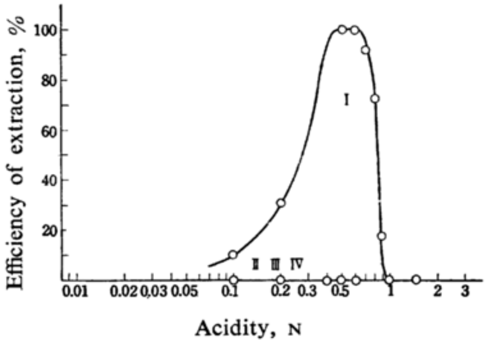

Fig. 2. Effect of the acidity on the extraction of heteromolybdates with the *n*-butanol-chloroform mixture.

I Phosphomolybdate
II Arsenomolybdate
III Germanomolybdate
IV Silicomolybdate

aim an appropriate solvent and the range of acidity where the solvent is most efficiently active were sought. Two sets of 50 ml. of arsenate, germanate and silicate solutions, each containing 10 μg. of one of the three elements, As, Ge and Si, were prepared and adjusted to various acidities with hydrochloric acid. Then, after the addition of 1 ml. of 10% ammonium molybdate solution, one of the sets was extracted with *n*-butanol, while another set with the *n*-butanol-chloroform mixture. The extracts were processed with 1.0% stannous chloride solution (1 N in hydrochloric acid) to reduce and strip the extracted heteropolyacids which were subjected to spectrophotometry to determine the efficiency of these extractions. As is seen from Figs. 1 and 2, the results point to the fact that the extraction is practically complete in a wider range of acidity from 0.1 to 0.8 N with *n*-butanol as compared with the range from 0.5 to 0.6 N with the mixed solvent. However, in these ranges, separation of phosphate from arsenate, germanate and silicate is not complete with *n*-butanol, while it is nearly complete with the mixed solvent. Thus the *n*-butanol-chloroform mixture is only acceptable as a solvent.

Standard Curve*.—Standard curves for three different methods are compared in Fig. 3, where the high sensitivity of the authors' method is quite evident.

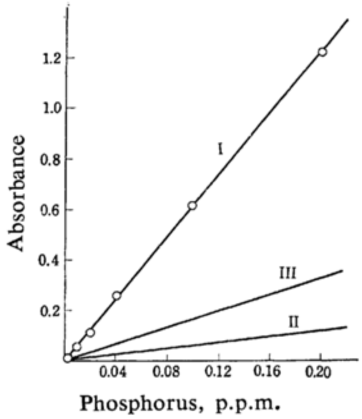

Fig. 3. Standard curves.

I The present method
II Molybdenum blue method (940 mμ)
III Simple phosphomolybdate extraction method (245 mμ)

Washing of the Extract.—This treatment is indispensable for removing the contamination of unbound moybdate which otherwise gives a higher apparent value at the final spectrophotometry. To check this point, samples containing 0.50 μg. of phosphorus and samples with

TABLE I. EFFECT OF THE WASHING ON THE BLANK VALUE AND RECOVERY OF PHOSPHORUS

Times of washing	Blank value absorbance	Recovery of 0.50 μg. of phosphorus μg.
1	0.005	0.49
1	0.001	0.51
2	0.000	0.50
2	0.001	0.50
3	0.000	0.50

TABLE II. THE INTERFERENCE OF VARIOUES IONS

Ion	Added as	Quantity mg.-element	PO_4^{3-} added μg.-P	PO_4^{3-} recovered μg.-P
AsO_4^{3-}	As_2O_5 in NaOH	0.1	0.50	0.52
GeO_3^{2-}	GeO_2 in NaOH	0.1	0.50	0.49
SiO_3^{2-}	SiO_2 in Na_2CO_3	5	0.50	0.50
Na^+	NaCl	800	0.50	0.52
K^+	KCl	500	0.50	0.50
Mg^{2+}	$MgSO_4$	400	0.50	0.50
Ca^{2+}	$Ca(NO_3)_2$	150	0.50	0.52
Fe^{3+}	$FeCl_3$	50	0.50	0.52
Al^{3+}	$Al_2(SO_4)_3$	25	0.50	0.51
Mn^{2+}	$MnSO_4$	10	0.50	0.50
Cl^-	NaCl	1200	0.50	0.52
SO_4^{2-}	$MgSO_4$	500	0.50	0.50
NO_3^-	$Ca(NO_3)_2$	100	0.50	0.52
Cu^{2+}	$CuSO_4$	1	0.50	0.49
Ag^{+}*	$AgNO_3$	1	0.50	0.48
Ba^{2+}	$BaCl_2$	1	0.50	0.50
Zn^{2+}	$ZnCl_2$	1	0.50	0.50
Cd^{2+}	$CdSO_4$	1	0.50	0.52
Hg^{2+}	$HgCl_2$	1	0.50	0.51
TiO^{2+}	TiO_2 in H_2SO_4	1	0.50	0.24
		0.1	0.50	0.47
		0.01	0.50	0.50
ZrO^{2+}	$ZrO(NO_3)_2$	1	0.50	0.52
Th^{4+}	$Th(NO_3)_4$	1	0.50	0.49
Pb^{2+}	$Pb(NO_3)_2$	1	0.50	0.50
VO_2^+	NH_4VO_3	1	0.50	0.20
		0.1	0.50	0.49
SbO^+	$K(SbO)C_4H_4O_6$	1	0.50	0.49
BiO^+	Nitrate	1	0.50	0.49
CrO_4^{2-}	K_2CrO_4	1	0.50	0.50
WO_4^{2-}	Na_2WO_4	1	0.50	0.37
		0.1	0.50	0.50
UO_2^{2+}	$UO_2(CH_3CO_2)_2$	1	0.50	0.50
SeO_3^{2-}	Na_2SeO_3	1	0.50	0.52
Co^{2+}	$CoSO_4$	1	0.50	0.50
Ni^{2+}	$NiSO_4$	1	0.50	0.51

* Sulfuric acid was used in place of hydrochloric acid for the extraction of phosphomolybdate.

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* The molar extinction coefficient of molybdenum thiocyanate under the present experimental condition was determined as 1.55×10^4 l. per mol. cm. and the corresponding value of phosphate 1.85×10^5 l. per mol. cm. Thus, the atom ratio of phosphorus to molybdenum is calculated to be 1/12 within an experimental error.

no phosphorus were processed according to the procedure in which they were subjected to one to three washings. The results listed in Table I show that the blank value is already nearly zero after one washing. This points to the fact that one washing is sufficient to completely remove free molybdate from the extract. On the other hand, the phosphate value remains constant even after three washings. This shows that there is no risk for the extracted phosphomolybdate to escape through the washing.

Interference. — The interference of various ions with the present determination of phosphate was examined with the results listed in Table II.

A) An error of 5% is found only with a concentration of 100 μg. As/50 ml. for arsenate, with 100 μg. Ge/50 ml. for germanate, and with 5 mg. Si/50 ml. for silicate. B) Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-} and NO_3^- cause no interference up to a considerable concentration. C) Tungstate and vanadate may be partly extracted in the mixed solvent to cause a considerable interference. However, the concentration of these elements in usual natural waters is too small to consider their interference. The same thing can be said in the case of titanium. In conclusion, the present method of phosphate determination can be safely applied to usual waters with no special consideration of the interference which might be caused by co-existing elements. Examples of the application of the method to several natural waters are listed in Table III.

TABLE III. APPLICATION OF THE METHOD TO NATURAL WATERS

Description	Sample taken ml.	P added μg.	P recovered μg.
Ground water from a well on the campus of Nagoya University	5.0		0.28
	5.0	0.10	0.39
Rain (March 20, 1956; Nagoya)	200		0.77
	200	0.05	0.83
Snow (Feb. 3, 1956; Nagoya)	100		0.48
	100	0.10	0.58

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