TRACE ANALYSIS OF PHOSPHATES IN SMALL AQUEOUS VOLUMES

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Abstract—The indirect determination of trace amounts of phosphate through estimation of the molybdenum in the phosphomolybdic acid complex has been improved. The lower limit of spectrophotometric determination is about $0.10 \mu\text{g P}$.

INTRODUCTION

The need to analyse phosphate in small volumes of interstitial water withdrawn from sediment cores provided the impetus for this investigation into a sensitive determination for small amounts of phosphate. The Murphy & Riley (1962) method using the blue coloured complex obtained by reducing a phosphomolybdic acid complex has been used extensively. A higher sensitivity can be achieved when the reduced complex is concentrated in an organic liquid. With sample volumes of 100 ml or more amounts of phosphorus on the order of $1 \mu\text{g l}^{-1}$ can be measured (Going *et al.*, 1975; Leyden *et al.*, 1975).

Since the Mo:P molar ratio in the phosphomolybdic acid complex is 12:1, an increased sensitivity can be obtained in principle by measuring the molybdenum in the phosphomolybdic acid complex (Umland & Wünsch, 1965). After formation, the complex is separated from the excess molybdic acid by extraction with an organic solvent and then decomposed by alkaline extraction. The molybdenum is then transferred to the aqueous phase and measured as the thiocyanate complex. Umland & Wünsch measured 1–10 μg of phosphorus using this procedure.

In order to extend the measurableness below $1 \mu\text{g}$ of phosphorus this method was starting point of the research reported herein. Special attention was given to the influence of the solvents used upon sensitivity and reproducibility. The Murphy & Riley method without extraction was used as a reference.

EXPERIMENTAL

Reagents

All chemicals used were of analytical grade.

Molybdate solution: 1.157 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 250 ml H_2O

Hydrochloric acid: concentrated, 3 N, 2 N and 0.4 N.

Sodium hydroxide: 0.1 N.

Solvents: diethyl ether/isobutanol (5:1), freshly distilled; isobutyl methylketone, freshly distilled; *N*-butylacetate, freshly distilled; acetone (A.R.), freshly distilled and stored in quartz bottle. Methanol, freshly distilled.

Thiocyanate solution: 50 g NH_4CNS in 100 ml H_2O or 5 g NH_4CNS in 100 ml H_2O or 5 g NH_4CNS in 100 ml freshly distilled acetone.

Coppersulfate solution: 0.04 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 1 ml 2 N H_2SO_4 in 100 ml H_2O .

Phosphate standards: prepared from KH_2PO_4 , dried at 110°C .

Apparatus

Spectrophotometer PMQ II, Zeiss. Separation Funnels: 100 ml separation funnels with Teflon stopcock, polyethylene stopper and a short delivery tube cut off at a 60° angle.

Glassware: steamed and rinsed with distilled water prior to use.

Procedure

The original Umland & Wünsch procedure consists of the following steps:

(1) In a separation funnel 5 ml 2 N HCl, 5 ml molybdate solution and at most 15 ml of the sample are mixed. If necessary, fill up with water to 25 ml.

(2) After 10 min, add 25 ml of the ether/isobutanol solvent and shake for 1 min.

(3) Remove the aqueous phase carefully by swinging the separation funnel after removal of the bulk of the water layer in order to drain away drops adhering to the wall and stopper.

(4) Wash the organic solution twice with 20 ml 0.4 N HCl for one minute and remove the aqueous phase carefully.

(5) Shake the ether solution for 1 min with 10 ml 0.1 N NaOH, separate the alkaline solution completely (see step 3) and repeat with 5 ml 0.1 N NaOH, shaking about 30 s.

(6) Mix the combined alkaline solutions with 1 ml of the copper-sulfate solution, 8.5 ml concentrated HCl, 7 ml methanol, 10 ml of the 50% thiocyanate solution and fill up with 3 N HCl to 50 ml. Mix thoroughly.

(7) Measure the absorbance after 45 min at 470 nm in a 1 cm cell, compare with standards after correction for the blank.

In this research the Umland & Wünsch procedure as such was only modified by application of a 4 cm cell instead of the 1 cm cell indicated in the original recipe. A modification described in the literature (Ehrenberger & Gorbach, 1973) is the use of a 5% aqueous thiocyanate solution in step 6 instead of the 50% solution. We compared both methods.

Further modifications were investigated by changing the organic solvent used in the extraction step 2. Ultimately the best results were obtained with a modification in which

Table 1. Calibration curve of Umland & Wünsch procedure with 50% thiocyanate solution (Sensitivity $A/\mu\text{gP} = 0.35$)

μgP added	Averaged Abs. of blank	Abs. after correction	Constant = $\mu\text{gP}/A$	Averaged constant
0.2		0.064	3.13	
0.4		0.138	2.90	
0.6	0.081	0.211	2.84	2.89
0.8	($n = 2$)	0.286	2.80	
1.0		0.358	2.79	

Table 2. Calibration curve of Umland & Wünsch procedure with 5% thiocyanate solution (Sensitivity $A/\mu\text{gP} = 0.23$)

μgP added	Averaged Abs. of blank	Abs. after correction	Constant = $\mu\text{gP}/A$	Averaged constant
0.2		0.054	3.70	
0.4	0.057	0.104	3.85	
0.6	($n = 4$)	0.122	4.92	4.26
0.8		0.196	4.08	
1.0		0.210	4.76	

Table 3. Calibration curve of present method (Sensitivity $A/\mu\text{gP} = 0.47$)

μgP added	Averaged Abs. of blank	Abs. after correction	Constant = $\mu\text{gP}/A$	Averaged constant
0.1		0.051	1.96	
0.2		0.094	2.13	
0.3		0.140	2.14	
0.4		0.190	2.11	
0.6	0.100	0.285	2.11	2.13
0.6	($n = 4$)	0.300	2.00	
0.7		0.320	2.19	
0.8		0.359	2.23	
0.9		0.411	2.19	
1.0		0.454	2.20	

the Umland & Wünsch method has been improved by replacing the methanol in step 6 by acetone and the thiocyanate solution by a 5% thiocyanate solution in acetone. This method is referred to as "present method".

EXPERIMENTAL RESULTS AND DISCUSSION

The sensitivities of the Umland & Wünsch method with 50% and 5% thiocyanate respectively and the present method were evaluated by comparing calibration curves: Tables 1-3.

It can be concluded from Tables 1-3 that the sensitivity of the present method is somewhat better than the original Umland & Wünsch procedure. The sensitivity is further decreased by lowering the thiocyanate concentration.

Based upon the calibration data presented in Tables 1-3 the reproducibility of the three methods was compared in Table 4. Table 4 shows that the present method has also the best reproducibility.

Before testing the present method we first investigated the effect of other organic solvents in step 2. Based on a suggestion by Simon *et al.* (1975) isobutyl-

methylketone and *N*-butylacetate were tested but the reproducibility did not improve whereas the sensitivity decreased considerably due to reduced efficiency of the extraction. The sensitivity based upon calibration curves as shown in Table 1 was reduced from 0.35 $A/\mu\text{gP}$ with ether-isobutanol to 0.12 $A/\mu\text{gP}$ for isobutylmethylketone and 0.05 $A/\mu\text{gP}$ for *N*-butylacetate.

The poor reproducibility of the Umland & Wünsch procedure turned out to be due to drift in the absorbance, resulting in an increase with time. Stabilisation of the colour can be achieved by acetone (Crouthamel & Johnson, 1954). Figure 1 shows observed changes in absorbance with different concentrations of acetone: the 0% acetone curve represents the original Umland & Wünsch method, in the 14% acetone curve the methanol in step 6 is replaced by acetone and in the 34% acetone also the thiocyanate is added as an acetone solution. The results indicate that the 34% acetone solution is stable after 45 min. After we decided upon the modifications in the original procedure we investigated the detection limit and the lower determination limit for the present method.

Fig. 1. Effect of acetone concentration on the stability of the colour of Mo-thiocyanate complex. Δ : 14% methanol, no acetone; $\circ$: 14% acetone; $\bullet$: 34% acetone in final solution.

Assuming a detection limit at three times the standard deviation of the blank and using the sensitivity $A/\mu\text{gP} = 2.13$ (Table 3), this limit was established to be $0.02 \mu\text{gP}$ (see Table 5).

The lower determination limit, which is the lowest amount which can be measured more or less quantitatively, is sometimes estimated as three times the detection limit. Hence the lower determination limit for the present procedure would be about $0.06 \mu\text{gP}$ or for 15 ml samples a concentration of 0.004 ppm. In order to verify this result 10 samples containing $4 \mu\text{gP}$ per litre were determined through the present method. The concentrations found were 2.7; 2.8 ($2 \times$); 3.4; 3.6 ($2 \times$); 3.8; 4.0; 5.7 and $7.5 \mu\text{gP l}^{-1}$ respectively with an average of 4.0 and a standard deviation of $1.5 \mu\text{gP l}^{-1}$.

The distribution however is rather skewed and one high value pushes up the average. Leaving out this value the average would be $3.6 \mu\text{gP}$ with a standard deviation of $0.8 \mu\text{gP}$. From this and similar results it can be concluded that the method becomes unreliable below a quantity of $0.1 \mu\text{gP}$ in the sample volume. We also tested the selectivity of this method with respect to interference by iron(III) and silica.

Table 6 summarizes the results. The small difference in sensitivity for the iron and silica samples was caused by differences in their respective calibration curves.

It can be concluded that a 100-fold greater concentration of Si and nearly 1000-fold of Fe by weight do not interfere. This is in accordance with the results of Umland & Wünsch. A comparison with the present method with the Murphy & Riley method without extraction in an organic solvent has been made for several samples, including rain water and interstitial water. Results obtained for interstitial water squeezed from an aerobic sediment core are summarized in Table 7.

It can be concluded from the results that both methods give equal results. The present method however is more laborious and should be applied only in situations where the sample volume is limited in such a way that less than about $1 \mu\text{gP}$ is available.

CONCLUSIONS

The sensitivity and reproducibility of the Umland & Wünsch indirect phosphate determination can be

Table 4. Reproducibility of three phosphate estimation procedures

Procedure	μgP added	μgP recovered	Averaged P-recovery	Standard deviation; % with respect to average
Umland & Wünsch 50% NH_4CNS	0.40	0.37; 0.40; 0.41; 0.45; 0.50.	0.43	11.6
Umland & Wünsch 5% NH_4CNS	0.30	0.19; 0.21; 0.26; 0.27 ($3 \times$) 0.29; 0.31; 0.32; 0.34.	0.27	18.5
Present method	0.30	0.28 ($4 \times$) 0.29 ($4 \times$) 0.30; 0.32	0.29	3.4

Table 5. Estimation of the detection limit of phosphate determination from standard deviation of blanks

Sample	Absorbance	$S_{\text{abs.}}$	Sensitivity $\mu\text{gP/A}$	S (μgP)	Detection (μgP) limit
blank ($n = 6$)	0.084 ($2 \times$) 0.085, 0.088 0.091 ($2 \times$)	0.0032	2.13	0.0068	0.02

Table 6. Interference of iron and silica in phosphate determination

μgP in sample	0.34	0.34	0.34	0.34	0.34
μgFe in sample	0	170	255	340	510
Abs. (corrected)	0.157	0.158	0.159	0.182	0.189
μgP recovered	0.34	0.34	0.35	0.40	0.41
μgP in sample	0.34	0.34	0.34	0.34	0.34
$\mu\text{g Si}$ in sample	0	17	25.5	34	51
Abs. (corrected)	0.163	0.168	0.161	0.168	0.205
μgP recovered	0.34	0.35	0.34	0.35	0.43

Table 7. Determination of phosphate in interstitial water with Murphy & Riley method and present method

Procedure	Sample volume (ml)	Abs. (corr.)	Sensitivity $\text{A}/\mu\text{gP}$	Phosphate conc. $\mu\text{gP/l}$
Murphy & Riley	40	0.162	0.0465	87
	40	0.167		90
	40	0.167		90
	40	0.164		88
	40	0.165		89
Present method	5	0.205	0.47	89
	5	0.204		89
	5	0.205		89
	5	0.201		87
	5	0.204		89

improved by measuring the molybdenum–thiocyanate complex in the final step in a 34% acetone solution.

This method can be applied for samples up to 15 ml containing 0.1–1.0 μgP . Above this range e.g. if more sample is available the less laborious Murphy & Riley method should be preferred.

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