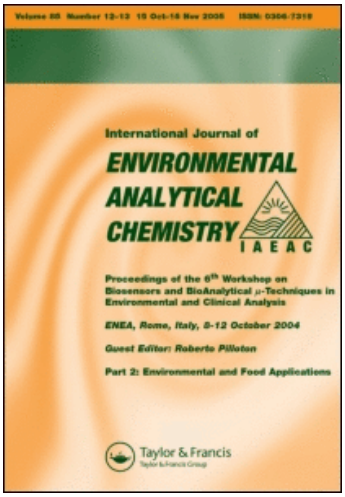

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Semi-Micro Determination of Total Phosphorus in Fresh Waters with Perchloric Acid Digestion

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SEMI-MICRO DETERMINATION OF TOTAL PHOSPHORUS IN FRESH WATERS WITH PERCHLORIC ACID DIGESTION

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A simple test-tube semi-micro method for the determination of total phosphorus in water is described. Samples are evaporated with perchloric acid at 115 °C overnight, digested at 170 °C for 40 minutes, diluted, hydrolyzed at 100 °C for 1 hour in order to decompose pyrophosphate and the liberated orthophosphate is determined without further neutralization and transfer steps by the phosphomolybdate blue method of Murphy and Riley (1962). The method can be applied directly to samples with phosphorus concentrations ranging from 4 to 1000 $\mu g.l^{-1}$. Comparability with the classic perchloric acid and sulphuric acid—persulphate digestion methods has been demonstrated using a wide range of fresh waters.

KEY WORDS: Total phosphorus, photometric semi-micro determination, perchloric acid digestion, fresh water.

INTRODUCTION

Total phosphorus (TP) in water is usually determined after converting all organic and inorganic forms of phosphorus to orthophosphate by an oxidative procedure. Water samples are digested using various techniques, e.g. by heating with perchloric acid[1, 2, 3, 4], with a combination of sulphuric and nitric acid, perchloric and nitric acid[5], sulphuric acid and hydrogen peroxide[6] or sulphuric acid and peroxydisulphate[5, 7], or by employing ultraviolet radiation[8]. The liberated orthophosphate is usually determined photometrically by reaction with molybdate to form a complex which is reduced using either stannous chloride or ascorbic acid[5] consequently increasing the stability and intensity of the colour. Because of the many disadvantages of stannous chloride[9, 10], most contemporary methods follow the procedure with ascorbic acid and potassium antimonyl tartarate described by Murphy and Riley[11]. In their procedure, a major factor in the reproducible formation of the blue colour of reduced molybdoantimonylphosphate complex is the ratio of the molar concentrations of H^+ and Mo(VI) ($[H^+]/[Mo(VI)]$) in the reaction mixture[12, 13]. This should be kept at 70 ± 10 for molybdate concentration in the range of 8×10^{-4} to 1×10^{-2} $mol.l^{-1}$[12]. Based on these results Eisenreich et al.[14] developed a simple procedure for TP determination using a sulphuric

acid—peroxydisulphate digestion which eliminates the neutralization step before colour development by ensuring a constant composition for the reaction mixture. The method, however, requires an autoclave.

This paper describes a simple procedure for the determination of TP on a semi-micro scale using perchloric acid digestion at 170 °C. The composition of the reagents was chosen so as to provide the optimum conditions for the formation of the molybdoantimonylphosphate complex[12, 14]. The procedure avoids neutralization and transfer steps. Test tubes with ground stoppers that are easy to keep clean were used as reaction vessels and a thermostated heating block allows an effectively large series of samples to be processed. Various disturbing effects that may occur during the digestion and colour development of natural samples have been evaluated and the method was compared with satisfactory results with two common procedures for TP determination.

EXPERIMENTAL

Materials and chemicals

Absorbance was measured with a Spekol 11 spectral colorimeter (Carl Zeis, Jena, Germany) at 710 nm. Redistilled water was used in the compensating cell.

An aluminium digestion block with 30 openings (19 × 100 mm), electronic temperature regulation and adjustable temperatures of (100, 115, 170) ± 0,5 °C was used to heat test-tubes fitted with ground glass stoppers (18 × 180 mm, product of Technosklo s.p., Držkov, Czechoslovakia).

Reagents were prepared from chemicals of analytical-reagent grade, supplied by Lachema, Brno, Czechoslovakia. Glycerol 2-phosphate, disodium salt, $(HOCH_2)_2CHOPO_3Na_2.5H_2O$, and triethyl phosphonoacetate, $(C_2H_5O)_2P(O)CH_2CO_2C_2H_5$, were obtained from Fluka AG. Lignin sulphonic acid "Marasperse N" was supplied by Marathon, Neenah, USA. Humic acid was isolated from peatbog water according to the procedure of Thurman and Malcolm[15]. Bovine serum albumin, hexametaphosphate $(NaPO_3)_x$, pyrophosphate $Na_4P_2O_7.10H_2O$, and polyphosphates $Na_5P_3O_{10}.6H_2O$ and $Na_4P_4O_{12}.4H_2O$ were obtained from Lachema.

All solutions were prepared from water obtained by redistilling distilled water with the addition of $KMnO_4$ and H_2SO_4.

Glassware was cleaned by soaking in hot (ca 70 °C) 15% m/v Na_2CO_3 solution for 30 minutes and rinsing with tap water, 10% m/m H_2SO_4 and redistilled water before use.

Reagents

Sulphuric acid (2.6 mol.l^{-1}) Mix 140 ml of concentrated H_2SO_4 (d_{20} = 1.84) with 800 ml of water, allow to cool and dilute to 1 l (reagent 1).

Ammonium molybdate (*50 g.l*$^{-1}$) Dissolve 25.0 g of $(NH_4)_6Mo_7O_{24}.4H_2O$ in water and dilute to 500 ml (reagent 2).

Ascorbic acid (*50 g.l*$^{-1}$) Dissolve 5 g of ascorbic acid in water and dilute to 100 ml. The solution may be stored at 5 °C for up to 2 weeks (reagent 3).

Potassium antimonyl tartrate (*3 g.l*$^{-1}$) Dissolve 1.5 g of $K(SbO)C_4H_4O_6.1/2H_2O$ in water and dilute to 500 ml (reagent 4).

Perchloric acid (*3.3 mol.l*$^{-1}$) Dilute 280 ml of concentrated $HClO_4$ (69–72% m/m) to 1 l with water (reagent 5).

Iso-butanol.

Mixed reagent I Mix 25 ml of reagent 1, 10 ml of reagent 2, 10 ml of reagent 3, and 5 ml of reagent 4 in 100-ml separatory funnel. Add 10 ml of iso-butanol and shake for 1 minute. After standing for 30 minutes separate the layers and discard the organic phase. Prepare daily.

Mixed reagent II Mix 25 ml of reagent 1, 10 ml of reagent 3, and 15 ml of water. This reagent may be stored at 5 °C for up to 2 weeks.

Procedure

Add 0.5 ml of perchloric acid (3.3 mol.l^{-1}) to 10 ml of the water sample in a test-tube. Stopper the test-tube and mix thoroughly. Place the open tube in the digestion block and allow the sample to evaporate at 115 °C overnight (12-20 hours). When only a small drop (less than 0.2 ml) of concentrated perchloric acid remains in the test-tube, increase the temperature to 170 °C and heat for 40 minutes. If the digest is still yellow or brown continue heating until it is colourless, however, if the coloration does not disappear within approximately three hours, repeat the digestion with diluted sample. Remove the test-tube from the block, allow it to cool and add 10 ml of redistilled water. Stopper the test-tube tightly, mix thoroughly, and heat at 100 °C for 60 minutes to decompose pyrophosphate. After cooling to room temperature, add 1 ml of mixed reagent I and allow to stand for 10 minutes. Measure the absorbance at 710 (or 882) nm in 1 or 5-cm cell against water. Standardize the method by means of a known concentration of phosphate and at least two blanks carried through the whole procedure with each series of samples.

Unfiltered samples containing suspended inorganic particles should be corrected for the turbidity error: Follow the procedure described above with another portion of sample but use the mixed reagent II instead of mixed reagent I. The TP value is then calculated using the corrected absorbance:

$$A = A_I - A_{II} - (A_{IO} - A_{IIO})$$

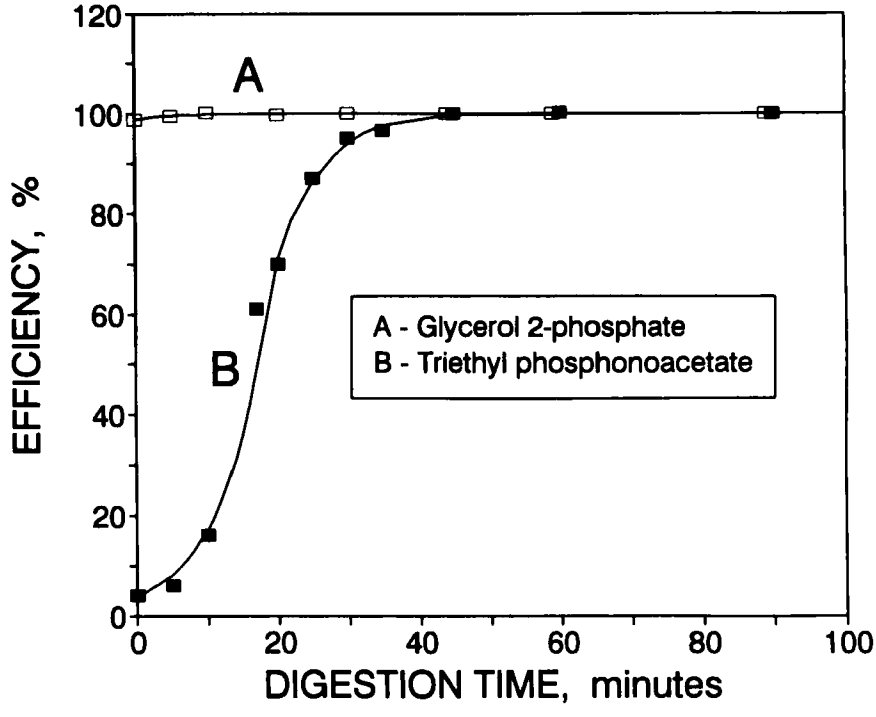

Figure 1 Kinetics of mineralization of glycerol 2-phosphate, disodium salt (TP=105 $\mu g.l^{-1}$) (A), and triethyl phosphonoacetate (TP=237 $\mu g.l^{-1}$) (B) during digestion with $HClO_4$ at 170 °C.

where, A_I and A_{II} are absorbances of the sample, and A_{IO} and A_{IIO} absorbances of the blank, with mixed reagents I and II, respectively.

RESULTS AND DISCUSSION

Digestion

The efficiency of digestion with perchloric acid at 170 °C was tested with glycerol 2-phosphate and triethyl phosphonoacetate, as representatives of the naturally most common types of organic phosphorus bonding P-O-C and P-C, respectively. The kinetics of orthophosphate release from these compounds are shown in Figure 1. Most orthophosphate (98%) had already been liberated from glycerol 2-phosphate after evaporating the sample and the complete oxidation of this compound occurred within 10 minutes of heating at 170 °C. Triethyl phosphonoacetate oxidation was complete after 40 minutes of digestion at 170 °C.

Orthophosphate liberation from glycerol 2-phosphate and triethyl phosphonoacetate was also tested in the presence of dissolved organic carbon (DOC) in the sample. The kinetics

of orthophosphate liberation and TP recoveries were not affected by glucose, peatbog humic acid, bovine serum albumin or lignin sulphonic acid with DOC concentrations of up to at least 200 $mg.l^{-1}$. However, with DOC concentrations above ca 50 $mg.l^{-1}$ the digest was still coloured after 40 minutes of digestion and the time to achieve the complete decolorisation had to be prolonged. A two to three hour digestion period was necessary for 200 $mg.l^{-1}$ of COD. This prolongation had no effect on the TP recovery.

The main advantage of the proposed procedure is that the loss of acidity during the digestion is negligible which provides a stable $[H^+]/[Mo(VI)]$ ratio in the following colorimetry without the necessity for carrying out a neutralization step. Existing methods of perchloric acid digestion use it at its boiling point[1, 2, 3, 4, 6, 16]. This leads to a rapid and uncontrollable decrease of acidity and, consequently, to the necessity to neutralize the digest.

The potential danger of explosion when hot concentrated perchloric acid comes in contact with organic materials is often mentioned as its disadvantage in its use for digestion[5, 6, 7]. In the present procedure, diluted perchloric acid is added to samples and the oxidation of organic matter occurs quietly at a constant temperature of 170 °C which is sufficiently below the boiling point of perchloric acid (203 °C).

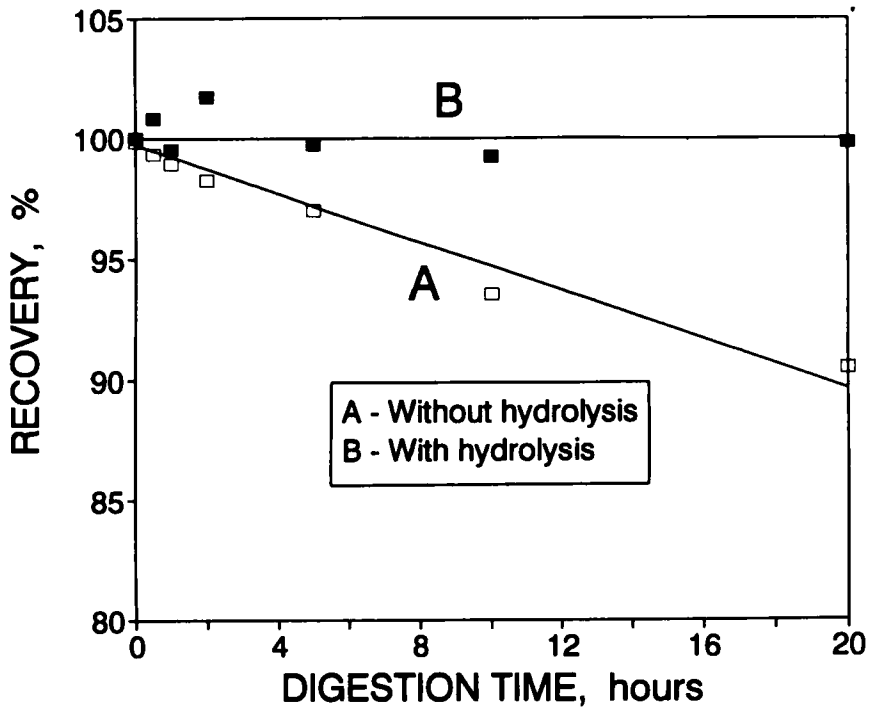

Figure 2 Orthophosphate recovery during digestion of standard KH_2PO_4 solution (TP=100 $\mu g.l^{-1}$) with $HClO_4$ at 170 °C. Samples determined without hydrolysis (A) and including hydrolysis (B).

Hydrolysis

Figure 2 shows orthophosphate recovery with hydrolyzed and non-hydrolyzed samples of standard KH_2PO_4 solution digested at 170 °C for various times from 0 to 20 hours. The difference between the results obtained with and without hydrolysis is believed to be caused by pyrophosphate which is formed from orthophosphate on heating with concentrated mineral acids and which does not produce colour in the phosphomolybdate blue method[17].

The necessity for hydrolysis has also been demonstrated with a set of 21 samples of various fresh waters digested for 40 minutes at 170 °C. The results obtained without hydrolysis were on average 1.4% lower than those obtained in the entire procedure. The paired t-test showed the difference was significant at the significance level (α) of 0.01.

The kinetics of the hydrolysis of condensed phosphates were examined for hexametaphosphate $(NaPO_3)_x$, pyrophosphate $Na_4P_2O_7.1OH_2O$, and polyphosphates $Na_5P_3O_{10}.6H_2O$ and $Na_4P_4O_{12}.4H_2O$ at 85 °C and 100 °C. All these compounds were completely hydrolyzed within 1 hour at 100 °C. However, more than 2 hours were required at 85 °C. Figure 3 shows the rate of hydrolysis for the quickest ($(NaPO_3)_x$) and the slowest ($Na_4P_4O_{12}.4H_2O$) hydrolyzable of the tested substances at 100 °C.

The presence of perchloric acid in the hydrolyzed mixture increased the boiling point of

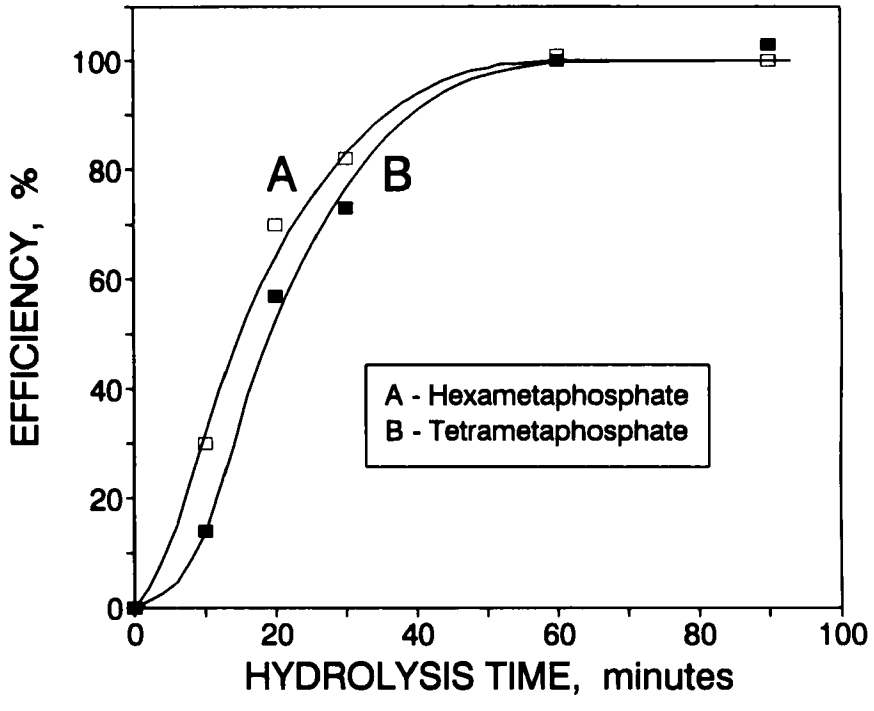

Figure 3 Kinetics of hydrolysis of sodium hexametaphosphate (TP=330 $\mu g.l^{-1}$) (A) and sodium tetrametaphosphate (TP=225 $\mu g.l^{-1}$) (B) at 100°C.

the mixture by about 1 °C diminishing thus the risk of bumping. Moreover, because of the test-tubes were inserted in the block only to the depth of 100 mm, the upper part of the test-tubes functioned as an air cooler and no clips were needed to hold the stoppers during the heating at 100 ± 0.5 °C. However, if the inaccuracy of the temperature regulation of the digestion block exceeds ± 0.5 °C, samples should be hydrolyzed at lower temperature (e.g. 2 hours at 85 °C).

Colour development

The formation of the reduced heteropoly acid complex was studied with various concentrations of perchloric acid in the reaction mixture (Figures 4 and 5). The absorbance was constant at a $[H^+]$ / [Mo(VI)] ratio between 70 and 80, increasing below 70 and decreasing above 80; moreover, both the increase and the decrease were time dependent. A similar optimum range for the $[H^+]$ / [Mo(VI)] ratio was found by Going and Eisenreich[12] with sulphuric acid. Based on these results and making use of the optimum conditions of reduced molybdoantimonylphosphate complex formation[12], the following final composition of the reaction mixture has been proposed: $[H^+] = 0.382$ mol.l^{-1}, [Mo(VI)] = 5.14×10^{-3} mol.l^{-1}, [Sb(III)] = 8.2×10^{-5} mol.l^{-1}, [ascorbic acid] = 5.6×10^{-3} mol.l^{-1}, with the ratio of $[H^+]$ / [Mo(VI)]

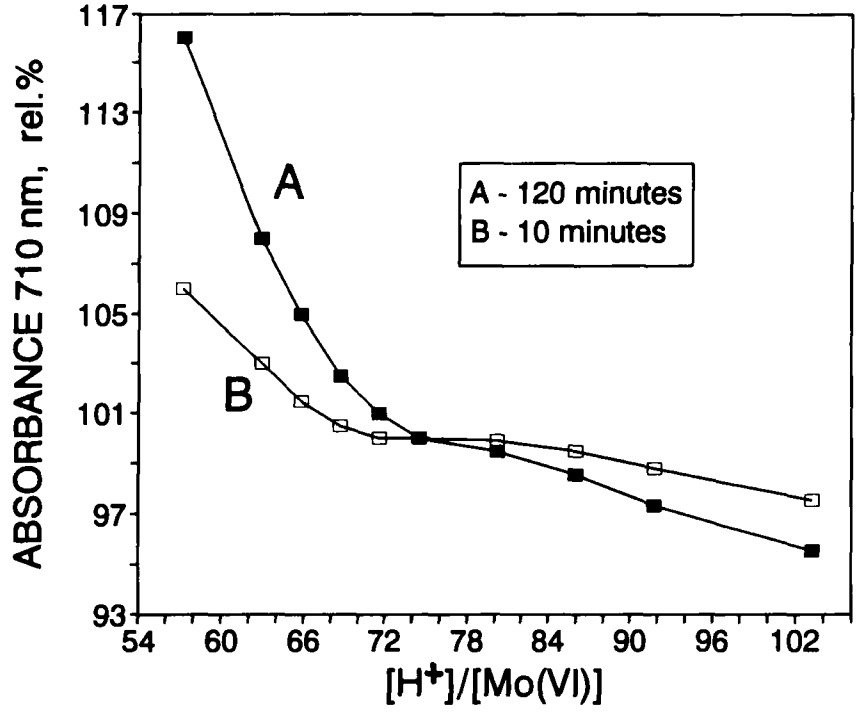

Figure 4 Relative absorbance of standard KH_2PO_4 solution (TP=200 µg. l^{-1}) as a function of $[H^+]$/[Mo(VI)] ratio. The $[H^+]$/[Mo(VI)] ratio of 74.3 corresponds to the proposed method.

= 74.3 for the total acidity of perchloric and sulphuric acids. At the ratio of $[H^+]$ / [Mo(VI)] = 74 the intensity of the colour was stable for at least 2 hours (Figure 5). However, with samples of natural waters the absorbance should be measured 10 minutes after adding the mixed reagent because the interference caused by silicate increases after a longer period of time[7, 13].

The molar absorptivity at 710 nm was determined to be $(1.65 \pm 0.06) \times 10^6$ $l.mol^{-1}.m^{-1}$ (mean ± standard deviation (SD); n = 150) which is in agreement with that found by Going and Eisenreich[12]. The molar absorptivity at 882 nm is about 30% higher than that at 710 nm[12, 14].

Interferences

The presence of inorganic colloids and particles in samples of natural waters causes a positive error by increasing the absorbance. Compensation for this error is an integral part of the procedure. Its efficiency was tested by adding a dispersion of diatomite and clay (10 NTU) to the standard solution of KH_2PO_4 (100 $\mu g.l^{-1}$ TP) and to ten different natural samples (42–345 $\mu g.l^{-1}$ TP). The mean of the ratio between TP values of the original samples and the samples after elimination of the added turbidity was 0.999 ± 0.020 (mean ± SD; n = 11).

Components of samples that change acidity of the reaction mixture in the photometric determination of orthophosphate by more than ± 7% may cause a decrease or increase in the

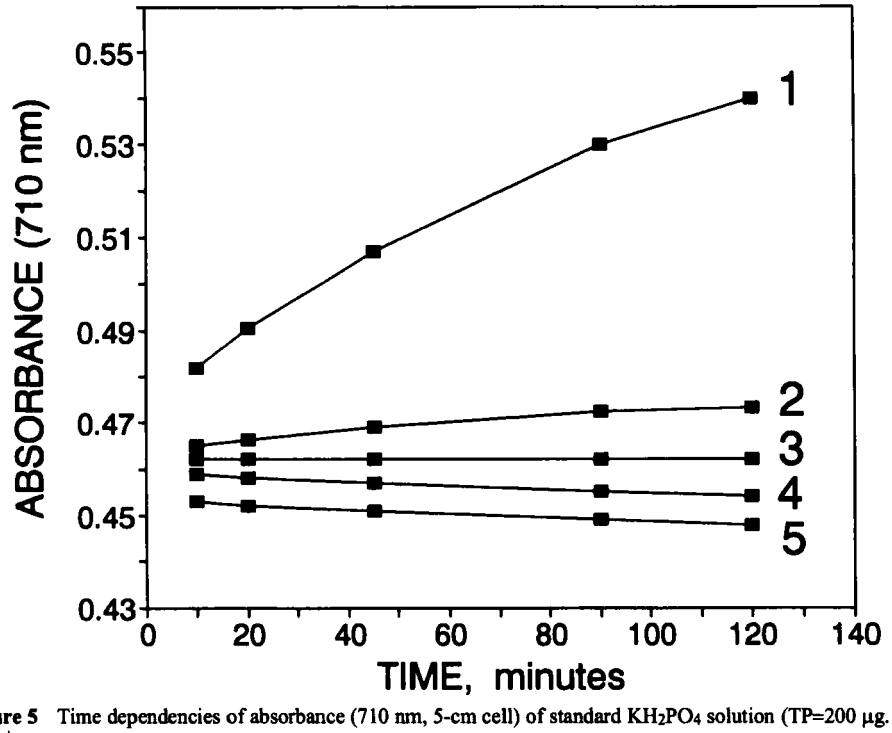

Figure 5 Time dependencies of absorbance (710 nm, 5-cm cell) of standard KH_2PO_4 solution (TP=200 $\mu g.l^{-1}$) at $[H^+]$/[Mo(VI)] ratios of 57 (1), 69 (2), 74 (3), 86 (4), and 103 (5).

colour intensity by shifting the $[H^+]/[Mo(VI)]$ ratio out of the optimum range between of 70 to 80, thus causing negative or positive error. Alkalinity, chloride and organic matter are the most often encountered components in fresh waters that may decrease the acidity. Alkalinity consumes H^+ ions directly, chloride vanishes during the digestion as HCl, and during organic matter oxidation, $HClO_4$ is probably partially reduced to Cl_2 or Cl^- which escape from the digestion mixture. The increase in the acidity may be caused by natural sample acidity and/or by addition of strong acids for sample conservation. The following recommendations can help to eliminate the errors:

1) A sample alkalinity of 10 $mmol.l^{-1}$ decreases the final acidity by approximately 2.5%. Alkalinity above 25 $mmol.l^{-1}$ should be neutralized, e.g. with perchloric or sulphuric acid.

2) A chloride concentration of 100 $mg.l^{-1}$ decreases the final acidity by approximately 0.7%. At chloride concentrations exceeding 1000 $mg.l^{-1}$ the effect can be eliminated by adding 0.1 ml of perchloric acid (reagent 5) per 1000 $mg.l^{-1}Cl^-$ to 10 ml of the sample.

3) A DOC concentration of 200 $mg.l^{-1}$ in the sample decreases the final acidity by 1.7 to 2.1% (tested for glucose, humic acid, bovine serum albumin, and lignin sulphonic acid).

In the case of joint effect of influences 1) to 3), 0.1 ml of perchloric acid (reagent 5) should be added to 10 ml of the sample per 7% of the calculated decrease of acidity.

4) A sample acidity of 10 $mmol.l^{-1}$ increases the final acidity by about 2.5%. Acidities greater than 25 $mmol.l^{-1}$ should be neutralized. Conservation of samples with H_2SO_4 in commonly used concentrations (up to about 20 mmol of H_2SO_4 per 1 l of the sample)[6,7] does not interfere.

Silicate, arsenate, and Cr(VI) interfere with the semi-micro method similarly as with the Murphy and Riley method[11]. For their effects and eliminations see e.g. ref. 7.

Detection limit, precision and accuracy

The detection limit of the semi-micro method was estimated as the sum of the mean value of the blanks and the threefold of the mean value of standard deviations (SD) of the samples. The mean value of the blank from duplicate determinations in 40 different series was 1.8 ± 0.9 $\mu g.l^{-1}$TP (mean ± SD). The average sample SD obtained from the analysis of 21 samples (6 parallel determination each sample) of river, reservoir and rain water with the TP range of 14 - 61 $\mu g.l^{-1}$ was 0.77 $\mu g.l^{-1}$TP. The detection limit was therefore $1.8 + 3 \times 0.77 = 4.1$ $\mu g.l^{-1}$TP. The precision estimated as the relative SD based on this set of 21 samples was 1.9% on average.

The accuracy of the semi-micro method was tested by adding a known quantity of KH_2PO_4 (0.5 µg TP per sample) to 150 different samples in 30 series. The percentage of TP recovery was 99.8 ± 3.5 (mean ± SD).

Comparison

The semi-micro method was compared with 1) the persulphate - sulphuric acid digestion method[5] and 2) the classic perchloric acid digestion method[1] in the modification according

Table 1 Comparison of semi-micro method with persulphate - sulphuric acid digestion method[5] (Method A) and classic perchloric acid digestion method[17, 18] (Method B).

Parameter	*Method A*	*Method B*
Number of samples	24	61
Pair t-test:		
computed t statistic	1.57	1.75
significance level (α)	0.13	0.085
Linear correlation analysis:[a]		
constant	2.14	-1.86
std error of y determination	2.30	5.78
x coefficient	0.952	1.046
std error of x coefficient	0.003	0.008
R^2	0.9997	0.997

[a]Semi-micro method as dependent variable

to Popovsky[17] followed by the extraction determination of orthophosphate according to Stephens[18]. The comparison with the persulphate - sulphuric acid method was performed on a set of 24 samples: rivers (n=8, 62–116 $\mu.1^{-1}$ TP), reservoirs (n=3, 50–100 $\mu g.1^{-1}$ TP), fish ponds (n=7, 6–121 $\mu g.1^{-1}$ TP), precipitation water (n=3, 19–31 $\mu g.1^{-1}$ TP) and purified municipal waste water (n=3, 80–670 $\mu g.1^{-1}$ TP). The comparison with the perchloric acid digestion method was performed on a set of 61 samples of various fresh water types: rivers (n=30, 17–267 $\mu g.1^{-1}$ TP), lakes (n=11, 3–14 $\mu g.1$-1 TP), reservoirs (n=5, 8–115 $\mu g.1^{-1}$ TP), fish ponds (n=5, 5–120 $\mu g.1^{-1}$ TP), precipitation water (n=5, 18–56 $\mu g.1^{-1}$ TP), tap water (n=2, 5$\mu g.1^{-1}$ TP), and purified municipal waste water (n=3, 80–670 $\mu g.1^{-1}$TP). A correlation analysis revealed good linearity between the semi-micro method and both reference methods (Table 1). The differences between the methods were examined using the paired t-test (Table 1). It showed no significant difference between the semi-micro method and the persulphate method ($\alpha > 0.1$). Only slight statistically significant difference ($0.05 < \alpha < 0.1$) was found between the semi-micro method and the classic perchloric acid digestion method. The results obtained by the semi-micro method were higher on average by 0.51%. This might have been caused by the interference of silicate that was more efficiently eliminated in the classic method using the extraction step[10].

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