

A PROCEDURE FOR THE SIMULTANEOUS DETERMINATION OF TOTAL NITROGEN AND TOTAL PHOSPHORUS IN FRESHWATER SAMPLES USING PERSULPHATE MICROWAVE DIGESTION

P. J. JOHNES¹ and A. L. HEATHWAITE²

¹Department of Environmental & Evolutionary Biology, University of Liverpool, Derby Bldg, P.O. Box 147, Liverpool L69 3BX and ²Department of Geography, University of Sheffield, Sheffield S10 2TN, England

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Abstract—In the absence of a suitable method for routine analysis of large numbers of natural river water samples for organic nitrogen and phosphorus fractions, a new simultaneous digestion technique was developed, based on a standard persulphate digestion procedure. This allows rapid analysis of river, lake and groundwater samples from a range of environments for total nitrogen and phosphorus.

The method was evaluated using a range of organic nitrogen and phosphorus structures tested at low, mid and high range concentrations from 2 to 50 mg l⁻¹ nitrogen and 0.2 to 10 mg l⁻¹ phosphorus. Mean recoveries for nitrogen ranged from 94.5% (2 mg l⁻¹) to 92.7% (50 mg l⁻¹) and for phosphorus were 98.2% (0.2 mg l⁻¹) to 100.2% (10 mg l⁻¹). The method is precise in its ability to reproduce results from replicate digestions, and robust in its ability to handle a variety of natural water samples in the pH range 5–8.

Key words—total nitrogen, total phosphorus, freshwater, water quality, simultaneous digestion, persulphate oxidation, microwave digestion unit, autoanalyser

INTRODUCTION

Traditionally, aquatic nutrient studies have focused on the concentrations of nitrate (NO₃⁻) and of soluble reactive phosphorus (PO₄³⁻) in natural waters. However, a number of studies have illustrated the dynamic nature of the nitrogen speciation and phosphorus fractionation balances in natural waters, and the significance of the organic nitrogen and particulate phosphorus fractions in contributing to the total nutrient loading in a water body (e.g. Heathwaite *et al.*, 1990; Johnes, 1990; Ryding and Forsberg, 1979; Stevens and Stewart, 1982). In order to assess the wider environmental significance of nutrient concentrations in natural waters, and to distinguish the relative contribution of catchment sources of nitrogen and phosphorus to aquatic loading, a relatively straightforward and rapid technique is needed for the digestion of organically bound nitrogen and phosphorus in natural waters.

The standard method for the determination of organic nitrogen in water samples in the past has been based on Kjeldahl's digestion technique which converts organic nitrogen into ammonia which can then be determined along with any ammonia originally present. However, this method is time consuming and the accuracy is variable (D'Elia *et al.*, 1977; Hosumi and Sudo, 1986; Smart *et al.*, 1981). An alternative method for determination of organic nitrogen devel-

oped by Koroleff (1972) and later modified by D'Elia *et al.* (1977) is the alkaline persulphate digestion technique. This oxidizes all nitrogen in the sample using potassium persulphate in a strongly alkaline environment under high temperature and pressure. Nitrate is the sole product and can easily be determined by reduction to nitrite (Hendriksen and Selmer-Olsen, 1970). This technique is well established for the analysis of freshwaters.

Determination of total phosphorus in natural water samples uses potassium persulphate as the oxidizing reagent, but requires an acidic environment (Jeffries *et al.*, 1979). A number of authors have suggested that a modification of the persulphate digestion technique will allow the simultaneous determination of total nitrogen and total phosphorus in freshwater samples (e.g. Ebina *et al.*, 1983; Hosumi and Sudo, 1986; Koroleff, 1977; Langner and Hendrix, 1982). This operates using the by-products of alkaline persulphate nitrogen digestion to create the acid environment necessary for breakdown of phosphorus compounds under conditions of high temperature and pressure. In previous studies, high temperature and pressure have been generated using autoclave units or domestic pressure cookers with a standard digestion time of 1.5 h (*ibid.*). To reduce digestion time, and to enable more complete breakdown of compounds containing HN=C groups, which are resistant to persulphate oxidation

(Ebina *et al.*, 1983; Nydahl, 1978), a modified technique has been developed for the simultaneous digestion of total nitrogen and total phosphorus in freshwaters. This utilizes a microwave digestion unit previously used for acid digestion (as reviewed by Kingston and Jassie, 1986) in place of an autoclave system.

MATERIALS

(1) Reagents

Analytical reagent grade chemicals were used for all experiments. Double-distilled deionized water (DDW) was found to provide least risk of nitrogen or phosphorus contamination and was used throughout for equipment cleaning and reagent preparation.

Oxidizing reagent. The oxidizing reagent was prepared using the following procedure. A stock solution of 3.75 M NaOH was made up. 15 ml of this solution was added to 50 ml DDW in a 100 ml volumetric flask. 10 g $K_2S_2O_8$ was then dissolved in this solution, and the reagent made up to 100 ml. The oxidizing reagent was prepared fresh daily.

Reagents for nitrate determination. Nitrate was determined in the sample digests using a colorimetric, automated technique based on hydrazine-copper reduction. Nitrate is reduced to nitrite in reaction with hydrazine-copper sulphate. Total nitrogen is then determined as NO_2-N by reaction with sulphanilamide coupled to *N*-1-naphthylethylene diamine dihydrochloride, forming a reddish-purple azo dye measured colorimetrically at 520 nm. The flow diagram for the autoanalytical procedure employed is shown in Fig. 1. The analytical reagents were prepared as follows: reagent 1, 0.4 M NaOH (16 g NaOH was dissolved in 1 litre DDW); reagent 2, 0.2 M NaOH (8 g NaOH was dissolved in 1 litre DDW); reagent 3, hydrazine-copper reagent (4 g copper sulphate was dissolved in 1 litre DDW. This was then stored as a stock solution. 2.2 g hydrazine sulphate was dissolved in 300 ml

DDW, 3 ml of the stock copper sulphate solution was added and the solution diluted with DDW to 1 litre); reagent 4, sulphanilamide reagent (100 ml concentrated phosphoric acid was added to 500 ml DDW. 10 g sulphanilamide was dissolved in, and 0.5 g naphthylethylene diamine dihydrochloride added to this solution which was then diluted to 1 litre with DDW). 10 g NaOH was also added to 5 l of the DDW diluent to buffer the system against the acidity of digested samples.

Reagents for orthophosphate. Total phosphorus concentrations in the sample digests were determined as orthophosphate, PO_4-P , using a colorimetric, automated technique. The orthophosphate reacts with ammonium molybdate in an acidic environment to form phosphomolybdic acid, with antimony as a catalyst. This is then reduced in reaction with ascorbic acid to produce a molybdenum blue complex measured colorimetrically at 660 nm. The flow diagram for the autoanalytical procedure is shown in Fig. 2. The analytical reagents were prepared as follows: reagent 1, sulphuric acid (10 ml concentrated sulphuric acid was added to 1 litre DDW); reagent 2, molybdate reagent (62 ml concentrated sulphuric acid was added to 800 ml DDW. 10 g ammonium molybdate was dissolved in the solution, followed by 0.2 g antimony tartrate. The solution was then made up to 1 litre with DDW); reagent 3, ascorbic acid (18 g ascorbic acid was dissolved in 950 ml DDW. 5 ml acetone was added, and the solution made up to 1 litre using DDW).

Stock standard reagents. Six stock standards of nitrogen and phosphorus were prepared in three concentration ranges for calibration of the total nitrogen/total phosphorus method, where N1 was combined with P1, N2 with P2 and so on, to provide a total of 18 dual N and P standards for calibration of the method. Reagent 1 contained no organic compounds, whereas reagents 2-6 all contained organically bound nitrogen and phosphorus. The compounds used are listed in Table 1. Reagents 1 and 2 were used for method calibration and optimization. All compounds were then used in the following steps to determine method precision, accuracy and the limits of detection. Standard N2/P2 (sulphanilamide/tetra-sodium pyrophosphate) was also used

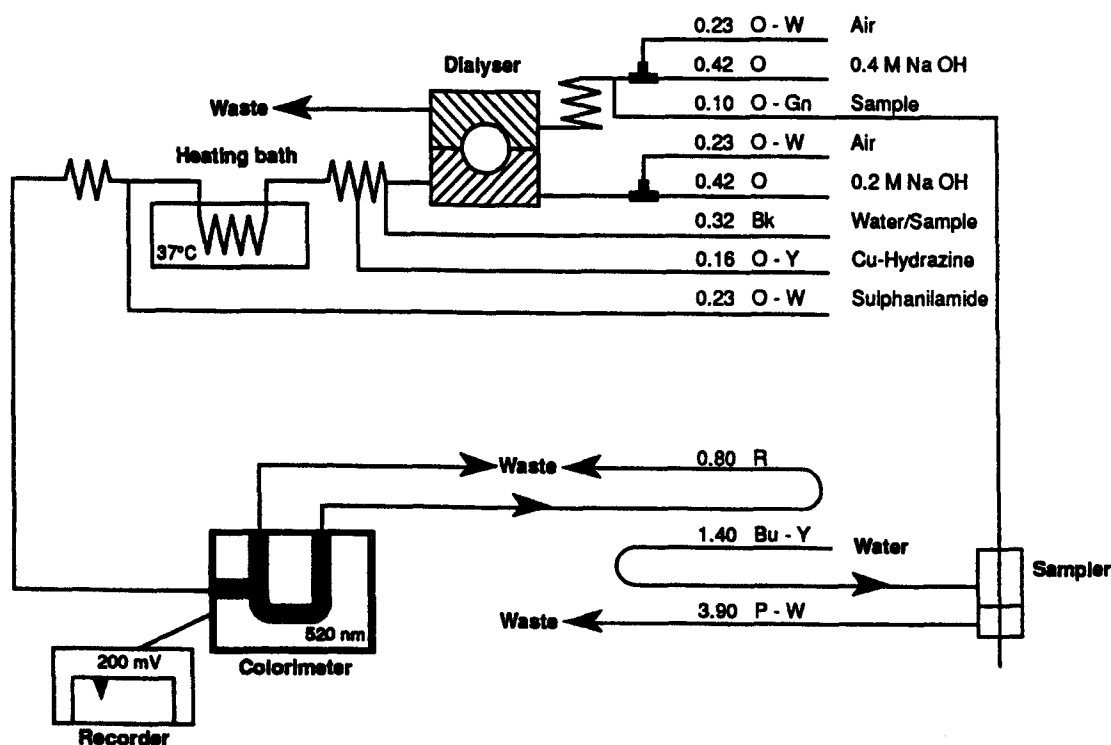


Fig. 1. Total oxidized nitrogen [modified from Chemlab Instruments Ltd (1984)].

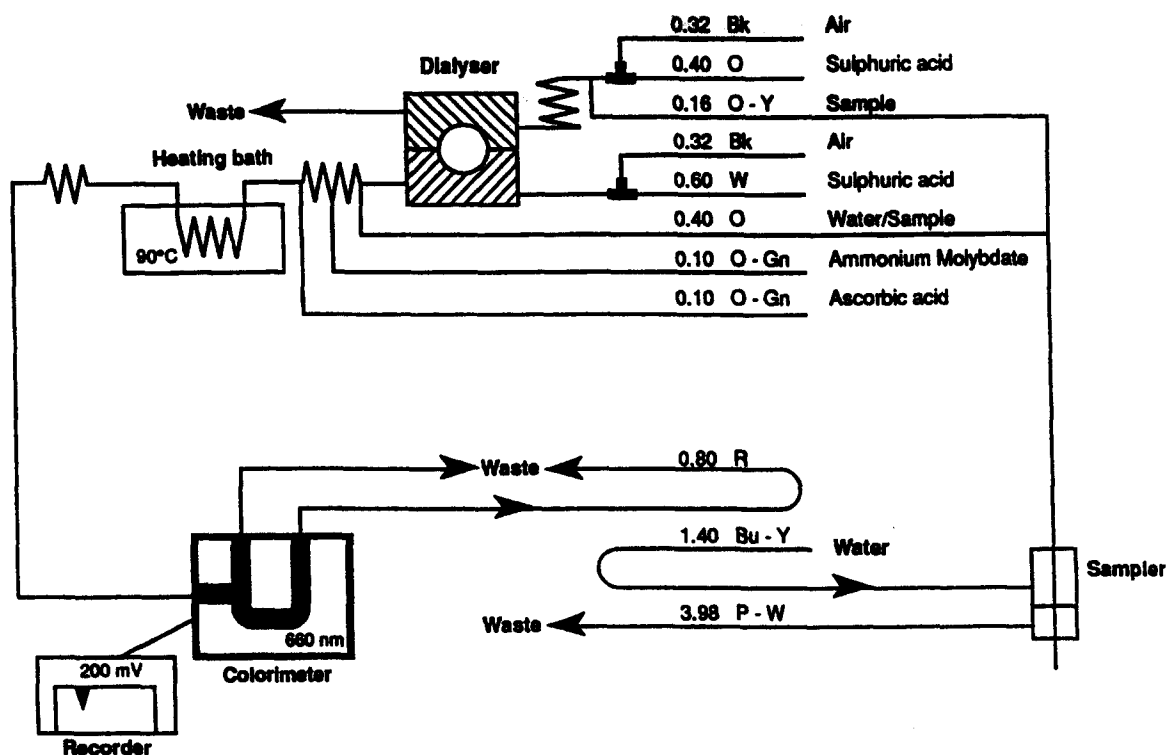


Fig. 2. Phosphate [modified from Chemlab Instruments Ltd (1983)].

for standard addition to natural water samples (Golterman *et al.*, 1979).

(2) Equipment

Digestions were carried out in closed 120 ml PTA Teflon vessels using a CEM Digestion System, Model MDS-81D (Oxford Laboratories) capable of 1% microwave power adjustments up to a maximum of 600 W. The vessels can operate up to internal pressures of 120 psig, above which an inbuilt safety valve will open, venting vaporized sample digests (see Fig. 3). The microwave unit operates using a 12-position sample carousel turntable operating at 3 rpm to ensure even temperature distribution in the digestion vessels.

A Chemlab System 4 Auto-analyser was used for determination of total digested nitrogen (as nitrite, $\text{NO}_2\text{-N}$) and total digested phosphorus concentrations (as orthophosphate, $\text{PO}_4\text{-P}$).

PROCEDURE

The procedure employed for chemical differentiation of nitrogen species and phosphorus fractions in freshwaters is outlined in Fig. 4.

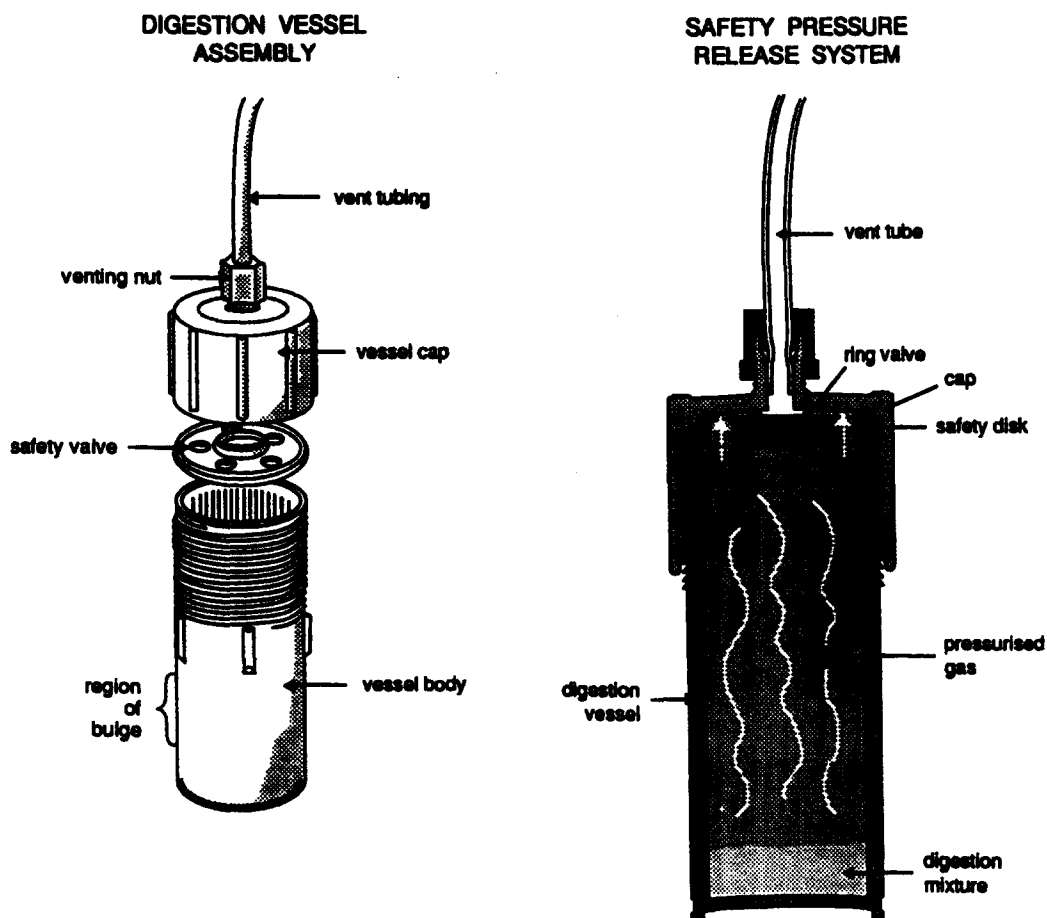
(1) Method optimization and calibration

Persulphate oxidation is temperature and pH sensitive (Langner and Hendrix, 1982). In order to determine total nitrogen using persulphate oxidation, it is necessary to maintain an alkaline environment for long enough to ensure complete digestion of all nitrogen compounds in the sample. This requires sufficient sodium hydroxide to be present in the oxidizing reagent. Determination of total phosphorus using persulphate oxidation requires that adequate potassium persulphate remains after the digestion of nitrogen compounds in the alkaline phase to ensure the complete digestion of all organically bound phosphorus in the liquid sample during the acid phase. Therefore, both the

Table 1. Nitrogen and phosphorus compounds used for calibration and evaluation of the persulphate digestion technique

Reagent number	Nitrogen
N1	Ammonium chloride (NH_4Cl)
N2	Sulphanilamide ($\text{NH}_2\text{C}_6\text{H}_4\text{SO}_2\cdot\text{NH}_2$)
N3	<i>N</i> -1-Naphthylethylene diamine dihydrochloride ($\text{C}_{10}\text{H}_7\text{NHCH}_2\text{CH}_2\text{NH}_2\cdot 2\text{HCl}$)
N4	Urea (NH_2CONH_2)
N5	4-Aminoantipyrine $\text{CH}_3\text{C}(\text{NH}_2)\cdot\text{CO}\cdot\text{N}(\text{C}_6\text{H}_5)_2\cdot\text{N}\cdot\text{CH}_3$
N6	Pyridoxine hydrochloride (vitamin B6) $\text{C}_8\text{H}_{11}\text{NO}_3\cdot\text{HCl}$
Phosphorus	
P1	Potassium dihydrogen phosphate (KH_2PO_4)
P2	Tetra-sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7\cdot 10\text{H}_2\text{O}$)
P3	Glucose-6-phosphoric acid dipotassium salt ($\text{C}_6\text{H}_{11}\cdot\text{O}\cdot\text{PO}_3\cdot\text{K}_2$)
P4	Glucose-1-phosphoric acid disodium salt ($\text{C}_6\text{H}_{11}\text{O}_5\cdot\text{O}\cdot\text{PO}_3\cdot\text{Na}_2\cdot 4\text{H}_2\text{O}$)
P5	Adenosine-5'-diphosphoric acid disodium salt ($\text{C}_{10}\text{H}_{13}\text{N}_5\text{O}_7\text{P}_2\cdot\text{Na}_2$)
P6	Adenosine-5'-triphosphoric acid disodium dihydrogen salt ($\text{C}_{10}\text{H}_{14}\text{N}_5\text{O}_{13}\text{P}_3\cdot\text{Na}_2\cdot 3\text{H}_2\text{O}$)

The concentration ranges used were: 2 mg TN 0.2 mg TP l^{-1} ; 15 mg TN 3.0 mg TP l^{-1} ; 50 mg TN 15.0 mg TP l^{-1} .



At pressures below 830 kPa ring valve sealed against the cap. Excess pressure will break ring valve seal and exhaust pressurised gases via the vent tube.

Fig. 3. Digestion vessel design for simultaneous total N and P determination using a microwave digestion technique.

concentration and relative proportion of sodium hydroxide and potassium persulphate in the oxidizing reagent are critical in determining the efficiency of the digestion technique.

A number of authors (e.g. D'Elia *et al.*, 1977; Ebina *et al.*, 1983; Hosumi and Sudo, 1986) confirm that digestion is complete where the initial pH 12 of the sample/oxidizing reagent mixture is reduced to approx. pH 2 in the digestion products. This indicator was used throughout to identify the degree of completion of the digestion.

Optimum sample:persulphate and persulphate:sodium hydroxide ratios for recovery of total N and P were determined. A range of oxidizing solutions was prepared from 5 to 150 g $K_2S_2O_8$ l^{-1} with 30 ml 3.75 M NaOH (the latter according to Ebina *et al.*, 1983). These were then run in the sample:oxidizing reagent ratio 2:10 ml at a standard microwave setting of 100% power for 15 min, fan setting 5. From this procedure, optimum reagent ratios were determined at 150 g $K_2S_2O_8$:30 ml 3.75 M NaOH per litre.

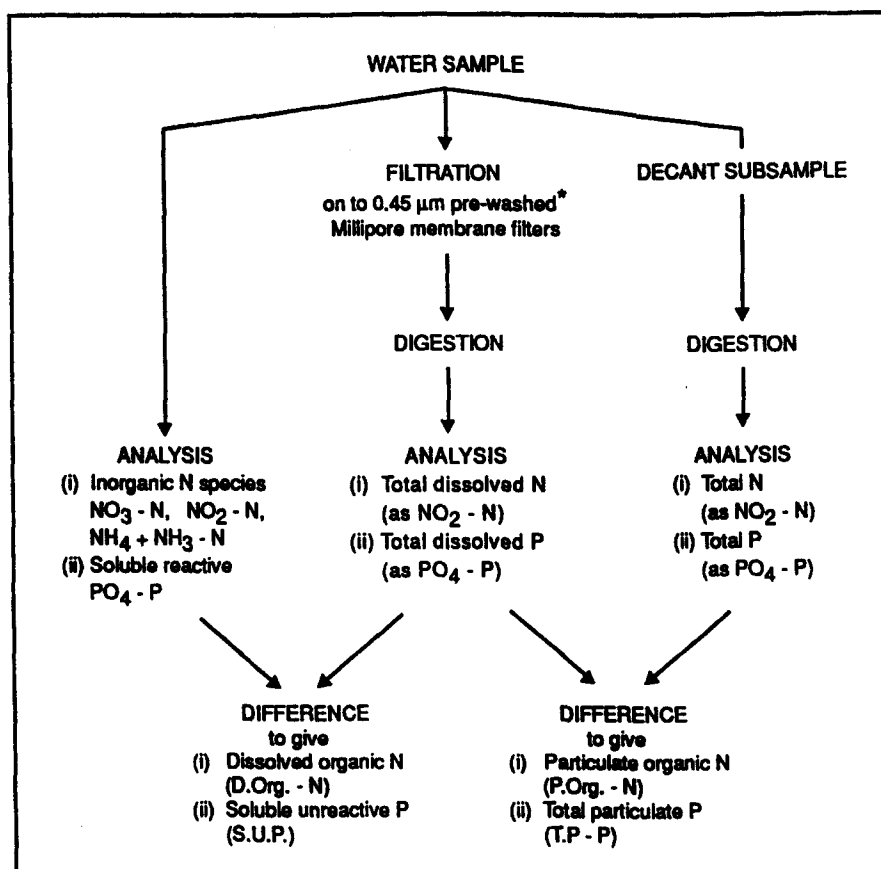
Optimum oxidizing reagent:sample ratio was derived using a range of sample digests made up in ratios from 1 ml oxidizing reagent:11 ml sample, to 6 ml oxidizing reagent:6 ml sample, at the same microwave settings using the optimum oxidizing reagent. This was determined to be a ratio of 8 ml sample:4 ml oxidizing reagent, and the final calculation takes account of this dilution.

Optimum microwave settings were determined by increasing the time in units of 5 min and decreasing power by 5%, whilst maintaining a fan setting of 5. From this the optimum was determined as 80% power for 45 min. It was also found that the fan setting was fairly critical, with sample recovery optimized at a setting between 4.5 and 5.5.

(2) Determination of method accuracy, precision and sensitivity

(a) *Synthetic nitrogen and phosphorus compounds.* The sensitivity of the digestion procedure to a range of organic nitrogen and phosphorus compounds was assessed for the six compound mixes shown in Table 1. These were prepared in three concentration ranges: 2, 15 and 50 mg N l^{-1} , and 0.2, 3 and 10 mg P l^{-1} . The compounds selected included sulphanilamide (N2); straight chain organics such as, urea (N4), pyridoxine (N6), glucose-6 (P3 and P4) and adenosine (P5 and P6); inorganic heterogeneous compounds represented by compounds N1 and P1; and also compounds containing $HN=C$ groups represented by N5 (amino-antipyrine) which have been found by a number of authors (e.g. Ebina *et al.*, 1983; Nydahl, 1978) to cause poor recoveries. The recoveries for all compounds are presented in Table 2.

Following method calibration and optimization, recoveries of N and P from synthetic standard 1 ranged from 98.9



* see Jay (1985), Lalande *et al.* (1986).

Fig. 4. Chemical differentiation of nitrogen and phosphorus in freshwaters.

to 102% for all concentration ranges. Recoveries of straight chain organics in standards 3, 4 and 6 ranged from 97.1 to 101.8% for nitrogen and 95.7 to 102.4% for phosphorus. For standards 2 and 5 recoveries were lower, particularly for aminoantipyrine (N5) recovered at 60.1–72.5%. However, phosphorus recoveries for compounds P2 and P5 were high. In compound N5, an insoluble colour complex formed at high concentration (50 mg N l⁻¹) interfering in the analytical procedure. However, at low and mid concentration ranges recoveries were higher at 72.5% (low) and 67.5% (mid). This improved on the recoveries obtained by Nydahl

Table 2. Recoveries of a range of nitrogen and phosphorus compounds (based on 6 replicates in each concentration range)

Nitrogen compound	Recoveries (%)		
	Low range (2 mg N l ⁻¹)	Mid range (15 mg N l ⁻¹)	High range (50 mg N l ⁻¹)
1	102.0 (0.0320)	99.6 (0.032)	98.9 (0.067)
2	97.5 (0.047)	95.0 (0.084)	99.2 (0.167)
3	98.3 (0.031)	100.9 (0.055)	97.7 (0.111)
4	99.3 (0.042)	98.0 (0.063)	101.8 (0.079)
5	72.5 (0.055)	67.5 (0.079)	60.1 (0.699)*
6	97.1 (0.039)	97.5 (0.069)	98.6 (0.117)
Phosphorus compound	Low range (0.2 mg P l ⁻¹)	Mid range (3 mg P l ⁻¹)	High range (10 mg P l ⁻¹)
1	100.6 (0.005)	103.0 (0.025)	101.6 (0.072)
2	96.7 (0.011)	100.7 (0.028)	100.2 (0.055)
3	102.4 (0.013)	96.6 (0.087)	100.1 (0.084)
4	95.7 (0.012)	96.3 (0.057)	99.3 (0.057)
5	96.1 (0.007)	96.2 (0.038)	101.2 (0.052)
6	97.8 (0.011)	96.7 (0.035)	98.5 (0.038)

*See text.

() indicates 1 SD (mg l⁻¹) for n = 6.

(1978) and Ebina *et al.* (1983) with 42.5 and 46.3%, respectively, at 2 mg N l⁻¹, with 72.5% for the 2 mg N l⁻¹ standard and 67.5% recovery for the 15 mg N l⁻¹ standard.

The results exhibit a high degree of precision for all compounds except compound N5 (see Table 2). For compound N5, the production of the colour complex at 50 mg N l⁻¹ may have caused interference in the colorimetric analysis, and potentially inhibited the digestion mechanism.

(b) *Freshwater samples.* The method was then examined for freshwater samples taken from lake, river and ground-water environments. Inorganic nitrogen and phosphorus concentrations were initially determined in pre-digest subsamples. The samples were then digested according to the method described above, and total nitrogen and total phosphorus concentrations were determined. Samples were then treated following methods of standard addition (Golterman *et al.*, 1979) using compounds N3 and P3 at 10, 20 and 30 mg N l⁻¹ and 1, 3 and 10 mg P l⁻¹. The results of this analysis are presented in Table 3. Recovery of nitrogen ranged from 101.7% (30 mg l⁻¹) to 102.8% (10 mg l⁻¹), and for phosphorus from 98.4% (10 mg l⁻¹) to 105.9% (1 mg l⁻¹). This procedure demonstrates the lack of interference in the digestion procedure by intrinsic chemical characteristics of the natural water samples. This is particularly important here where the river water samples were taken from a limestone catchment with a high buffering potential for acidity.

(3) Range of applicability

The effective range of the procedure was determined by running digests up to a maximum concentration of 200 mg N and P l⁻¹. The technique proved accurate for concentrations of up to 50 mg N or P l⁻¹. Above this level, recovery decreased markedly, with method precision becoming poor

Table 3. Recoveries of nitrogen and phosphorus compounds added to the natural water sample

N added (mg N l ⁻¹)	River water digest (mg N l ⁻¹)	Spiked sample digest (mg N l ⁻¹)	Recovery (%)
10	15.42	26.13	102.8
20	15.42	35.87	101.3
30	15.42	46.21	191.7

P added (mg P l ⁻¹)	River water digest (mg P l ⁻¹)	Spiked sample digest (mg P l ⁻¹)	Recovery (%)
1	0.34	1.42	105.9
3	0.34	3.41	99.7
10	0.34	10.18	98.4

at concentrations above 100 mg l⁻¹. The lower limits of detection were determined based on digestions of double-distilled deionized water, which produced consistently low concentrations of 0.05 mg N l⁻¹ and <0.01 mg P l⁻¹ (the latter being at the limits of auto-analytical detection).

CONCLUSIONS

The results illustrate the accuracy and precision of this digestion technique for both nitrogen and phosphorus within the range 0.05–50 mg N l⁻¹ and 0.01–50 mg P l⁻¹. River water, groundwater and lake water samples digested using this method have confirmed its suitability for freshwater samples. The rapid simultaneous digestion and analysis of waters for total nitrogen and total phosphorus, combined with chemical differentiation of nitrogen species and phosphorus fractions, allow full evaluation of the impact of increased nutrient loadings on aquatic environments (see, for example, Heathwaite and Burt, 1991; Johnes, 1990; Johnes and Burt, 1991). The adaptation of the standard persulphate digestion procedure to a microwave digestion unit has improved the digestion time of earlier techniques, whilst maintaining accuracy and precision, allowing more efficient sample digestion. The microwave digestion technique will be a valuable tool in future aquatic nutrient studies. A complete summary of the digestion procedure is given in the Appendix.

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APPENDIX

- (1) Ensure all digestion equipment is chromic acid washed and rinsed in double-distilled deionized water. Wear sterile gloves at all times to prevent phosphorus contamination. Use analytical reagent grade chemicals, and only make up reagents using double-distilled deionized water.
- (2) Dissolve 75 g NaOH (e.g. BDH "Aristar") in 500 ml double-distilled deionized water, dilute to 1000 ml to produce a 3.75 M NaOH solution.
- (3) Add 15 ml of the 3.75 M NaOH solution to 500 ml double-distilled deionized water. Dissolve 50 g $K_2S_2O_8$ in the solution, using gentle heating to $<40^\circ\text{C}$ if necessary. Dilute to 1000 ml. Prepare this oxidizing reagent fresh as required.
- (4) Add 4 ml of oxidizing reagent to 8 ml of sample in 120 ml capacity Teflon vessels. Cap immediately ensuring that the pressure disc is correctly positioned inside the cap. Tighten to the pre-set pressure using the microwave digestion system capping station and place in carousel. Connect vent tube to the central receptable (see Fig. 3).
- (5) Place the completed carousel in the microwave unit and run at 80% for 45 min with a fan setting of 5. When the digestion run is completed, allow samples to cool to room temperature before uncapping. If necessary, the sample vessels can be placed unopened in cold water.
- (6) Decant the digested samples into 2 ml cuvettes and place uncovered onto the auto-analyser sample tray. Total nitrogen is then measured as nitrate following the hydrazine-copper reduction method. Total phosphorus is measured as orthophosphate by the ascorbic acid reduction method. The sample digests may equally be analysed using standard batch analyti-

cal procedures where autoanalytical systems are not available.

Note 1

For non-automated procedures, owing to the acidity of the digested samples (pH 1.8–2.2), the pH of sample digests should be gradually adjusted to pH 7 by addition of 1 ml aliquots of 0.4 M NaOH to the sample digests. On auto-analytical systems, it is necessary to buffer both the TON and orthophosphate systems of the auto-analyser to ensure machine quality control. On the TON module, the reduction of nitrate to nitrite takes place under strongly alkaline conditions. To compensate for sample acidity add 10 g NaOH to 5000 ml of the water diluent. Phosphate is determined in a strongly acidic environment. The concentration of reagent 1 (H_2SO_4) should therefore be diluted to half strength.

Note 2

The actual concentration of total nitrogen and total phosphorus is determined by multiplying auto-analyser results by 1.5, since 8 ml of sample is initially diluted in this ratio when 4 ml of oxidizing reagent is added.

Note 3

Standards must be digested prior to sample digestion in order to ensure comparable conditions with respect to interferences and acidity. Sufficient volumes should be digested to provide for the number of scheduled analyses.

Note 4

The oxidizing reagent will recrystallize when the temperature falls below 30°C . It can be redissolved by gentle heating to $<40^\circ\text{C}$ as required.