Review

Procedures for the storage and digestion of natural waters for the determination of filterable reactive phosphorus, total filterable phosphorus and total phosphorus

W. Maher*, L. Woo

CRC for Freshwater Ecology, University of Canberra, Applied Ecology Research Group, Canberra, ACT 2601, Australia

Received 2 January 1998; received in revised form 15 April 1998; accepted 22 April 1998

Abstract

An overview of the forms of phosphorus species likely to be encountered in natural waters and the implications for the measurement of filterable and total phosphorus is given. Procedures reported in the literature for the storage and digestion of water samples for filterable reactive phosphorus (FRP), total filterable phosphorus (TFP) and total phosphorus (TP) measurements are summarised and the advantages and limitations of methods discussed.

Water samples for FRP and TFP measurements need to be filtered immediately on collection as exchange on and off particles may occur in the sample container. Slow freezing of filtered or turbid water samples in acid washed low density polyethylene bottles appears to be satisfactory for the long term storage (years) of a variety of water sample types. Storage of water samples at room temperature or refrigeration (1–5°C) with a preservative is suitable only for short term storage (days-months). If water samples contain < 20 μg P/l, adsorption to containers may be significant.

Batch digestion of samples with alkaline or acid peroxidisulphate using autoclave or microwave heating offers the advantages of ease, simplicity and precision. Good recoveries of phosphorus from a range of phosphorus compounds containing P–O–P, C–O–P and C–P bonds expected in natural waters have been reported. If turbid samples are to be analysed, caution must be exercised to ensure that the carbon or suspended solids concentration does not exceed the capacity of the digestion procedure to oxidise the carbon present and release occluded phosphorus from particulate materials. Better recoveries of phosphorus from turbid water samples are achieved using microwave heating with closed vessels, probably because of the higher temperatures and pressures generated.

The use of on-line heating (microwave, thermal induced) coupled with flow injection analysis and using peroxidisulphate or an oxidising acid mixture should also allow the automation of TFP and TP measurements. Reliable procedures for the removal of unwanted particulate material prior to or after the digestion step need to be developed. © 1998 Published by Elsevier Science B.V. All rights reserved.

Keywords: Phosphorus compounds; Natural waters; Filterable reactive phosphorus; Total filterable phosphorus and total phosphorus; Storage; Digestion
1. Introduction

Eutrophication of natural waters is a worldwide problem. It is generally accepted that excess nutrients, particularly phosphorus are responsible for algal blooms [28,38,128,129,140,143].

Vollenweider [143] has shown that algal biomass in water systems can be predicted from total phosphorus (TP) measurement. Empirical correlations between phytoplankton (chlorophyll a) and the concentrations of TP in lakes are used taking into account the residence time of phosphorus in the system, to predict algal biomass. TP refers to all phosphorus forms in an unfiltered water sample (Fig. 1).

Many workers believed that filterable reactive phosphorus (FRP) or total filterable phosphorus (TFP) (Fig. 2) better represents bioavailable phosphorus in predicting algal growth rates [16,58]. FRP and TFP are normally defined as the phosphorus present in the filtrate of a water sample passed through a 0.45 μm membrane filter before and after digestion, although there is considerable controversy over what filtration through a 0.45 μm filter actually measures [14,34,133].

Because of their importance in predicting algal biomass and growth rates these operationally defined phosphorus fractions are extensively monitored. Two important considerations need to be made to obtain accurate measurements; how samples are stored, and how samples are digested to release phosphorus from soluble phosphorus compounds and phosphorus sorbed to particles in a form suitable for analysis.
Water samples may undergo changes over time by several mechanisms including adsorption, precipitation, hydrolysis, complexation, bacterial usage and algae uptake [71,86,89], and if not stored correctly, will not be representative of the original sample. Many procedures have been advocated for preserving samples including acidification, addition of chloroform, mercuric chloride or alkali, refrigeration and freezing (see Table 1). Numerous procedures for digesting samples for TFP and TP have also been reported in the literature (see Table 4). However, no systematic review is available that evaluates these procedures in terms of the phosphorus species likely to be encountered in natural waters and the operationally defined phosphorus fractions to be measured.

This paper presents an overview of the phosphorus species likely to be encountered in natural waters and the implications for the measurement of FRP, TFP and TP. Procedures reported in the literature for the storage and digestion of water samples for FRP, TFP and TP measurements are summarised and the advantages and limitations of methods discussed.

2. Types of phosphorus compounds in aquatic environments

Phosphorus compounds occurring in natural waters are classified as orthophosphates, condensed phosphates (pyro-, meta-, and other polyphosphates), organically bound phosphates and phosphonates. These compounds may contain \( \text{PO}_4^{3-} \), P–O–P, C–O–P and C–P bonds (Fig. 3). Phosphorus compounds occur in solution, in living aquatic organisms (e.g. algae, bacteria) and adsorbed to, or incorporated into abiotic and dead biotic particles [10,29,30]. The distribution between these compartments will be highly variable and dependent on the processes operating in aquatic systems [102].

2.1. Phosphorus in solution

Phosphorus forms likely to be encountered in dissolved forms are shown in Fig. 1. Orthophosphate is the oxidised state of phosphorus (\( \text{H}_2\text{PO}_4^- \), \( \text{HPO}_4^{2-} \), \( \text{PO}_4^{3-} \)) and its form is pH dependent. Orthophosphate is also the form in which phosphorus is most readily available for biological utilisation [80]. Orthophosphate applied to agricultural or residential cultivated land as fertilisers (e.g. \( \text{Ca(H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O} \), \( \text{NH}_4\text{H}_2\text{PO}_4 \), \( \text{NH}_4\text{PO}_4 \cdot 3\text{H}_2\text{O} \), etc.) carried into surface waters mainly via storm run-off often attached to particles [30]. Organic compounds containing esters of orthophosphoric acid (C–O–P) and phosphonates (C–P) are also present from the breakdown of organic material or excreted as metabolic products [31,66,85,97,120,138].

Condensed inorganic phosphates (P–O–P) such as triply phosphates, which are produced for use in detergents, are discharged with domestic and industrial wastewaters [90]. Organic condensed phosphates are generated by all plants and animals, e.g. ATP, ADP [26,44]. Condensed phosphates are only slowly hydrolysed in natural waters to orthophosphate [80,114,134,150].

2.2. Phosphorus associated with particles

Phosphorus forms likely to be encountered in natural waters associated with particles are shown in Fig. 1.

Broberg and Pettersson [18] have categorised three main sources of particulate phosphorus as:

- weathering products such as primary or secondary minerals,
- authigenic mineral formation by direct precipitation of inorganic phosphorus, and
- plant, animal and bacterial cellular material.

Thus, phosphorus in particles may be found incorporated in minerals, both crystalline and amorphous forms (e.g. \( \text{Ca}_4\text{Ti}(\text{PO}_4)_6(\text{OH})_2 \), \( \text{AlPO}_4 \cdot 2\text{H}_2\text{O} \), \( \text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O} \)), adsorbed/absorbed to particles and associated with organic matter [10]. Refractory phosphorus compounds are formed primarily as a result of microbial activity and the accumulation or of non-biodegradable plant material in sediments [1,127].

The ability of particles to adsorb/absorb phosphorus vary significantly [10,17,145]. Smaller particles (e.g. clay or silt) have larger surface areas and tend to adsorb more phosphorus [40,103]. In general, particles derived from neutral or alkaline soil/sediments contain calcium phosphate as the dominant phosphorus phase as water soluble phosphates react to form calcium.
<table>
<thead>
<tr>
<th>Phosphorus compound</th>
<th>Chemical formula</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO₄³⁻</td>
<td>AlPO₄·2H₂O</td>
<td><img src="image" alt="Structure PO₄³⁻" /></td>
</tr>
<tr>
<td>Variscite</td>
<td>Fe₅(PO₄)₃·8H₂O</td>
<td><img src="image" alt="Structure Variscite" /></td>
</tr>
<tr>
<td>Vivianite</td>
<td>Ca₁₀(PO₄)₃F₂</td>
<td><img src="image" alt="Structure Vivianite" /></td>
</tr>
<tr>
<td>Fluoroapatite</td>
<td>Ca₁₀(PO₄)₃(OH)₂</td>
<td><img src="image" alt="Structure Fluoroapatite" /></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td></td>
<td><img src="image" alt="Structure Hydroxyapatite" /></td>
</tr>
<tr>
<td>P-O-P</td>
<td></td>
<td><img src="image" alt="Structure P-O-P" /></td>
</tr>
<tr>
<td>Linear condensed phosphates</td>
<td>[-PO₄³⁻]</td>
<td><img src="image" alt="Structure Linear condensed phosphates" /></td>
</tr>
<tr>
<td>Cyclic condensed phosphates</td>
<td>P₂O₇⁵⁻</td>
<td><img src="image" alt="Structure Cyclic condensed phosphates" /></td>
</tr>
<tr>
<td>C-O-P</td>
<td></td>
<td><img src="image" alt="Structure C-O-P" /></td>
</tr>
<tr>
<td>Glucose-1-phosphoric acid</td>
<td>C₆H₁₁O₇P₂H₂</td>
<td><img src="image" alt="Structure Glucose-1-phosphoric acid" /></td>
</tr>
<tr>
<td>Inositolhexaphosphate (Phytic acid)</td>
<td>C₄H₁₈O₂₄P₆</td>
<td><img src="image" alt="Structure Inositolhexaphosphate" /></td>
</tr>
<tr>
<td>Phospho(enol)pyruvic acid</td>
<td>C₃H₄O₂P</td>
<td><img src="image" alt="Structure Phospho(enol)pyruvic acid" /></td>
</tr>
<tr>
<td>DL-α-glycerophosphate</td>
<td>C₅H₄O₃P</td>
<td><img src="image" alt="Structure DL-α-glycerophosphate" /></td>
</tr>
<tr>
<td>O- phosphoserine</td>
<td>C₃H₈N₂O₃P</td>
<td><img src="image" alt="Structure O- phosphoserine" /></td>
</tr>
<tr>
<td>C-P or N-P</td>
<td></td>
<td><img src="image" alt="Structure C-P or N-P" /></td>
</tr>
<tr>
<td>2- methylaminophosphonic acid</td>
<td>C₅H₁₀N₂O₃P</td>
<td><img src="image" alt="Structure 2- methylaminophosphonic acid" /></td>
</tr>
<tr>
<td>2-Aminoethylphosphonic acid</td>
<td>C₅H₈N₂O₄P</td>
<td><img src="image" alt="Structure 2-Aminoethylphosphonic acid" /></td>
</tr>
<tr>
<td>Phosphonoformic acid</td>
<td>CO₂PH₃</td>
<td><img src="image" alt="Structure Phosphonoformic acid" /></td>
</tr>
<tr>
<td>O-Phosphonylethanol amine</td>
<td>C₃H₈N₂O₄P</td>
<td><img src="image" alt="Structure O-Phosphonylethanol amine" /></td>
</tr>
</tbody>
</table>

Fig. 3. Chemical form and structure of P compounds.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Nutrients analysed (operationally defined)</th>
<th>Sample type</th>
<th>Filtered/not filtered</th>
<th>Storage</th>
<th>Storage container and preparationa</th>
<th>Maximum storage time</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collier and Marvin [27]</td>
<td>FRP</td>
<td>Sea water</td>
<td>Filtered</td>
<td>Quick freezing (−5 °F and room temperature)</td>
<td>Glass culture tubes washed with distilled water</td>
<td>61 days</td>
<td>Unfiltered samples lost FRP within 3 h. No change in FRP for 61 days if filtered samples frozen.</td>
</tr>
<tr>
<td>Murphy and Riley [112]</td>
<td>FRP</td>
<td>Sea water</td>
<td>F (Whatman No. 1)</td>
<td>Dark, 20°C with/without NaF, with/without CHCl₃ (7ml/l) with/without Al(OH)₃ and Th(CO₃)₂</td>
<td>Soda glass (150 ml) and polyethylene</td>
<td>4 days</td>
<td>CHCl₃ was the only effective preservative if samples stored in glass containers. Losses of FRP occurred when water stored in polyethylene containers.</td>
</tr>
<tr>
<td>Heron [65]</td>
<td>FRP</td>
<td>Lake water</td>
<td>Centrifuged at 3000 rpm or F (Pyrex no. 2 sintered glass funnel)</td>
<td>Room temperature with toluene, CHCl₃ (7ml/l) or dichloroethane and quick freezing</td>
<td>Polyethylene (1 l) treated with iodide solution</td>
<td>70 days</td>
<td>Frozen filtered lake samples stored in polyethylene containers treated with iodine stabilised phosphorus concentration for up to 70 days. Erratic changes if samples stored at room temperature with preservatives.</td>
</tr>
<tr>
<td>Jones [81]</td>
<td>FRP</td>
<td>Sea water</td>
<td>NF</td>
<td>Room temperature with CHCl₃ (0.1%) added</td>
<td></td>
<td>&lt;10 min</td>
<td>Release of phosphorus from particulate matter on addition of CHCl₃.</td>
</tr>
<tr>
<td>Gilmartin [49]</td>
<td>FRP</td>
<td>Estuarine</td>
<td>NF and F (Whatman No. 1)</td>
<td>Quick frozen (−5 to −10°C) and room temperature (18–21°C) with/without CHCl₃ (7 ml/l)</td>
<td>Polyethylene (112 ml)</td>
<td>4 days</td>
<td>No loss of FRP in samples stored at room temperature with CHCl₃ for short periods (6 h). Frozen samples with added CHCl₃ could be stored for at least four days. The best preservation technique was storage at −10°C with the addition of HgCl₂. CHCl₃ addition produced a dramatic reduction in FRP and TFP.</td>
</tr>
<tr>
<td>Jenkins [76]</td>
<td>FRP, TFP</td>
<td>Estuarine</td>
<td>NF and F (0.45 μm Millipore)</td>
<td>4°C with/without HgCl₂ (40 mg/l) or CHCl₃ (5 ml/l), −10°C with/without HgCl₂ (40 mg/l).</td>
<td>Plastic</td>
<td>30 days</td>
<td>Quick freezing with/without CHCl₃ stabilised nutrient concentrations.</td>
</tr>
<tr>
<td>Thayer [137]</td>
<td>FRP, TFP, TP</td>
<td>Estuarine</td>
<td>F (No. 10 bolting cloth)</td>
<td>Quick frozen (−10°C), or refrigeration (5°C) with/without CHCl₃</td>
<td>Polyethylene bags (1000 ml)</td>
<td>4–10 days</td>
<td></td>
</tr>
<tr>
<td>Authors</td>
<td>Nutrients analysed (operationally defined)</td>
<td>Sample type</td>
<td>Filtered/not filtered</td>
<td>Storage</td>
<td>Storage container and preparation&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Maximum storage time</td>
<td>Conclusions</td>
</tr>
<tr>
<td>------------------------------</td>
<td>--------------------------------------------</td>
<td>-------------</td>
<td>-----------------------</td>
<td>---------</td>
<td>-----------------------------------------------</td>
<td>---------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Nelson and Romkens [113]</td>
<td>P&lt;sup&gt;?&lt;/sup&gt;</td>
<td>Surface runoff</td>
<td>Centrifugation at 2500 rpm</td>
<td>Slow freezing at –20°C with/without removal of sediment, quick freezing with liquid N&lt;sub&gt;2&lt;/sub&gt; and storage at 2°C</td>
<td>Not specified</td>
<td>3 days</td>
<td>Storage of unfiltered samples at 2°C for three days was suitable. Freezing was suitable for preserving runoff samples provided sediment was removed prior to freezing.</td>
</tr>
<tr>
<td>Ryden et al. [124]</td>
<td>FRP</td>
<td>Distilled water, tap water and lake water</td>
<td>NF and centrifuged or F (0.45 μm Membrane filter)</td>
<td>4°C</td>
<td>Glass, polypropylene and polycarbonate, containers rinsed with combinations of distilled water, tap water, dilute HCl and phosphate solution</td>
<td>1 day</td>
<td>Phosphated polycarbonate and acid washed polypropylene and polycarbonate containers were suitable for storage of filtered lake samples at 4°C. Acid washed glass containers sorbed phosphorus (&lt;20 μg P/l) from distilled (80%) and lake waters (10–20%) within 1–6 h.</td>
</tr>
<tr>
<td>Latterell et al. [95]</td>
<td>RP</td>
<td>Standards in distilled water</td>
<td>NF</td>
<td>Room temperature at 23°C</td>
<td>Polyethylene with/without iodine impregnation (8 oz and 1 quart) and borosilicate glass (250 ml) washed with 1 N HCl and deionised water</td>
<td>1 day</td>
<td>Samples containing more than 0.03 mg/l orthophosphate can be stored in either polyethylene or borosilicate containers for only one day.</td>
</tr>
<tr>
<td>Johnson et al. [79]</td>
<td>FRP, TDP</td>
<td>Stream samples</td>
<td>NF and centrifugation at 17 000 rpm followed by F (0.05 μm membrane filter)</td>
<td>Freezing and refrigeration at 5°C</td>
<td>Glass</td>
<td>1 day</td>
<td>Freezing and refrigeration were found to be unsatisfactory for NF and centrifuged/filtered samples.</td>
</tr>
<tr>
<td>Bowditch et al. [15]</td>
<td>RP</td>
<td>Standards in distilled water</td>
<td>NF</td>
<td>Refrigeration at 4°C</td>
<td>HDPE (230 and 290 ml) and LDPE (290 ml) with/without iodine impregnation and soda glass (200 ml). Containers rinsed with dilute HCl</td>
<td>14 days</td>
<td>Storage in LDPE containers with/without iodine impregnation and HDPE with iodine impregnation showed no significant changes in RP concentrations.</td>
</tr>
<tr>
<td>Reference</td>
<td>FRP</td>
<td>Sample Type</td>
<td>Storage Conditions</td>
<td>Storage Container</td>
<td>Duration</td>
<td>Notes</td>
<td></td>
</tr>
<tr>
<td>--------------------</td>
<td>--------------</td>
<td>------------------------------</td>
<td>------------------------------------------------------------------------------------</td>
<td>-------------------</td>
<td>----------</td>
<td>----------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Klingaman and Nelson [86]</td>
<td>FRP</td>
<td>Surface runoff, tile drainage water and stream samples</td>
<td>Freezing at $-20^\circ$C, refrigeration at $4^\circ$C with/without HgCl$_2$ (40 mg/l) and phenyl mercuric acetate (20 mg/l)(PMA). Refrigeration at $4^\circ$C and room temperature ($23^\circ$C with PMA. Plastic (250 ml) sealed with rubber stoppers</td>
<td>84 days</td>
<td>No change in FRP if samples stored at $-20^\circ$C (84 days) or if stored at $4^\circ$C (84 days) with HgCl$_2$ or PMA (42 days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goltermen et al. [51]</td>
<td>FRP</td>
<td>Fresh water</td>
<td>Deep freezing at $-20^\circ$C. Room temperature with HgCl$_2$ (0–50 mg/l) in Polyethylene.</td>
<td>3 days</td>
<td>Recommended conditions given but no data for HgCl$_2$ interferes with the molybdenum blue method if ascorbic acid is used as the reductant. Samples showed no decrease in FRP if chloroform added and samples stored at $4^\circ$C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skjemstad and Reeve [130]</td>
<td>FRP</td>
<td>Standards added to rain water</td>
<td>F(0.45 μm Millipore)</td>
<td>Not specified</td>
<td>14 days</td>
<td>Samples showed no decrease in FRP if chloroform added and samples stored at $4^\circ$C.</td>
<td></td>
</tr>
<tr>
<td>Pichete et al. [118]</td>
<td>FRP</td>
<td>River water</td>
<td>F(0.45 μm Millipore)</td>
<td>Polyethylene, Teflon and glass (125 ml)</td>
<td>60 days</td>
<td>No significant change in TP concentration when samples frozen with/without acid.</td>
<td></td>
</tr>
<tr>
<td>Morse et al. [109]</td>
<td>FRP, TP</td>
<td>Open ocean</td>
<td>F (0.45 μm Nucleopore)</td>
<td>Polystyrene, glass (15 ml)</td>
<td>365 days</td>
<td>Little change in FRP if samples are frozen provided turbid samples are filtered. Quick freezing reduces losses and increased precision. Cooled filtered samples could be stored for at least 56 days if CHCl$_3$ added. If no CHCl$_3$ added unfiltered/filtered samples could be stored for 1–6 days. Coated samples containing HgCl$_2$ showed no loss in FRP and TFP after 16 days. Sulphuric acid caused an increase in FRP.</td>
<td></td>
</tr>
<tr>
<td>MacDonald and McLaughlin [101]</td>
<td>FRP</td>
<td>Coastal and estuarine</td>
<td>NF and F (0.45 μm Millipore) and NF</td>
<td>Polyethylene (20 ml)</td>
<td>56 days</td>
<td>Cooled filtered sample could be stored for at least 56 days if CHCl$_3$ added. If no CHCl$_3$ added unfiltered/filtered samples could be stored for 1–6 days.</td>
<td></td>
</tr>
<tr>
<td>Hagebo and Rey [56]</td>
<td>FRP</td>
<td>Open ocean and standards</td>
<td>NF and F (Whatman GF/C)</td>
<td>Polyethylene (20 ml)</td>
<td>56 days</td>
<td>Cooled filtered sample could be stored for at least 56 days if CHCl$_3$ added. If no CHCl$_3$ added unfiltered/filtered samples could be stored for 1–6 days.</td>
<td></td>
</tr>
<tr>
<td>Fishman et al. [45]</td>
<td>FRP, TFP</td>
<td>Lake, river, tap water and standards added to distilled water</td>
<td>Ambient temperature with/without CHCl$_3$ (2 ml/l) and 4°C with/without HgCl$_2$ (52 mg/l), H$_2$SO$_4$ (0.072 M) and CHCl$_3$ (2 ml/l). Polyethylene amber (250 ml)</td>
<td>16 days</td>
<td>Coated samples containing HgCl$_2$ showed no loss in FRP and TFP after 16 days. Sulphuric acid caused an increase in FRP.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Authors</td>
<td>Nutrients analysed (operationally defined)</td>
<td>Sample type</td>
<td>Filtered/not filtered</td>
<td>Storage</td>
<td>Storage container and preparation$^a$</td>
<td>Maximum storage time</td>
<td>Conclusions</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>--------------------------------------------</td>
<td>--------------------</td>
<td>-----------------------</td>
<td>---------</td>
<td>--------------------------------------</td>
<td>----------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Anon [2]</td>
<td>TP</td>
<td>Natural water</td>
<td>NF</td>
<td>Freezing with 1% HCl −18°C, 4°C with/without HgCl$_2$ (10 mg/l)</td>
<td>Polyethylene</td>
<td>28 days 109 days</td>
<td>Frozen samples showed a slight FRP decrease (5–7%) with time. Cooled samples showed a marked FRP decrease with time</td>
</tr>
<tr>
<td>Kremling and Wenck [89]</td>
<td>FRP</td>
<td>Open ocean</td>
<td>NF</td>
<td></td>
<td>Polypropylene (60 ml). Detergent washed, stored in 0.1% HCl and rinsed with sample</td>
<td></td>
<td>No change in TP occurred in frozen samples for up to six months</td>
</tr>
<tr>
<td>Lambert et al. [92]</td>
<td>TP</td>
<td>Turbid lakes</td>
<td>NF</td>
<td>Frozen (−10°C)</td>
<td>LDPE (250 ml), soaked in 1 M HCl and rinsed in deionised water</td>
<td>180 days</td>
<td>No change in TP occurred in frozen samples for up to six months</td>
</tr>
<tr>
<td>Clementon and Wayte [25]</td>
<td>FRP</td>
<td>Sea water</td>
<td>F (0.45 μm Millipore)</td>
<td>Initially frozen at −40°C and then stored at −20°C</td>
<td>HDPE (250 ml), HDPE (50 ml), HDPE (20 ml) and polypropylene (10 ml), soaked in 10% HCl for 48 h and rinsed in deionised water</td>
<td>147–210 days</td>
<td>Concentration of FRP steadily decreased in samples stored for longer than four months. HDPE containers were preferred for water storage</td>
</tr>
<tr>
<td>Kirkwood [84]</td>
<td>P?</td>
<td>Sea water and standards added to sea water</td>
<td>NF</td>
<td>Room temperature and refrigeration with HgCl$_2$ (20 mg/l)</td>
<td>Polypropylene (20 ml) and polycarbonate (20 ml)</td>
<td>365 days</td>
<td>No change in P if samples stored in polypropylene with HgCl$_2$ added</td>
</tr>
<tr>
<td>Avanzino and Kennedy [9]</td>
<td>FRP</td>
<td>Stream water</td>
<td>F (0.45 μm Millipore HATF membrane)</td>
<td>Freezing at −16°C</td>
<td>LDPE soaked in 1 M HCl for 16 h and rinsed in distilled water</td>
<td>4–8 years</td>
<td>No significant change in FRP concentration provided precipitation does not occur</td>
</tr>
<tr>
<td>Haygarth et al. [63]</td>
<td>FRP</td>
<td>Soil leachates</td>
<td>F (0.45 μm Millipore membrane)</td>
<td>Room temperature (5–19°C), refrigeration (4°C) frozen (−20°C) with/without HgCl$_2$ (40–400 mg/l) and H$_2$SO$_4$ (0.04 M)</td>
<td>Polyethylene, polystyrene and Teflon (30 ml and 201) pre-washed in 10% H$_2$SO$_4$ and deionised water. One set of containers were iodine impregnated with iodide</td>
<td>1–2 days</td>
<td>Changes occurred within two days for all samples. Smallest changes occurred in samples stored at room temperature or in a refrigerator. Preservatives were not recommended</td>
</tr>
</tbody>
</table>

$^a$Container preparation stated when given.
F=filtered.
NF=not filtered.
HDPE=high density polyethylene.
LDPE=low density polyethylene.
RP=reactive phosphorus
phosphate precipitates [73,127]. Particles derived from acid soils and sediments contain phosphorus combined with iron and aluminium phases because of the positive charges on the soil particles [29]. Phosphorus associated with iron and aluminium phases is relatively insoluble as under oxidising conditions rates of dissolution are slow [127,142]. Phosphorus adsorbed to iron phases is released under reducing conditions [110,111].

Organic phosphorus compounds are formed primarily by biological processes and may be produced in situ (e.g. algal products) [97] or enter via sewage effluent containing body wastes and food residues [90]. Organic phosphorus compounds in soils entering natural waters are principally esters of orthophosphoric acid [1,17]. The most common form is myo-inositol phosphate, also known as phytic acid. The most likely source of these compounds is plant material [94] and it is believed that other forms of inositol hexaphosphates are the result of microbial utilisation of myo-inositol phosphate [21,32,94]. Other organic phosphate compounds found in soil organic matter are nucleic acids, phosphoproteins, sugar phosphorus compounds (glucose-1-phosphate, glucose-6-phosphate) and glycerol phosphates [1,117]. Unweathered organic materials are usually higher in nucleic acids than inositol phosphates, but nucleic acids are rapidly broken down to orthophosphate [10,17]. Many organisms also produce relatively refractory organic phosphorus compounds containing C–P bonds [31,66,138].

From the proceeding discussion it is clear that any digestion procedure used to convert all the potential forms of phosphorus in natural waters to a form suitable for analysis must be able to release phosphorus incorporated in or adsorbed to mineral phases of particles, into solution.

3. Storage

Many techniques are described in the literature for the storage of water samples prior to nutrient determination (Table 1). We have indicated where possible the operationally defined phosphorus fraction measured. These fractions are described in Fig. 3. Other authors have further sub-divided these fractions (e.g. see [76,104,116,121,122]) but their measurement is not considered routine and are not considered in this review.

Ideally samples should be analysed immediately after collection to minimise any changes in the concentrations of phosphorus over time. This is not always possible when analytical equipment fails during an intensive field program or sample collection is from remote locations, and samples must be stored before analysis.

Storage techniques are usually variants of methods for preserving samples by reducing the activity of micro-organisms present in the sample [86]. The most widely used methods of preservation include storage at room temperature, refrigeration, freezing, biocide amendment or acidification. Often samples are filtered before storage. Filtering has been used to remove bacteria and plankton that may alter nutrient concentrations. However, filtration may not eliminate colloidal particulate matter that can adsorb or release nutrients on standing [25,92]. Filtration is not appropriate for TP measurements as it fundamentally alters the composition of samples.

3.1. Room temperature storage

3.1.1. Filterable reactive phosphorus

Collier and Marvin [27] stored sea water samples with added orthophosphate at room temperature and showed that rapid changes in FRP occurred in filtered and unfiltered samples (3 and 1.5 h, respectively). Murphy and Riley [112] added chloroform to filtered sea water samples which were stored in glass containers at 20°C. They concluded that this procedure...
stabilised FRP concentrations for four days. Jones [81] showed that unfiltered samples could not be preserved with chloroform for FRP measurements. Addition of chloroform resulted in the immediate release of phosphorus from particulate matter. Gilmartin [49] showed that estuarine samples with added chloroform could only be stored in polyethylene containers for 6 h. Heron [65] compared the storage of unfiltered lake water samples in polyethylene containers treated with iodine at room temperature with the addition of toluene, chloroform and dichloroethane. He found that the addition of toluene and chloroform resulted in an increase in FRP concentration while the addition of dichloroethane prevented significant decreases in FRP concentration for three days. Klingaman and Nelson [86] added phenyl mercuric acetate to preserve unfiltered run-off water samples stored in plastic bottles (unspecified) at 23°C. An increase in FRP concentration was found after three days. They suggested that this increase was caused by the lysis of bacteria cells. Skjemstad and Reeve [130] showed that phosphorus standards mixed with filtered rain water with mercuric chloride added could be stored in dark glass containers for three days. They noted that mercuric chloride interferes with the molybdenum blue method if ascorbic acid is used as the reductant. Pichet et al. [118] added potassium fluoride, thymol, sulphuric acid, tributyltin chloride and chloroform to filtered river water samples which were stored at 20°C. The container composition was not specified. A decrease in FRP occurred in all samples after one day. Fishman et al. [45] showed that filtered freshwater samples could be stored for only one day for FRP measurements if chloroform was added.

3.1.2. Total filterable phosphorus

Fishman et al. [45] showed that filtered freshwater samples could be stored at room temperature for 1–8 days for TFP measurements if chloroform was added.

3.1.3. Other (phosphorus fraction analysed not specified)

Latterell et al. [95] have stored synthetic water samples in polyethylene and borosilicate glass containers at 23°C for one day with negligible phosphorus losses (0.5%), but losses increase to 3–36% after 14 days. Fishman et al. [45] showed that orthophosphate standards added to distilled water could be stored at room temperature for 1–8 days for phosphorus measurements if chloroform was added. Kirkwood [84] found that sea water samples stored in polyethylene containers with the addition of mercuric chloride showed no change in phosphorus concentration after 365 days. Kirkwood also compared the storage of orthophosphate added to unfiltered sea water samples in polycarbonate containers at room temperature and showed that on the addition of mercuric chloride no differences in phosphorus concentrations were found between the storage procedures. The maximum period of storage was not specified.

3.2. Refrigeration

3.2.1. Filterable reactive phosphorus

Jenkins [76] compared the storage of filtered estuarine water samples in plastic containers at 4°C with/without the addition of chloroform or mercuric chloride. Immediate changes in FRP concentrations occurred, i.e. within one day. Thayer [137] when storing gross filtered estuarine water samples in polyethylene bags at 5°C with and without chloroform found that changes in FRP occurred within 3 h. Nelson and Romkens [113] reported no changes in FRP in unfiltered surface run-off samples stored at 2°C for three days. Ryden et al. [124] showed that filtered lake and tap water samples could be stored at 4°C for one day in polyethylene and polycarbonate containers. Johnson et al. [79] reported that refrigeration of centrifuged/filtered stream samples in glass containers at 5°C was not satisfactory for storing samples even for one day, 50% of FRP was lost within two days. Klingaman and Nelson [86] examined methods for preserving the concentrations of FRP in unfiltered surface run-off, tile drainage water, and river water samples in plastic containers (unspecified) stored at 4°C. They found that the addition of mercuric chloride or phenylmercuric acetate stabilised FRP concentrations for 42 days. Pichet et al. [118] found that for
filtered river water samples, FRP concentrations were stable for at least 14 days at 4°C when chloroform was added as a preservative. The container material was not specified. Morse et al. [109] presented contradictory results for filtered open ocean samples stored in glass, polyethylene or Teflon containers at 2°C. Changes in FRP occurred after one day for one sampling run while no changes occurred for at least seven days after another sampling run. Hagebo and Rey [56] showed that no decrease of FRP in filtered/non-filtered open ocean samples occurred for 1–6 days when samples were stored in polyethylene containers at 1–3°C. If chloroform was added, filtered samples could be stored for at least 56 days. Kremling and Wenck [89] reported that when unfiltered ocean water samples were stored in polyethylene containers with and without the addition of mercuric chloride losses of FRP occurred within five days. 50% of FRP was lost after 60 days. Fishman et al. [45] found that for filtered lake, river, creek and tap water samples stored in polyethylene containers at 4°C, no decrease in TFP occurred for 16 days. Samples to be preserved for longer periods (16 days) needed mercuric chloride added. The addition of sulphuric acid caused an increase in FRP. Haygarth et al. [63] investigated the storage of filtered soil leachates in polyethylene containers refrigerated at 4–5°C. Changes in FRP occurred within 24–48 h.

3.2.2. Total filterable phosphorus

Jenkins [76] compared the storage of filtered estuarine water samples in plastic containers at 4°C with/without the addition of chloroform or mercuric chloride. Samples with mercuric chloride or chloroform added could be stored for five days. Thayer [137] when storing gross filtered estuarine water samples in polyethylene bags at 5°C, with and without the addition of chloroform, found that changes in TFP occurred within 3 h. Johnson et al. [79] reported that refrigeration of centrifuged/filtered stream samples in glass containers at 5°C was not to be satisfactory for storing samples even for one day. Morse et al. [109] have shown that filtered ocean water samples can be stored at 2°C in polyethylene, glass or Teflon containers for at least 60 days. Fishman et al. [45] found that for filtered lake, river, clear creek and tap water samples stored in polyethylene containers at 4°C, with and without the addition of mercuric chloride, no decrease in TFP occurred for 16 days.

3.2.3. Other (phosphorus fraction analysed not specified)

Bowditch et al. [15] showed that phosphorus standards in distilled water could be stored in low density polyethylene containers with and without iodine impregnation and high density polyethylene containers with iodine impregnation for 14 days. Kirkwood [84] reported no changes in the phosphorus concentrations of refrigerated sea water samples stored in polyethylene containers with chloroform added for 365 days.

3.3. Freezing

3.3.1. Filterable reactive phosphorus

Collier and Marvin [27] quick froze filtered sea water samples to which orthophosphate had been added and found that samples could be stored for at least 61 days. Unfiltered samples could be stored frozen for at least seven days without changes in FRP occurring. Heron [65] found that when filtered or centrifuged lake water samples stored in polyethylene containers were frozen no change in FRP concentration occurred for up to 70 days. His study also showed that unfiltered centrifuged samples and samples filtered through sintered glass funnels (Pyrex No. 2) before storage had no difference in the measured FRP concentration. Jenkins [76] showed that estuarine water samples could be stored in plastic containers at −10°C for FRP analyses if mercuric chloride was added as a preservative. The use of chloroform as a preservative resulted in a large loss of FRP. Gilmartin [49] showed that no loss of FRP occurred for at least four days occurred in estuarine water samples stored frozen (−5°C to −10°C) in polyethylene containers with chloroform added. Thayer [137] showed that gross filtered estuarine water samples could be stored in polyethylene bags frozen (−10°C) with or without the addition of chloroform for 4–10 days. Klingaman and Nelson [86] found that unfiltered water samples from tile drains and rivers could be stored at −20°C in plastic bottles (unspecified) for up to 84 days. MacDonald and McLaughlin [101] analysed both filtered and unfiltered estuarine and sea water samples immediately on collection and after freezing in glass and
polyethylene containers for a period of 0.5, 1, 2, 5, and 12 months. Turbid samples needed to be filtered before storage. On average, the samples that were quick frozen on collection had FRP concentrations 1% lower than the original sample. Water samples that had been slowly frozen were on average 3% lower in FRP concentration. Kremling and Wenck [89] found that if samples of unfiltered river water, surface run-off and agricultural drainage were stored in polyethylene containers frozen (−18°C), no change in FRP concentrations occurred for up to 109 days. Clementson and Wayte [25] found that filtered sea water samples quick frozen at −40°C and subsequently stored at −20°C in high density polyethylene containers showed no changes in FRP for 147–210 days. Avanzino and Kennedy [9] found that filtered stream water samples could be frozen in low density polyethylene containers for 4–8 years without significant changes in FRP concentration occurring.

Some studies have reported the loss of FRP from solution when water samples are frozen. Johnson et al. [79] found that the centrifuged stream water samples stored in glass containers lost 33–78% of FRP upon freezing within 24 h. The FRP loss was attributed to the formation of calcite residues during the freezing process. Pichet et al. [118] preserved filtered river water samples with potassium fluoride, thymol, sulphuric acid, tributyltin chloride or chloroform which were stored at −10°C. A decrease in FRP occurred in all samples within three days and the decrease was similar to that of samples frozen without a preservative. Morse et al. [109] showed that when filtered open ocean samples were frozen and stored in glass, polyethylene or Teflon containers, changes in TFP occurred after one day for one sampling run while no changes occurred for at least seven days after another sampling run. Haygarth et al. [63] investigated the storage of filtered soil leachates in polyethylene containers frozen (−20°C). Changes in FRP occurred within 24–48 h.

3.3.2. Total filterable phosphorus

Jenkins [76] showed that estuarine water samples could be stored in plastic containers for 30 days at −10°C if mercuric chloride was added as a preservative. Chloroform could not be used as a preservative. Thayer [137] showed that gross filtered estuarine water samples stored in polyethylene bags and frozen (−10°C) with or without chloroform could be stored for 4–10 days. Johnson et al. [79] reported that freezing of centrifuged/filtered stream samples in glass containers was not satisfactory for storing samples even for one day. Morse et al. [109] showed that when filtered open ocean samples were frozen and stored in glass, polyethylene or Teflon containers, no changes in TFP occurred for at least 60 days.

3.3.3. Total phosphorus

Lambert et al. [92] found that when unfiltered turbid lake water samples were stored in low density polyethylene containers frozen (−10°C), no change in TP concentrations occurred for up to 150 days.

3.3.4. Other (phosphorus fraction analysed not specified)

Nelson and Romkens [113] have shown that quick freezing of centrifuged run-off samples has no advantage over slow freezing unless suspended sediment was removed prior to freezing. Centrifuged water samples could be stored for at least three days.

3.4. Filtration before storage (filterable reactive and total filterable phosphorus measurements)

Fitzgerald and Faust [46] found that freezing or the addition of chloroform appeared to release phosphorus from algal cells and suggested samples be filtered prior to preservation or freezing. Lambert et al. [92] showed that if filtration was not conducted within an hour of collection then FRP and TFP concentrations reported may be meaningless because of exchange on and off particles in the sample container.

Most studies that have separated suspended materials from water samples prior to storage have used 0.45 μm membrane filters (Table 1) as a compromise between the slow rate of filtration and poor retention of particles [76]. The use of cellulose filter papers are not recommended for filtration because of the contamination from the fibres and the uncertainty of their pore sizes (around 2 and 0.8 μm for the types cited) [13,72].

Problems associated with membrane filtration are well documented. Bickford and Willett [13] found that membrane filters containing wetting agents can interfere in filterable phosphorus measurements. They recommended that membrane filters be washed with
acid prior to analysis to remove interfering materials. Danielsson [34] studied the effect of filtration on the colloid content in natural water samples using 0.45 μm Millipore and 0.40 μm Nucleopore membrane filters. He found that these membrane filters could not be used to separate particles, colloidal material and truly dissolved material as the effective pore size changed during filtration. Hughes and Macphee [72] and Tarapchak et al. [136] showed that FRP measured in filtered water samples was dependent on the volume of water filtered and the vacuum pressure used. Both these factors influenced the amount of phosphorus released from particles and retained on filters. Bloesch and Gavrieli [14] found that filtration using pressure could cause the breakdown of algal cells, resulting in the increase of FRP concentration. Broberg and Pettersson [18] have listed clogging, contamination, variation in pore-size and destabilisation of colloids, as factors which cause variability in measured FRP concentration. Stockner et al. [133] stated that the results of dissolved organic phosphorus measurements may be exaggerated by inclusion of particulate femto- and picoplankton in the filtrate caused by the leakage through the membrane filter. Eisenreich et al. [43] showed that prior centrifugation of water samples eliminated problems caused by membrane filtration, e.g. clogging. They suggested that centrifugation should be used for the separation of particulates from samples containing large quantities of suspended materials.

3.5. Storage containers and pretreatment

3.5.1. Filterable reactive phosphorus

Murphy and Riley [112] recommended the use of soda glass for the storage of filtered sea water samples prior to FRP analysis. Large losses of FRP occurred when samples were stored in polyethylene bottles. They suggested that adsorption of phosphate on to the wall of the polyethylene containers was occurring. Heron [65] recommended the use of polyethylene bottles treated with iodide solution for the storage of filtered or centrifuged lake water samples prior to FRP analysis. They attributed any reduction in FRP concentrations to bacterial uptake not adsorption to polyethylene. Hassenteufel et al. [62] found that the uptake of FRP (60 μgP/l, pH 7.5–8) was three times greater for polyethylene and polyvinylchloride containers than glass containers with Teflon adsorbing the least amount of FRP. Treatment of glass with 0.5–1% hydrofluoric acid in 2 M hydrochloric acid or distilled water reduced FRP uptake. Ryden et al. [124] studied the sorption of orthophosphate standards by glass, polypropylene and polycarbonate containers. They found that when containers were only rinsed with distilled water, that phosphorus sorption occurred from standards in distilled water (i.e. 100% at 2 μgP/l; 52–100% at 5 μgP/l, 24–52% at 10 μgP/l, 18–28% at 20 μgP/l and 7–13% at 60 μgP/l). When containers were rinsed with dilute hydrochloric acid, a decrease in phosphorus sorption by the polycarbonate containers occurred and an increase in phosphorus sorption by glass containers was observed. Acid washed polypropylene containers adsorbed 2.5–3.2 μgP/l while acid washed glass adsorbed 5–17 μgP/l solutions, respectively. For lake water samples little adsorption of phosphorus by acid washed polypropylene and polycarbonate containers occurred while 1–2 μgP/l of FRP was adsorbed within 1–6 h when samples were stored in acid washed glass containers. Polycarbonate bottles previously rinsed in 3 μg/l potassium dihydrogen phosphate then tap water and distilled water were also found to be satisfactory for the storage of lake water samples for at least one day and were recommended for the storage of water samples with low phosphorus concentrations. Adsorption by phosphated glass and phosphated polypropylene was variable indicating sorption and release of phosphorus by the treated containers. Clementson and Wayte [25] studied the effect of freezing on the storage of open ocean sea water samples (33 μg P/l) in high density polyethylene bottles over a period of 700 days. Three bottle sizes (20, 50 and 250 ml) with different surface area/volume ratios were examined. They found that the concentration of FRP steadily decreased while 1–2 μgP/l of FRP after this time could not be explained, but their study showed that surface adsorption was not important. Their study also showed that non-acid washed polypropylene containers were unsuitable for the storage of water samples. Subsequently they showed that FRP concentrations decrease more slowly in acid washed containers. Haygarth et al. [63] investigated the storage of spiked filtered soil leachates in polyethylene, polystyrene and PTFE containers which had been prewashed in 10% sulphuric acid and deionised.
water. Smaller losses occurred in PTFE containers and larger containers vs smaller containers (20 l vs 30 ml). It was concluded that the effect of container material was not as important as the storage environment.

3.5.2. Total phosphorus
Lambert et al. [92] showed that no change in TP concentrations occurred in frozen unfiltered turbid lake samples (30–100 µgP/l) for up to 168 days after collection when acid washed low density polyethylene containers were used. They concluded that adsorption of phosphorus on to the sample container was not significant as this would also have resulted in a decrease in the TP concentration measured.

3.5.3. Other (phosphorus fraction analysed not specified)
Latterell et al. [95] studied the sorption of orthophosphate by borosilicate glass and polyethylene containers. They showed that aqueous solutions containing 30–112 µgP/l of orthophosphate may be stored in either borosilicate or polyethylene bottles with less than 0.5% of the orthophosphate being adsorbed during a 24 h storage period and 3–36% adsorption after 14 days storage. Sorption of orthophosphate by polyethylene containers impregnated with iodine was 2.6–4% compared to 7–17% by untreated containers. More adsorption occurred as the ratio of surface area to solution volume increased. Bowditch et al. [15] compared the use of low density polyethylene and high density polyethylene bottles with/without iodine impregnation and soda glass containers for the storage of phosphorus in synthetic water samples containing orthophosphate (10 µgP/l). They found that storage of phosphorus standards in low density polyethylene bottles with/without iodine impregnation and high density polyethylene with iodine impregnation showed no significant changes in phosphorus concentrations after 14 days.

3.6. Discussion
3.6.1. Introduction
Many processes can occur in the sample container when water samples are stored that may increase or decrease the measured phosphorus fraction. A summary of the reactions that may occur and possible effect on operationally defined phosphorus fractions is given in Table 2.

Factors influencing whether these reactions occur and that ultimately determine the effectiveness of any particular storage technique include:

- the composition of the sample,
- whether the sample was filtered, the type of filter and filtration procedure,

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactions that may occur in containers and effect on operationally defined phosphorus fractions</td>
</tr>
<tr>
<td>Reaction</td>
</tr>
<tr>
<td>Physical</td>
</tr>
<tr>
<td>Temperature dissolution</td>
</tr>
<tr>
<td>Sorption</td>
</tr>
<tr>
<td>Destabilisation of colloids</td>
</tr>
<tr>
<td>Chemical</td>
</tr>
<tr>
<td>Chemical hydrolysis</td>
</tr>
<tr>
<td>Enzymatic hydrolysis</td>
</tr>
<tr>
<td>Oxidation/reduction</td>
</tr>
<tr>
<td>Precipitation</td>
</tr>
<tr>
<td>Complexation</td>
</tr>
<tr>
<td>Biological</td>
</tr>
<tr>
<td>Microbial growth</td>
</tr>
<tr>
<td>Microbial death</td>
</tr>
<tr>
<td>Algal growth</td>
</tr>
<tr>
<td>Algal cell disruption</td>
</tr>
</tbody>
</table>
• container composition and pretreatment,
• the presence or absence of light,
• the storage temperature and how it was achieved, and
• the time taken to analyse the sample after storage.

In this review, information on these factors (when available) has been given in Table 1 and the text. However, rarely in these studies has more than one factor been studied. Notable exceptions are the studies conducted by Morse et al. [109] and MacDonald and McLauglin [101]. Thus the studies reviewed can only be used to give a general indication of what procedures are likely to be effective for storing water samples. In the discussion that follows we have attempted to determine the influence of each factor on the storage of water samples.

3.6.2. Sample composition

Freshwater, groundwater, estuarine and sea water samples will have varying chemical compositions and biological communities. Chemical and biological characteristics may change from season to season and change storage characteristics (see [49,109]).

Ionic strength will influence the magnitude of surface adsorption as competition between phosphorus and major anions occurs for adsorption sites. The presence of particles and colloids provides surfaces on which adsorption–desorption reactions can occur. Acidity (pH) will influence the type of complexes phosphorus can form in solution. Precipitation of calcite or iron flocs containing phosphorus may occur during aeration of water samples, alternatively dissolution of particle phases can also occur. Different assemblages of phytoplankton, zooplankton, bacteria, extra cellular enzymes and abiotic particles may increase or decrease the measured FRP, TFP and TP concentration depending on the dominant processes operating. For example bacteria may take up phosphorus if in a growth phase but if dying, lysis of bacteria cells will release phosphorus into solution.

The implications for water sample storage is that careful consideration should be given to the composition of water samples to be stored and appropriate action taken to minimise expected changes. For example if precipitation of calcite or iron flocs is likely, acid should be added to prevent the formation of precipitates either on freezing or aeration [79]. Water samples of low ionic strength may require containers specifically prepared to prevent phosphorus adsorption. Filtration may need to be carefully carried out to remove plankton, etc. without rupturing cells and releasing phosphorus in to solution if FRP measurements are being made.

3.6.3. Filtration of samples

Many studies report that a significant decrease in FRP occurs if samples are not filtered before storage (see [27,124]) even if suspended solid concentrations are low. Filtration will eliminate particles that adsorb phosphorus and will also remove bacteria, which may utilise phosphorus and adhere to container surfaces (see Section 3.6.5).

The studies of Fitzgerald and Faust [46] and Lambert et al. [92] conclusively show that if any other operationally defined fraction other than TP is to be measured then filtration must be carried out immediately upon sampling, and hence prior to storage. Exchange of phosphorus on and off particles can occur in the container as well as release from algal cells. The addition of preservatives such as chloroform can result in the immediate release of phosphorus from particles [81], while filtration after freezing is not advisable as freezing may rupture cells and release phosphorus into solution [14,46,65].

The reported dependence of FRP (and probably TFP) measurements on the filter type used, the volume of water filtered and the vacuum pressure used [72,136], the destabilisation of colloids by filtration [18] and the rupture of algal cells [14,65] seriously brings into question the value of FRP and TFP measurements. It is our opinion that if FRP and TFP are measured that considerable thought be given to what the measured values mean. If they are to be used as a measure of algal bioavailable phosphorus then filtration procedures should be rigorously standardised and results compared to those estimated using algal bioassays [108].

3.6.4. Container composition and pretreatment

The magnitude of adsorption of phosphorus from a stored water sample will be dependent on the container material, the container pretreatment, the concentration of phosphorus in solution and the sample matrix Glass, low and high density polyethylene, polypropylene and polycarbonate containers have
been used for storage of water samples. Where poly-
ethylene containers have been used it is not clear in
many reported studies if low or high density material
has been used. Often how containers have been
cleaned prior to usage has not been stated. When
container preparation is reported, rinsing with either
deionised water or dilute acid followed by deionised
water is commonly used [9,15,25,89,92,124,130].

All containers sorb some phosphorus [62,124].
Plastics contain a range of stabilisers, fillers, trace
metals (Cd, Pb) and polarised bonds; glass contains
positive charged sites, e.g. $\text{Al}^\text{I} \cdot \text{OH}^\text{II}$, $\text{Si}^\text{I} \cdot \text{OH}^\text{II}$ that
may adsorb phosphorus. Rinsing with dilute acid has
been shown to reduce adsorption by some plastics
[25,124]. Rinsing with hydrofluoric acid has been
shown to reduce/eliminate phosphorus adsorption
by glass containers [62]. The efficiency of fluorine
ions in preventing the adsorption of phosphorus is
probably due to fluorine occupying positions held by
adsorbed hydroxide ions. Rinsing of glass containers
with dilute acid increases phosphorus adsorption pos-
sibly by the creation of active adsorption sites
$\text{Al}^\text{I} \cdot \text{OH}^\text{II}$, $\text{Si}^\text{I} \cdot \text{OH}^\text{II}$ [124]. Several authors [15,65]
have shown that if containers are impregnated with
iodine adsorption of phosphorus decreases. The
authors suggested that this reduction is due to the
reduction in bacterial uptake of phosphorus. However,
addition of iodine/iodide may also saturate adsorption
sites on the container walls. The question raised is that
if bacterial utilisation of phosphorus is important how
should bottles be sterilised? Rinsing with deionised
water may be insufficient and a more rigorous washing
with iodine or dilute acid may be required. Acid
washing seems to have the advantage of killing bac-
teria and reducing phosphorus adsorption by plastic
containers.

Surface adsorption by containers may only be
important for samples containing low concentrations
of phosphorus and only for water samples of low ionic
strength. Many papers [65,112,124] have shown large
losses of FRP for samples containing low concentra-
tions of FRP (1.3–9.3 $\mu$gP/l). Adsorption of phos-
phorus by containers is also greater for phosphorus
standards prepared in distilled water than in natural
waters during storage [124]. Generally lower losses
are reported for samples containing higher phosphorus
concentrations [95,124], although it may be that rela-
tive losses are reduced but absolute losses may not
have decreased, and samples with high ionic strength,
e.g. sea water [25,101]. If water samples that have low
phosphorus concentrations are to be stored, to reduce
adsorption, consideration should be given to selecting
containers with the lowest volume to surface ratio [95]
or rinsing containers with orthophosphate or iodide to
saturate adsorption sites [15,65].

Acid washed low density polyethylene containers
appear to be suitable for the storage of most types of
water samples. Low density polyethylene bottles have
been reported to be suitable for storage of frozen
samples for TP analysis for up to 168 days [92] and
for FRP analysis for up to 4–8 years [9]. Little
published literature is available on the use of high
density polyethylene bottles for the storage of water
samples. Bowditch et al. [15] showed that significant
changes in phosphorus occurred over short times (<1
day) compared to low density polyethylene containers
(14 days) when these containers were used to store
phosphorus standards. Changes of FRP can be reduced
by impregnating containers with iodine. Clementson
and Wayte [25] have shown that filtered sea water
samples could be stored for 147–210 days in high
density polyethylene containers. The use of high
density polyethylene containers needs to be further
evaluated before use.

3.6.5. Preservatives

Preservatives are added for three purposes [2]:

- to stop metabolic processes,
- to prevent precipitation and flocculation, and
- to reduce surface adsorption.

Mercuric chloride, chloroform, hydrochloric acid
and sulphuric acid are commonly used as preserva-
tives, but the use of iodine, alkali, phenylmercuric
acid, tributyl tin and dichloromethane have been
reported (Table 1).

Various concentrations of preservatives have been
recommended in the literature without any explana-
tion of why particular concentrations were chosen (see
Table 1). Kirkwood [84] has a good summary of the
various concentrations of mercury compounds used to
preserve natural water samples. The optimum amount
of preservative will probably depend on the composi-
tion of the sample, i.e. the amount of flora and fauna to
be killed.
Chloroform is a good example of the advantages/disadvantages and contradictions reported in the literature associated with the use of preservatives. Chloroform appears to be an effective biocide only if samples are saturated [49,112] and seems to help stabilise phosphorus concentrations during freezing and thawing. However, some studies have shown that the addition of chloroform produces a dramatic change in FRP and TFP in filtered samples [76,137]. The addition of chloroform can result in the immediate release of phosphorus from particles such as algal cells [46,81,137], thus water samples to be analysed for FRP and/or TFP must be filtered before the addition of chloroform. Chloroform can also interfere in the colourimetric determination of phosphorus [2,130].

The use of mercuric chloride and alkali as preservatives offers additional problems as these compounds can precipitate bacteria and proteins. Mercury has also been shown to interfere in the colourimetric determination of phosphorus [63,130,139].

We believe that the use of preservatives should be avoided where possible because of the potential release of phosphorus from sediment particles, bacteria and algae even in filtered water samples [46,63], contamination and colourimetric method interference problems. However, the addition of acid may be needed sometimes to prevent precipitation in water samples during freezing [2,71,79].

3.6.6. The presence or absence of light

It is unclear from the papers reviewed whether water samples stored at room temperature are stored in the presence or absence of light. It is assumed that storage in refrigerators and freezers will effectively exclude light, although this may not be the case if samples are being frequently removed. Light exclusion is essential at room temperature and desirable at reduced temperature to prevent algal growth. Photosynthesis will reduce FRP and TFP concentrations. As shown by Stockner et al. [133] filtration through a 0.45 μm filter may not remove femto and picoplankton from water samples.

3.6.7. Storage temperature and how it was achieved

From the literature, three temperature regimes are used to store water samples, room temperature (20–25°C), refrigeration (1–5°C) and freezing (−5 to −20°C) (Table 1). A summary of storage times for water samples at the three temperature regimes is presented in Table 3.

If samples are stored at room temperature (20–25°C), preservatives are normally added to retard biological changes. Because of the rapid increase of bacterial activity in water samples at room temperature, even in the presence of preservatives, storage at room temperature is used only for very short periods of storage (i.e. <3 days) (Tables 1 and 3).

Refrigeration of water samples at 2–5°C, that normally have been filtered, is often used to reduce biological changes [125]. Bacteria can still function at these temperatures, so that, changes in samples can still occur [9]. Because of the diversity of sample types, refrigeration has generally not been recommended as a long term preservation procedure [35,79,125]. On examination of Table 3, refrigeration with the addition of a preservative does not appear to offer any advantage over room temperature storage with preservatives. However, when reported results for storage of water samples at room temperature and refrigeration are compared [45,86,118] a significant increase in storage time is observed for refrigerated samples.

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>FRP</th>
<th>TFP</th>
<th>TP</th>
<th>Other (not specified)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–25</td>
<td>P</td>
<td>6 h–3 days</td>
<td>1–8 days</td>
<td>3–8 days (365a)</td>
</tr>
<tr>
<td>NP</td>
<td>&lt;3 h–8 days</td>
<td>3 h–16 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–5</td>
<td>P</td>
<td>3 h–42 days (60 daysa)</td>
<td>3 h–5 days (60 daysa)</td>
<td>14–365 days</td>
</tr>
<tr>
<td>NP</td>
<td>1–8 years</td>
<td>1–10 days</td>
<td>60–150 days</td>
<td>3 days</td>
</tr>
<tr>
<td>−5 to −20</td>
<td>P</td>
<td>3–30 days</td>
<td>4–30 days</td>
<td></td>
</tr>
</tbody>
</table>

Seawater.
NP—not preserved.
P=preserved.
water samples (i.e. 3 days vs 42 days; 1 day vs 14 days and 1 day vs 16 days, respectively).

Various researchers have recommended freezing or freezing after filtration as the preferred method of storage of samples for phosphorus analysis. In general, slow freezing of filtered and turbid water samples appears to be satisfactory for the long term storage of a wide variety of water sample types. It should be noted that once samples are frozen, filtering of thawed samples to measure FRP and TFP is meaningless as freezing will rupture cells and release phosphorus into solution [46]. Where comparisons between freezing and either room temperature or refrigeration have been made, a marked increase in storage time when samples are frozen is evident. For example the study of Collier and Marvin [27] compared storage at room temperature and freezing and showed longer storage times for freezing for both unfiltered (7 days vs 1.5 h) and filtered (61 days vs 3 h) water samples. Some reports have advocated quick freezing as being preferable to slow freezing [101,109] as losses of phosphorus are less and results have better precision. It is possible that if slow freezing is used, bacteria may still be able to utilise phosphorus and attach themselves to walls causing a loss of phosphorus.

A problem encountered by some workers is calcite formation and occlusion of phosphorus on freezing [71,79]. If using freezing, it will need to be demonstrated that no loss of phosphorus on freezing occurs because of precipitate formation.

3.6.8. The time taken to analyse samples after storage

It has been demonstrated by many authors that rapid changes in phosphorus can occur if water samples are left at room temperature especially without preservatives [45,49,118]. If samples are refrigerated or frozen, they are thawed and normally allowed to return to room temperature before analysis. In most of the papers reviewed no mention was made of how long samples were left at room temperature before analysis and some losses of phosphorus attributed to storage may be due to losses at room temperature. Freezing and thawing is unlikely to kill all bacteria and at room temperature (or lower temperatures) bacteria will again utilise phosphorus, attach themselves to walls and cause a decrease in phosphorus.

Ryden et al. [124] have shown that losses of phosphorus from lake samples can be minimised if samples are stored in vessels in which phosphorus is to be determined. They used a manual phosphorus determination method in which colour development was performed in the sample storage vessel. With modern analytical techniques, i.e. flow injection analysis and segmented flow analysis this is not possible, but to minimise losses samples should be transferred and analysed as soon as possible after reaching room temperature. If samples are to be digested, samples should be stored in the vessels to be used for digestion to minimise phosphorus losses.

4. Digestion procedures

The determination of TP and TFP in aqueous samples involves the conversion of particulate, organic and condensed phosphates into orthophosphate by a digestive or oxidative procedure followed by determination of the released orthophosphate by a modification of the “molybdenum blue” procedure [41,43,112], i.e.

\[
\text{PO}_4^{3-} + 12\text{MoO}_4^{2-} \rightarrow \text{PMo}_{12}\text{O}_{40}^{3-} + 12\text{O}^{2-}
\]

Trading with Sb

\[
\text{Sb} \rightarrow \text{PSb}_2\text{MoO}_{10}\text{O}_{30}^{3-}
\]

The use of high temperatures, high acidity and the creation of an oxidising environment are essential for the complete release and conversion of phosphorus [51,69,148]. Digestion methods that have been used include fusion, ignition, dry ashing, conventional heating using a hot plate, sand bath or aluminium block, UV-photo-oxidation, and autoclave or microwave heating, employing various oxidising agents such as perchloric acid, hydrogen peroxide, sulphuric acid–nitric acid or peroxodisulphate (Table 4). Most of the methods found in the literature for analysing TP and TFP in natural waters are based on conventional heating, UV-photo-oxidation, autoclave or microwave digestion (see Table 4). Although dry ashing with magnesium nitrate has been used to determine TFP and particulate phosphorus in natural waters [23,61,116,131], fusion, ignition and dry ashing techniques are normally only recommended for soil/sediment analysis [5] and are not considered in this review.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Phosphorus fraction</th>
<th>Sample type</th>
<th>Digestion technique – oxidising agent</th>
<th>Digestion time</th>
<th>Digestion temperature</th>
<th>Recoveries studies (if applicable)</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvey [60]</td>
<td>TP</td>
<td>Sea water</td>
<td>0.14 M H2SO4</td>
<td>5–6 h</td>
<td>135–140°C</td>
<td>Nucleic acids, phosphoproteins, glycoproteins</td>
<td>No data given, but reports the complete hydrolysis of these compounds and presumably full recovery of phosphorus</td>
</tr>
<tr>
<td>Menzel and Corwin [105]</td>
<td>TFP, TP</td>
<td>Sea water</td>
<td>Autoclave – 0.026 M K2S2O8, borosilicate flasks</td>
<td>30 min</td>
<td>120°C</td>
<td>5-Adenylic acid, lecithin, phosphorocholine, zooplankton</td>
<td>Recoveries of phosphorus compounds from zooplankton, an algae culture, sea water samples and added to distilled water were quantitative (96–101%). Incomplete recoveries (80%) were obtained from suspensions of bottom sediments</td>
</tr>
<tr>
<td>Gales et al. [47]</td>
<td>TP</td>
<td>Freshwater</td>
<td>Boiling – 0.03 M K2S2O8, 0.11 M H2SO4</td>
<td>30 min</td>
<td>100°C</td>
<td>Fructose-6-phosphate (barium salt), adenosine-5‘-monophosphate, glucose-1-phosphate (dipotassium salt, dihydrate), calcium phytate, sodium β-glycerophosphate (pentahydrate), sodium desoxyribonucleate, lecithin, sodium tripolyphosphate, sodium metaphosphate, sodium pyrophosphate</td>
<td>Recoveries of phosphorus compounds from standards were quantitative (83–113%)</td>
</tr>
<tr>
<td>Grassholf [55]</td>
<td>TP</td>
<td>Sea water</td>
<td>UV-photo-oxidation (900 W)</td>
<td></td>
<td></td>
<td>Phosphoethanolamine, calcium glycerophosphate Riboflavine-5‘-phosphate ester, guanosine-2‘(3’) phosphate</td>
<td>Recoveries of phosphate obtained were between 92–105%</td>
</tr>
<tr>
<td>Armstrong et al. [8]</td>
<td>RP, TFP</td>
<td>Sea water</td>
<td>UV-photo-oxidation (1200 W) – 1–2 drops of 30% H2O2</td>
<td>1–2 h</td>
<td>60–80°C</td>
<td>β-Glycerophosphate, ribose-5-phosphate, choline phosphate, 2-aminoethanephosphonic acid, ribonucleic acid</td>
<td>Recoveries of phosphorus compounds added to sea water were quantitative. Inorganic polyphosphates are not hydrolysed to orthophosphate by this procedure</td>
</tr>
<tr>
<td>Sanning [126]</td>
<td>TP</td>
<td>Lake, river water and effluents</td>
<td>Boiling – 0.18 M H2SO4, boiling – 0.29 M K2S2O8 and 0.18 M H2SO4</td>
<td>1.5 h, 1.5 h</td>
<td></td>
<td>Uridine diphosphate disodium salt trihydrate</td>
<td>Better recoveries when peroxydisulphate used</td>
</tr>
<tr>
<td>Authors</td>
<td>Phosphorus fraction</td>
<td>Sample type</td>
<td>Digestion technique – oxidising agent</td>
<td>Digestion time</td>
<td>Digestion temperature</td>
<td>Recoveries studies (if applicable)</td>
<td>Conclusions</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td>----------------------------------------</td>
<td>----------------</td>
<td>-----------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Jankovic et al. [74]</td>
<td>TP/TFP</td>
<td>Wastewater and sewage</td>
<td>Boiling – 0.06–0.14 M H₂SO₄ and 0.06–0.15 M K₂S₂O₈, 0.017–0.03 M KMnO₄, 0.06–0.14 M KClO₃ or 0.056-1 M HClO₄</td>
<td>30–50 min</td>
<td>55 °C</td>
<td>Addition of K₂S₂O₈ gave similar recoveries to evaporation and dryashing (93–100%). Other oxidants gave lower recoveries, i.e. KMnO₄ (89–90%), KClO₃ (70–90%), HClO₄ (95–99%). Recoveries of phosphorus compounds incomplete (no results given).</td>
<td></td>
</tr>
<tr>
<td>Armstrong and Tibbits [7]</td>
<td>TP</td>
<td>Sea water</td>
<td>(1) UV-photo-oxidation (380 W) – 1–2 drops of 30% H₂O₂, (2) UV-photo-oxidation (380 W) alone or followed by hydrolysis with 0.025 M H₂SO₄</td>
<td>15–16 h,</td>
<td>46–61 °C</td>
<td>Little phosphorus recovered by UV-photo-oxidation alone. When followed by acid hydrolysis 80% of phosphorus was recovered</td>
<td></td>
</tr>
<tr>
<td>Jenkins [76]</td>
<td>TFP</td>
<td>Estuarine</td>
<td>Ignition 800 °C, boiling with 30% H₂O₂, boiling with HNO₃–H₂SO₄ and autoclave with K₂S₂O₈</td>
<td>1 h</td>
<td>110–120 °C</td>
<td>K₂S₂O₈ digestion was the simplest method and suitable for routine analysis. Quantitative recovery of organic phosphorus compounds containing P–C bonds was obtained at the 50 µgP/l level using this method. Polyphosphates did not hydrolyse under UV-photolysis if the temperature was below 70 °C. All five methods gave similar recoveries of phosphorus from particulate and liquid samples.</td>
<td></td>
</tr>
<tr>
<td>Solorzano and Strickland [132]</td>
<td>TP</td>
<td>Sea water</td>
<td>UV-photo-oxidation – 1–2 drops of 30% H₂O₂, hydrolysis in 0.094 M HCl, (1) Mg(NO₃)₂ fusion, (2) boiling with HClO₄–H₂SO₄, (3) autoclave 0.03 M K₂S₂O₈–0.11 M H₂SO₄, (4) mixed nitrate fusion, (5) boiling with 50% H₂O₂/H₂SO₄</td>
<td>&lt;1 h,</td>
<td>70 °C, 2 h, 100 °C, 550 °C, 100 °C</td>
<td>Polyphosphates did not hydrolyse under UV-photolysis if the temperature was below 70 °C. All five methods gave similar recoveries of phosphorus from particulate and liquid samples.</td>
<td></td>
</tr>
<tr>
<td>Harwood et al. [61]</td>
<td>TFP, TP, PP</td>
<td>Freshwater and solid samples</td>
<td>UV-photo-oxidation (900 W) – a few drops of 30% H₂O₂ and 0.004 M H₂SO₄</td>
<td>1.5–2 h</td>
<td>100 °C</td>
<td>UV-photo-oxidation was suitable for determining low concentrations of total phosphorus (TP) in freshwater samples with low turbidities. Polyphosphates were only partially hydrolysed. The recovery of particulate phosphorus (74–85%) decreased with increasing particulate material.</td>
<td></td>
</tr>
<tr>
<td>O’Connor and Syers [115]</td>
<td>TP</td>
<td>Particulates and effluent</td>
<td>Autoclave – K₂S₂O₈</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Author(s)</td>
<td>Method/Type</td>
<td>Water Type</td>
<td>Conditions</td>
<td>Recoveries</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>-------------</td>
<td>--------------------------</td>
<td>-----------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goulden and Brooksbank [54]</td>
<td>TP</td>
<td>Freshwater (1) Autoclave – 0.013 M K₂S₂O₈ and 0.001 M H₂SO₄ and 0.0006 M HNO₃, (2) on-line UV-photo-oxidation – 0.031 M K₂S₂O₈ and 1.26 M H₂SO₄</td>
<td>30 min 85°C</td>
<td>Similar results by both methods when lake samples analysed. Recovery of standards was 100%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canelli and Mitchell [22]</td>
<td>TFP</td>
<td>Freshwater/sewage Boiled – 0.006–0.02 M K₂S₂O₈ and 0.11–0.42 M H₂SO₄</td>
<td>100°C</td>
<td>Recoveries of phosphorus were between 98% and 105%.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jirka et al. [77]</td>
<td>TP</td>
<td>Waste water and sewage Boiled – HgO, K₂S₂O₈ and H₂SO₄</td>
<td>200–370°C</td>
<td>Recoveries of 84–118% when added to waste water samples. Results in agreement with those obtained by APHA/AWWA/WPCF [4] standard methods</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gilbert et al. [48]</td>
<td>TP</td>
<td>Freshwater Autoclave – 0.015 M K₂S₂O₈–0.045 M NaOH</td>
<td>30 min 100–110°C</td>
<td>Quantitative recoveries of organically bound phosphorus in 0–5 μgP/l range, were achieved (96–102%). HClO₄ or K₂S₂O₈ were both suitable for digestion of fresh and saline waters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Golterman [51]</td>
<td>TP</td>
<td>Fresh and saline water Boiling with H₂SO₄ with/without H₂O₂, boiling with HClO₄, autoclave – K₂S₂O₈</td>
<td>1.5 h 145°C</td>
<td>Complete recoveries of phosphorus compounds (95–101%). Acid hydrolysis of polyphosphates gave moderate recoveries (79–100%).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goossen and Kloosterboer [52]</td>
<td>TFP, PP</td>
<td>Fresh and wastewater UV-photo-oxidation (75 W) – 0.32 M H₂SO₄</td>
<td>30 min 100°C</td>
<td>Complete recoveries of organic phosphorus compounds were obtained (95–100%) except for methyltriphenylphosphonium bromide (30%). Acid hydrolysis of polyphosphates gave moderate recoveries (79–100%).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lennox [98]</td>
<td>TDP</td>
<td>Freshwater and wastewater Boiled – 0.028 M K₂S₂O₈ and 0.21 M H₂SO₄, glass test tubes</td>
<td>1.5 h, 1.5 h 145°C, 250°C</td>
<td>Complete recoveries of phosphorus compounds (95–101%). Recoveries of potassium orthophosphate added to river water were 100%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Authors</td>
<td>Phosphorus fraction</td>
<td>Sample type</td>
<td>Digestion technique – oxidising agent</td>
<td>Digestion time</td>
<td>Digestion temperature</td>
<td>Recoveries studies (if applicable)</td>
<td>Conclusions</td>
</tr>
<tr>
<td>------------------</td>
<td>---------------------</td>
<td>-------------------------------</td>
<td>---------------------------------------</td>
<td>----------------</td>
<td>-----------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Jeffries et al.</td>
<td>TP</td>
<td>Freshwater and sewage</td>
<td>Autoclave – 0.015 M K$_2$S$_2$O$_8$ and 0.067 M H$_2$SO$_4$, pyrex screw top culture tubes with Teflon lined caps</td>
<td>1 h</td>
<td>200–300°C</td>
<td>Glucose-1-phosphoric acid (di potassium salt), glucose-6-phosphoric acid (di potassium salt), DNA (sodium salt), adenosine-5'-monophosphoric acid, adenosine-5'-diphosphate di sodium salt, adenosine-5'-triphosphate di sodium salt, phosphoserine, sodium-β-glycerophosphate, tetrasodium pyrophosphate, sodium tripolyphosphate, sodium metaphosphate, disodium hydrogen orthophosphate</td>
<td>Recoveries of phosphorus were between 95% and 115%. This technique was not suitable for samples with high levels of suspended solids such as raw sewages and flooded rivers</td>
</tr>
<tr>
<td>Crowther et al.</td>
<td>TP</td>
<td>Freshwater</td>
<td>0.009 M HgO, 0.36 M H$_2$SO$_4$ and 0.077 M K$_2$SO$_4$</td>
<td>1–1.5 h</td>
<td>200–300°C</td>
<td>Potassium dihydrogen phosphate, pyrophosphate</td>
<td>High recoveries of phosphorus compounds (99.8±0.1%) from compounds</td>
</tr>
<tr>
<td>Hirai et al.</td>
<td>TP</td>
<td>Standards</td>
<td>On-line digestion with 7.2 M H$_2$SO$_4$, 4.8 M HCl, 0.2 M Mo(V)-Mo(VI) and 0.05 M Zn</td>
<td>3 min</td>
<td>140°C</td>
<td>Pyrophosphate, tripolyphosphate</td>
<td>Recoveries of phosphorus compounds greater than 98%</td>
</tr>
<tr>
<td>Valderrama</td>
<td>TP</td>
<td>Marine</td>
<td>Autoclave – 0.022 K$_2$S$_2$O$_8$, 0.06 bout acid and 0.04 M NaOH, sovirel bottles, polypropylene caps</td>
<td>30 min</td>
<td>100–120°C</td>
<td>Potassium dihydrogen phosphate</td>
<td>High recoveries of standards (8–11 µgP/l) were obtained (95–108%)</td>
</tr>
<tr>
<td>Cabrera et al.</td>
<td>TFP, TP, PP</td>
<td>River and marsh water</td>
<td>Boiling with 0.18 M H$_2$O$_2$–0.36 M H$_2$SO$_4$</td>
<td>1 1/2 h</td>
<td>200–300°C</td>
<td>Phosphoglyceric acid (calcium salt)</td>
<td>This method was suitable for water samples containing high iron and aluminium oxides as precipitation of oxides on neutralisation avoided</td>
</tr>
<tr>
<td>Langner and Hendrix</td>
<td>TP</td>
<td>Orchard leaves and Aufwuchs suspensions in distilled water</td>
<td>Autoclave – 0.05–0.123 M K$_2$S$_2$O$_8$–0.15–0.37 M NaOH</td>
<td>1 h</td>
<td>100–110°C</td>
<td>NIST 1571 Orchard leaves, Aufwuchs</td>
<td>Recoveries of phosphorus from Orchard leaves (5–15 mg) and Aufwuchs material (2 mg) were 85–89% and 94–101%, respectively</td>
</tr>
<tr>
<td>Ebina et al.</td>
<td>TP</td>
<td>River water</td>
<td>Autoclave – 0.037 M K$_2$S$_2$O$_8$–0.038 M NaOH, glass test tubes (10 ml) fitted with Teflon lined screw caps</td>
<td>30 min</td>
<td>120°C</td>
<td>Adenosine-5'-monophosphate, adenosine-di-phosphate, adenosine-5'-triphosphate, potassium dihydrogen phosphate, sodium pyrophosphate, sodium hexametaphosphate, glucose-1-phosphate, sodium-β-glycerophosphate, sodium tripolyphosphate</td>
<td>Good recoveries of phosphorus compounds (up to 1 mgP/l) from standards (96.5–100.8%) and added to samples (96.7%–103.3%)</td>
</tr>
<tr>
<td>Author(s) and Method</td>
<td>Sample Type</td>
<td>Digestion Method</td>
<td>Recovery Notes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------</td>
<td>-----------------</td>
<td>----------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hosomi and Sudo [69]</td>
<td>Freshwater and suspensions</td>
<td>Autoclave –0.05 M K₂S₂O₈–0.073 M NaOH 1 h 120°C</td>
<td>Adenosine-5'-triphosphate (disodium salt), adenosine-5'-diphosphate (sodium salt), sodium pyrophosphate, sodium metaphosphate, sodium polyphosphate, α-D-glucose-6-phosphoric acid (dipotassium salt), recoveries of phosphorus compounds were 94–102%. Recoveries of phosphorus from suspensions (50 mg/l) were 96–100%. Similar results to APHA/AWWA/WPCF standard methods (96.5–100.8%).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cembella et al. [24]</td>
<td>Sea water</td>
<td>Autoclave – 0.026 M K₂S₂O₈ UV-photo-oxidation (1200 W) – 1–2 drops of 30% H₂O₂ 30 min 120°C, 60–80°C</td>
<td>Phosphoformate, 1-aminoethylphosphonate, 2-aminoethylphosphonate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zaiyou and Limin [149]</td>
<td>Freshwater and sewage</td>
<td>UV-photo-oxidation (1000 W) – 0.04 M K₂S₂O₈–0.15 M H₂SO₄ 50 min 100°C</td>
<td>Glycerol-2-phosphate, O,O-dimethyl-2,2-dichlorovinyl phosphate, O,O-dimethyl-O-(1-chloro-1,N,N-diethylcarbamoyl) phosphate, tributylphosphine oxide, O,O-dimethyl-S-(N-methylcarbomyl) methyl phosphorodithioate, O,O-dimethyl-S-(1,2-dicarboxyethyl) dithiophosphate, sodium hexametaphosphate, sodium pyrophosphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aoyagi et al. [3]</td>
<td>Sea water and waste water</td>
<td>On-line Teflon capillary digestor containing Pt with 0.049 M K₂S₂O₈ &lt;4 min 160°C</td>
<td>Triphenylphosphine, sodium metaphosphate, sodium pyrophosphate, sodium tripolyphosphate, di sodium phenylphosphate, adenosine-5'-triphosphate, fenitrothion, casein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McKelvie et al. [104]</td>
<td>Dissolved organic P</td>
<td>On-line UV-photo-oxidation (40 W) – alkaline 0.15 M K₂S₂O₈ (pH 9.2) and 0.17 M Ba₄Na₃O₇ &lt;2 min</td>
<td>Potassium dihydrogen orthophosphate, sodium tripolyphosphate, myo-inositol hexakis (dihydrogen phosphate), disodium salt dihydrate, phosphoenolpyruvic acid (disodium salt), 2-aminoethylphosphonic acid (disodium salt), adenosine 5'-monophosphate (sodium salt), adenosine 5'-triphosphate (disodium salt), D-glucose-6-phosphate (disodium salt)</td>
<td>High recoveries of all phosphorus compounds (99–100%) except adenosine-5'-triphosphate and sodium tripolyphosphate. Results for freshwaters, sewage effluent and an algae culture were in agreement with the APHA/AWWA/WPCF batch acid persulphate procedure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Authors</td>
<td>Phosphorus fraction</td>
<td>Sample type</td>
<td>Digestion technique – oxidising agent&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Digestion time</td>
<td>Digestion temperature</td>
<td>Recoveries studies (if applicable)</td>
<td>Conclusions</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------------</td>
<td>--------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>----------------</td>
<td>-----------------------</td>
<td>-----------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Ridal and Moore [121]</td>
<td>TFP</td>
<td>Sea water</td>
<td>(1) UV-photo-oxidation (1200 W), pH 3, (2) autoclave – 0.015–0.15 M K₂S₂O₈ (pH 3)</td>
<td>6 h, 90 min</td>
<td>65–70 °C, 125 °C</td>
<td></td>
<td>UV-photo-oxidation gave marginally higher results than autoclaving. Combination gave the best results</td>
</tr>
<tr>
<td>Littau and Engelhart [99]&lt;sup&gt;g&lt;/sup&gt;</td>
<td>TP</td>
<td>Plant tissue, animal tissue, dried sewage sludge and river sediment</td>
<td>Microwave – 0.166 M K₂S₂O₈ and 0.24 M NaOH, closed PTFE vessels Two stages: (i) 10 min, (ii) 30 min</td>
<td>Two stages: (i) 30 min at 961 W – 100% power, (ii) 135 psig at 961 W – 100% power</td>
<td></td>
<td>High recoveries of phosphorus from plant and animal tissues, dried sewage sludge, and river sediment were obtained (77–103%). The method was effective for the oxidation of compounds with difficult to oxidise phosphorus bonds</td>
<td></td>
</tr>
<tr>
<td>Hinkamp and Schwedt [67]&lt;sup&gt;g&lt;/sup&gt;</td>
<td>TFP</td>
<td>Wastewater</td>
<td>On-line microwave – 0.05 M K₂S₂O₈ and 0.08 M HClO₄ &lt;3 min</td>
<td>650 W</td>
<td></td>
<td>Tetrasodium diphosphate decahydrate, pentasodium triphosphate, hexamethylenediaminetetramethylene phosphonic acid, 1-hydroxy-ethane-1,1-diphosphonic acid, adenosine-5'-monophosphoric acid, α-D-glucose-1-phosphate (disodium salt tetrahydrate), disodium phenyl phosphate dihydrate, diethyl phosphate, triethyl phosphate</td>
<td>Recoveries of organic phosphates and condensed phosphates added to distilled and wastewater samples (5 mgP/l) were 87–100% and 62–80%, respectively</td>
</tr>
<tr>
<td>APHA-AWWA-WPCF [6]&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>TFP, TP</td>
<td>Freshwater, sea water and waste water</td>
<td>Boiling with HClO₄, boiling with H₂SO₄ and HNO₃, autoclave – K₂S₂O₈ and H₂SO₄</td>
<td>45 min 480 W</td>
<td></td>
<td>Potassium dihydrogen phosphate, tetra-sodium pyrophosphate, glucose-6-phosphoric acid (dipotassium salt), glucose-1-phosphoric acid (disodium salt), adenosine-5'-diphosphoric acid (disodium salt), adenosine-5'-triphosphoric acid (disodium dihydrogen salt)</td>
<td>The use of H₂SO₄– HNO₃ acid is recommended for most samples</td>
</tr>
<tr>
<td>Johnes and Heathwaite [78]&lt;sup&gt;c&lt;/sup&gt;</td>
<td>TP</td>
<td>River, lake and ground-water</td>
<td>Microwave – 0.25 M K₂S₂O₈–0.038 M NaOH</td>
<td>45 min 480 W</td>
<td></td>
<td>Potassium dihydrogen phosphate, tetra-sodium pyrophosphate, glucose-6-phosphoric acid (dipotassium salt), glucose-1-phosphoric acid (disodium salt), adenosine-5'-diphosphoric acid (disodium salt), adenosine-5'-triphosphoric acid (disodium dihydrogen salt)</td>
<td>Recoveries of phosphorus compounds added to distilled and turbid water (0.2–10 mgP/l) were 96–103% and 98–106%, respectively. The method was suitable for the analysis of water samples (0.01–50 mgP/l) in the pH range 5–8</td>
</tr>
<tr>
<td>Reference</td>
<td>Application</td>
<td>Method</td>
<td>Time</td>
<td>Temp</td>
<td>Recoveries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>-------------</td>
<td>--------</td>
<td>------</td>
<td>------</td>
<td>------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ron Vaz et al. [122]&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Soil solutions</td>
<td>On-line UV-photocatalysis with 0.24 M K₂S₂O₈ and 0.26 M NaF</td>
<td></td>
<td></td>
<td>High recoveries of phosphorus from all compounds (99–101%) except polyphosphates (6–8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Williams et al. [147]&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Wastewater</td>
<td>On-line microwave – 0.70 M HNO₃, pretreatment with pyrophosphate phosphohydrolase</td>
<td>&lt;2 min</td>
<td>540 W</td>
<td>Potassium dihydrogen phosphate, tri sodium trimetaphosphate, tri sodium tetrametaphosphate, sodium tetrapyrrophosphate, tetra sodium pyrophosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benson et al. [11]&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Freshwater and wastewater</td>
<td>On-line microwave – 0.018 M K₂S₂O₈·H₂SO₄ (pH=0.6)</td>
<td>&lt;2.5 min</td>
<td>700 W</td>
<td>Myo-inositol hexakis (dihydrogen phosphate), phosphonofumaric acid, 2-aminoethylphosphonic acid, β-glucose-6-phosphate, α,α'-glycerolphosphate, O-phosphorylethanolamine, adenosine 5'-monophosphate, adenosine 5'-diphosphate, adenosine 5'-triphosphate, sodium pyrophosphate, sodium tripolyphosphate, nitrophenylphosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Williams et al. [146]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Soil litter extracts</td>
<td>Autoclave – 0.03 M K₂S₂O₈ and 0.18 M NaOH</td>
<td>30 min</td>
<td>110°C</td>
<td>Inositol hexaphosphate (dodeca sodium salt)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lambert and Maher [91]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Freshwater</td>
<td>Autoclave – 0.025 M K₂S₂O₈ and 0.038 M NaOH</td>
<td>1 h</td>
<td>120°C</td>
<td>Potassium dihydrogen phosphate, sodium tripolyphosphate, adenosine-5'-monophosphate, sodium β-glycerophosphate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

High recoveries of phosphorus compounds from distilled (10 mgP/l) and wastewater (2 mgP/l), i.e. 99–101% and 99.5%, respectively. Pretreatment of samples containing pyrophosphate was needed in order to release phosphorus. High recoveries of phosphorus compounds (2-10 mgP/l), i.e. 88–106% except condensed phosphates (29–40%) and adenosine-5-di, and triphosphates (41–49%). The method was suitable for the analysis of sewage effluents containing low amounts of condensed phosphates without additional sample pretreatment. The method was in excellent agreement with the results obtained using sulphuric acid–nitric acid digestion. Recoveries of phytic acid standards (0.1-1 mg/l) were 96–106%. Above 100–200 mgC/l recoveries reduced to 10–25%. High recoveries of phosphorus from suspensions of Chlorella (up to 100 μgP/l) and phosphorus compounds added to lake water (92–109% and 94–103%, respectively). Incomplete recoveries of phosphorus from Pond sediment suspensions were found at concentrations above 50 μgP/l.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Phosphorus fraction</th>
<th>Sample type</th>
<th>Digestion technique – oxidising agent a</th>
<th>Digestion time</th>
<th>Digestion temperature</th>
<th>Recoveries studies (if applicable)</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woo and Maher [148]</td>
<td>TP</td>
<td>Turbid lake water</td>
<td>0.045 M K₂S₂O₈ and 0.04 M NaOH</td>
<td>Autoclave 1 h, 120 °C, 450 W</td>
<td>1 h, 10 min</td>
<td>Potassium dihydrogen phosphate, DL α-glycerophosphate, phytic acid, 2-aminoethylphosphonic acid, O-phosphonylethanol, phospho(enol)pyruvate, phosphonoformic acid</td>
<td>High recoveries of phosphorus from suspensions of Chlorella (up to 100 µg P/l) and phosphorus compounds added to lake water (99–103% and 99–107%, respectively) using autoclave or microwave heating. Incomplete recoveries of phosphorus from Pond sediment suspensions were found at concentrations above 60 µg P/l when autoclave heating was used (91–92%). Complete recoveries were achieved using microwave heating (96–107%)</td>
</tr>
<tr>
<td>Halliwell et al. [57]</td>
<td>Condensed polyphosphates</td>
<td>Waste waters</td>
<td>0.24 M H₂SO₄ and 0.0016 M Mo</td>
<td>On-line heating with 120 °C</td>
<td>120 °C</td>
<td>Sodium tripolyphosphate, tetra sodium pyrophosphate</td>
<td>High recoveries of phosphorus added to unfiltered sewage (95–103%).</td>
</tr>
<tr>
<td>Kerouel and Aminot [82]</td>
<td>TP</td>
<td>Freshwater, sea water</td>
<td>0.016 M K₂S₂O₈ and 0.133 M H₂SO₄, autoclave – 0.016 M K₂S₂O₈, 0.05 M H₂BO₃ and 0.035 M NaOH, UV-photo-oxidation, 1–2 drops H₂O₂, dry ashing with Mg(NO₃)₂</td>
<td>30 min, 2 h, 115 °C, 480 °C</td>
<td>60–80 °C, 480 °C</td>
<td>Phytic acid, 2-aminoethylphosphonic acid, Phospho(enol)pyruvic acid, acid, tri(cyclohexylamine) salt, sodium β-glycerophosphate, adenosine-5'-monophosphate, monohydrate, guanosine-5'-monophosphate (disodium hydrate), phosphoryl choline chloride, riboflavin-5'-monophosphate, glycerophosphate, glucose-6-phosphate (sodium salt), ribose-5-phosphate (disodium salt dihydrate)</td>
<td>Good recoveries of phosphorus compounds (31 µg P/l) from distilled water (98–109%). Incomplete recoveries from sea water (53–101%). Alkaline persulphate gave the best recoveries (92–101%) from both fresh and sea waters except for 2-aminoethylphosphonic acid (88%)</td>
</tr>
<tr>
<td>Ormaza-Gonzalez and Statham [116]</td>
<td>TFP</td>
<td>Fresh and sea water</td>
<td>0.014 M K₂S₂O₈ and 0.017 M H₂SO₄, Autoclave – 0.02 M K₂S₂O₈, 0.06 M H₂BO₃ and 0.04 M NaOH, UV-photo-oxidation with a few drops of H₂O₂, dry ashing with Mg(NO₃)₂</td>
<td>30 min, 2 h, 100–120 °C, 300 °C</td>
<td>60–80 °C, 300 °C</td>
<td>4-Nitrophenylphosphate, glycerophosphate, glucose-6-phosphate, triphosphosphate, trimetaphosphate, adenosine-5'-triphosphate, guanosine-5'-diphosphate, 2-aminoethylphosphonate</td>
<td>Variable recoveries of phosphorus compounds from samples distilled and spiked sea water (0–100%). Only methods with a hydrolysis step gave high recoveries of P from polyphosphates and nucleosides. Similar recoveries of TFP in river waters by all procedures (96–98%). Recoveries of TFP in sea waters by UV-photo-oxidation (22–83%), acid peroxodisulphate (65–88%) and alkaline peroxodisulphate (54–90%) were lower than those obtained using dry ashing</td>
</tr>
</tbody>
</table>
| Benson et al. [12] | TP | On-line UV (8 W) thermal induced with 0.026 M NH$_4$S$_2$O$_8$ and 2.1 M HClO$_3$ | <2.5 min | 90°C | 0-$\alpha$-
-glycerophosphate, phytic acid, 2-aminoethylphosphonic acid, $O$-phosphorylethanolamine, phosphonoformic acid, sodium triplyphosphate, $\alpha$-glucose-6-phosphate, adenosine 5'-diphosphate, adenosine 5'-triphosphate, adenosine 5'-monophosphate | High recoveries for all phosphorus compounds (98–108%) except polyphosphates (85–89%)

Rowland and Haygarth [123]$^b$ | TFP | Soil solutions (1) 0.72 M H$_2$SO$_4$ and 0.14 M HNO$_3$ (Kjedhal flask), (2) 0.079 M H$_2$SO$_4$, 0.005 M Se, 0.013 M Li$_2$SO$_4$ and 0.32 M H$_2$O$_2$ (conical flask), (3) 0.024 M H$_2$SO$_4$ and 0.026 M K$_2$S$_2$O$_8$ (Teflon bottle, 60 ml) | 1 h | 120°C | Adenosine-5'-triphosphate (disodium salt), adenosine-5'-monophosphate (sodium salt), glucose-6-phosphoric acid (disodium salt), inositol hexaphosphoric acid dodeca sodium salt, tetra potassium pyrophosphate | Peroxydisulphate gave high recoveries of phosphorus from standards (89–102%) and a river water sample (95%) compared to nitric–sulphuric acid (32–102% and 110%) and sulphuric acid–hydrogen peroxide (89–101%, 75%) procedures. Comparison of the analysis of soil solutions showed similar recoveries of phosphorus by all three methods

$^a$Final solution concentrations.
$^b$Colourimetric determination by molybdenum blue method – batch.
$^c$Colourimetric determination by molybdenum blue method – on-line FIA.
$^d$Colourimetric determination by vanadomolybdophosphoric acid.
$^e$Colourimetric determination by molybdophosphate and malachite green – on-line FIA.
$^f$Molybdophosphate – amperometric detection.
$^g$Colourimetric determination by molybdenum blue method/isobutanol extraction – on-line segmented flow.
4.1. Conventional heating

A general problem with methods using conventional heating and a volume reduction is that the precipitation of large quantities of salts occurs which may cause problems when analysing phosphorus by colourimetric procedures [76].

Other disadvantages of using conventional heating with strong acids are summarised by notes that accompany these procedures. For example the use of sulphuric and nitric acids (Ref. [107] cited in [123]); “the procedure is slow, requires almost continuous attention from the analyst and markedly worsens the precision of the analytical result”. A problem with heating sulphuric acid to fumes may be the formation of condensed phosphates by dehydration of phosphoric acid [52].

4.1.1. Perchloric acid

The use of perchloric acid with/without sulphuric acid as an oxidising agent has been widely used with heating by a hot plate, sand bath or aluminium block for the digestion of fresh and saline waters [51,59,61,76]. These procedures have been found to be tedious, dangerous and time consuming [105,119,135] and are not generally recommended for the routine analysis of large numbers of water samples.

4.1.2. Hydrogen peroxide

Jenkins [76] reported incomplete recoveries of FRP and TFP from filtered estuarine water samples boiled with 30% hydrogen peroxide. He suggested that the low recoveries were caused by the presence of unreacted peroxide which interfered during the subsequent colourimetric procedure by reacting with molybdic acid to form permolybdic acid. Harwood et al. [61] tested five different digestion methods on six solids and five liquid samples for TP analyses and concluded that relative to a number of other techniques, boiling with 50% hydrogen peroxide and 2 M sulphuric acid was the quickest and most effective technique. They also noted that errors in phosphorus concentration occurred when unreacted peroxide was present, and suggested that the excess peroxide present should be completely destroyed prior to colourimetric determination. Cabrera et al. [20] found that the preferred method of Harwood et al. [61] under estimated the orthophosphate concentration when natural water samples contained high levels of iron and/or aluminium. They modified the method of Harwood et al. [61] by using 0.36 M^2 sulphuric acid and 0.18 M hydrogen peroxide with the addition of 0.5 ml of 18 M sulphuric acid after neutralisation to avoid loss of phosphorus by adsorption on hydrous iron and aluminium oxides. Rowland and Haygarth [123] used a combination of 0.026 M hydrogen peroxide and 0.079 M sulphuric acid (with 0.005 M selenium and 0.013 M lithium sulphate as catalysts) to determine TFP in soil solutions. Results were in agreement with those obtained when water samples were digested with sulphuric–nitric acids or peroxodisulphate–sulphuric acid. Recoveries of phosphorus from adenosine 5-triphosphate disodium salt, adenosine 5-monophosphate sodium salt, tetra sodium pyrophosphate and inositol hexaphosphoric acid dodeca sodium salt standards were 89–101%. The recoveries from a quality assurance river water sample were low (75%).

4.1.3. Sulphuric acid

One of the earliest reported procedures for the determination of TP in sea water was by Harvey [60]. He digested sea water samples in 0.14 M sulphuric acid at 135–140°C for 5–6 h. Although no data were given in the paper he reports the complete hydrolysis (and presumably recovery of phosphorus) from nucleic acids, phospho- and glycoproteins.

Jirka et al. [77] and Crowther et al. [33] reported the use of 0.36 M sulphuric acid in combination with 0.009 M mercuric oxide and 0.077 M potassium sulphate for the analysis of TP in surface waters, waste waters and sewage. High temperatures were used (200–370°C) to evaporate samples and the procedure takes 1–3 h. Crowther et al. [33] showed that the recoveries of phosphorus from potassium orthophosphate and pyrophosphate standards were quantitative (99.8%) using this procedure.

4.1.4. Sulphuric acid–nitric acid

The American Standard Methods for Water and Wastewater Analysis [6] has recommended the use of a sulphuric acid–nitric acid digestion as being suitable for most types of water samples. Goossen

---

2All concentrations reported are those in the final digest solution.
and Kloosterboer [52] found this method to give low recoveries of orthophosphate if measurements were made shortly after vigorous fuming. They suggested that this may have been caused by the formation of small quantities of condensed phosphates by dehydration of phosphoric acid in hot concentrated sulphuric acid. Good recoveries of phosphorus were found after dilution and standing of digested water samples for 90 min.

A recent evaluation of the use of 0.72 M sulphuric and 0.14 M nitric acids to determine TFP in soil solutions [123] gave erratic results and was vulnerable to contamination. Recoveries of phosphorus from adenosine 5-triphosphate disodium salt, adenosine 5-monophosphate sodium salt, tetra sodium pyrophosphate and inositol hexaphosphoric acid dodeca sodium salt standards were 37–102%. The recoveries from a quality assurance river water sample were 110%. However, results were in agreement with those obtained for water samples digested with sulphuric acid–hydrogen peroxide or peroxodisulphate–sulphuric acid. Aoyagi et al. [3] showed that for organic phosphorus compounds that gave low recoveries when digested with sulphuric–nitric acids, recoveries could be increased by the addition of a piece of platinum gauze to the digest mixture. The recoveries of phosphorus from disodium phenylphosphate, triphenylphosphine and casein standards could be increased from 7–66% to 94–102%.

4.1.5. Sulphuric acid–peroxodisulphate

Gales et al. [47] boiled water samples containing 0.11 M sulphuric acid and 0.03 M peroxodisulphate for 30 min. Recoveries of phosphorus from solutions of fructose 6-phosphate, adenosine 5-monophosphate, glucose 1-phosphate, calcium phytate, sodium β-glycerophosphate, sodium desoxyribonucleate and lecithin were between 83% and 113%. Sanning [126] compared boiling of acidified (0.18 M sulphuric acid) lake, river and effluent samples with and without 0.29 M peroxodisulphate for TP analysis. In general higher recoveries were obtained with the addition of peroxodisulphate (increases of 3–308%) for water samples and uridine diphosphate standard solutions. It should be noted that some samples gave lower recoveries of TP (1–67%) when peroxodisulphate was used. Jankovic et al. [74] boiled waste water and sewage samples with 0.06–0.14 M sulphuric acid and 0.06–0.15 M potassium peroxodisulphate for 35–50 min. Similar results for water samples were obtained to those obtained by evaporation and dry ashing at 600°C. Two pesticides containing phosphorus, O, O-Diethyl-O-2 [ethylthio] ethylphosphorothioate and O, O-Diethyl-O-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl-phosphorothioate, were only partially oxidised. Canelli and Mitchell [22] reported the use of sulphuric acid and peroxodisulphate to determine TFP in waste waters from sewage treatment plants. Filtered samples (5–25 ml) were made 0.11–0.42 M in sulphuric acid and 0.006–0.02 M potassium peroxodisulphate added and boiled on a hot plate until the volume was reduced to 7–10 ml. High recoveries of phosphorus from sodium hexametaphosphate (50–1000 μgP/l) and disodium phenylphosphate (50–1700 μgP/l) standards were obtained (98–105%). Results for TFP analyses of waste waters (1.6–9.9 mgP/l) were in agreement with those found using the APHA-AWWA-WPCF [4] sulphuric–nitric acid procedure.

Lennox [98] used acid peroxydisulphate digestion to determine TP in surface freshwater samples. Samples were digested for 1.5 h at 145°C and 1.5 h at 250°C with 0.28 M of potassium peroxydisulphate and 0.21 M sulphuric acid. High recoveries of phosphorus from adenosine-5-monophosphoric acid, sodium hexametaphosphate, disodium phenyl phosphate, glucose-1-phosphoric acid and fructose-6-phosphoric acid standards (50–1000 μgP/l) were obtained (95–100%).

4.1.6. Other

Jankovic et al. [74] boiled waste water and sewage samples with 0.06–0.14 M sulphuric acid and 0.017–0.03 M potassium permaganate, 0.06–0.14 M potassium perchlorate or 0.056–1 M perchloric acid for 35–50 min. Lower recoveries (71–99%) were obtained relative to those obtained by evaporation and dry ashing of water samples at 600°C.

4.2. Ultraviolet (UV)-photo-oxidation

The use of UV-photo-oxidation of organic phosphorus compounds combined with thermal hydrolysis of acid-hydrolysable phosphates has been reported for the analysis of sea water [8] and filtered freshwaters [52,64,100,104]. Grassholf [55] showed that high
recoveries of phosphorus from phosphoethanolamine, calcium glycerophosphate, riboflavin 5'-phosphate ester and guanosine 2' (3') phosphate sea water standards could be obtained (92–105%). Armstrong et al. [8] showed that quantitative recoveries of phosphorus could be obtained from β-glycerophosphate, ribose-5-phosphate, RNA, choline phosphate and 2-aminoethane-phosphonic acid (100 µgP/l) filtered sea water standards, if samples were irradiated with hydrogen peroxide for 2 h at 60–70°C. Cembellia and Antia [24] using this procedure showed that high recoveries of phosphorus from phosphorus compounds containing C–P bonds, 1 and 2 aminoethylphosphate and phosphoformate added to sea water (10 µgP/l), could also be achieved (96–101%). Henriksen [64] using a similar procedure showed that for complete recoveries of phosphorus from low turbid freshwaters, samples needed to be acidified to at least 0.004 M before irradiation. When this was done, similar results were obtained to those using a peroxodisulphate and a thioglycollic acid hydrolysis procedure. Armstrong et al. [8] noted that UV-photo-oxidation alone was not sufficient to convert condensed phosphates (P–O–P) to orthophosphate and Solorzano and Strickland [132] have suggested that the use of UV-photo-oxidation provides a basis for the discrimination between dissolved organic phosphorus and condensed phosphorus fractions. Dissolved organic phosphorus is determined by UV-photolysis with hydrogen peroxide and polyphosphates are subsequently hydrolysed with hydrochloric acid at 100°C. Armstrong and Tibbitts [7] in a later paper showed that if irradiation without acid was extended to 15–16 h still only small amounts of polyphosphates were hydrolysed. If samples were made 0.025 M in hydrochloric acid and boiled about 80% of phosphorus added to sea water as pyrophosphate was recovered. Goulden and Brooksbank [54] reported an on-line UV digestion system for measuring TP in lake water samples. Samples were digested at 85°C with 1.26 M sulphuric acid and 0.031 M potassium peroxodisulphate. Complete recoveries of phosphorus from tripolyphosphate and adenosine 5-monophosphate standards were obtained (100%). Results for lake waters were in agreement with a batch autoclave procedure using sulphuric acid–peroxodisulphate. Zaiyou and Limin [149] used UV-photo-oxidation with 0.15 M sulphuric acid and 0.04 M potassium peroxodisulphate to determine TP in freshwater and sewage samples. Quantitative recoveries of phosphorus from glycerol 2-phosphate, o,o-dimethyl-2,2-dichlorovinylphosphonic acid, o,o-dimethyl-2,2-dichloroethoxyphosphate, tri-butylphosphine oxide, o,o-dimethyl-S-(N-methylcarbamoyl)methyl phosphorodithioate, o, o-dimethyl-S-(1,2 dicarboxethoxyethyl) dithiophosphate, sodium pyrophosphate and hexametaphosphate standards (400 µgP/l) were obtained. Goossen and Kloosterboer [52] used UV-photo-oxidation with 0.32 M sulphuric acid to determine TFP and TP in river water samples. High recoveries of phosphorus from solutions of disodium phenylphosphate, adenosine 5-monophosphoric acid, adenosine 5'-diphosphate sodium salt, adenosine 5' triphosphate disodium salt, adenosine 5' tetraphosphate trisodium salt, flavine-mononucleotide disodium salt, glycerol 2-phosphate and tris (β-propionic acid)phosphonium chloride standards (266 µgP/l) were obtained (95–100%). Low recoveries of phosphorus were obtained for solutions of methyltriphenylphosphonium bromide (30%). Recoveries of phosphorus from sodium pyrophosphate, sodium tripolyphosphate and sodium hexametaphosphate standards were intermediate (79–101%). Results for the analysis of river waters were similar to those obtained by wet digestion with sulphuric acid and hydrogen peroxide on a steam bath at 100°C. Turbid samples needed to be diluted before analysis. Mc Kelvie et al. [104] used an in-line UV-photo-oxidation procedure with 0.15 M potassium peroxodisulphate and 0.17 M sodium tetrahydroborate to determine organic phosphorus in freshwaters. They showed that quantitative recoveries of phosphorus from inositol hexaphosphate, phosphoenolpyruvic acid, 2-aminoethyll phosphonic acid, adenosine 5'-monophosphate and glucose 6-phosphate standards (69–1000 µgP/l) could be obtained (90–100%). They reported no appreciable recoveries of phosphorus from sodium tripolyphosphate and adenosine 5-tri-phosphate standards. Results for freshwaters, sewage effluents and an algae culture were in good agreement with those obtained using the APHA-AWWA-WPCF batch acid peroxodisulphate procedure. Ridal and Moore [121] re-evaluated the use of UV-photo-oxidation with hydrogen peroxide to determine TFP in sea water samples (pH 3). UV-photo-oxidation gave higher recoveries of TFP from sea waters than an autoclave procedure using peroxodisulphate. Best
results were obtained when a combination of UV-photo-oxidation followed by autoclaving with peroxydisulphate was used. Ron Vaz et al. [122] reported the use of an on-line UV-photo-oxidation procedure with and without 0.24 M peroxydisulphate to determine TFP in soil solutions. Sodium fluoride (0.26 M) was added to digests to prevent the formation of aluminium hydroxide and the scavenging of phosphorus. High recoveries of phosphorus from phytic acid, β-glycerophosphate, α-D-glucose 1-phosphate, 2-amino-nophosphonic acid and adenosine 2′ (3′) phosphonic acid standards (200 μgP/l) were obtained by UV-photo-oxidation alone (99–101%). Recoveries of phosphorus from nicotinamide, adenine dinucleotide, adenosine 5′-triphosphoric acid, trimetaphosphate and pyrophosphate standards were low (6–8%). Higher TFP values were obtained for soil solutions if UV-photo-oxidation with peroxydisulphate was used to digest soil solutions (15–78% increase). Kerouel and Aminot [82] using a continuous flow UV-photo-oxidation procedure with the addition of 1–2 drops of hydrogen peroxide have confirmed that high recoveries of phosphorus from 2-aminoethylphosphonic acid, phytic acid, guanosine-5-monophosphate, phosphorylcholine, riboflavine-5-monophosphate, glucose-6-phosphate, adenosine-5-monophosphate, ribose-5-phosphate, phosphoenolpyruvic acid and sodium β-glycerophosphate (31 μgP/l) distilled deionised water standards could be obtained (100–106%). Lower recoveries from sea water standards were reported (53–101%) especially for 2-aminoethylphosphonic acid (53%), phytic acid (57%) and phosphoryl choline chloride (69%).

Ormaza-Gonzalez and Statham [116] using Armstrong et al. [8] procedure but with a lower irradiation time (30 min vs 2 h) have reported that high recoveries of phosphorus were obtained from 2-aminoethylphosphonic acid, phytic acid, guanosine-5-monophosphate, phosphorylcholine, riboflavine-5-monophosphate, glucose-6-phosphate, adenosine-5-monophosphate, ribose-5-phosphate, phosphoenolpyruvic acid and sodium β-glycerophosphate (31 μgP/l) distilled deionised water standards could be obtained (100–106%). Lower recoveries from sea water standards were reported (53–101%) especially for 2-aminoethylphosphonic acid (53%), phytic acid (57%) and phosphoryl choline chloride (69%).

Ormaza-Gonzalez and Statham [116] using Armstrong et al. [8] procedure but with a lower irradiation time (30 min vs 2 h) have reported that high recoveries of phosphorus were obtained from 2-aminoethylphosphonic acid, phytic acid, guanosine-5-monophosphate, phosphorylcholine, riboflavine-5-monophosphate, glucose-6-phosphate, adenosine-5-monophosphate, ribose-5-phosphate, phosphoenolpyruvic acid and sodium β-glycerophosphate (31 μgP/l) distilled deionised water standards could be obtained (100–106%). Lower recoveries from sea water standards were reported (53–101%) especially for 2-aminoethylphosphonic acid (53%), phytic acid (57%) and phosphoryl choline chloride (69%).

4.3. Autoclave digestion

4.3.1. Peroxydisulphate

Menzel and Corwin [105] advocated the use of autoclave heating with the addition of peroxydisulphate to determine TP in sea water. Samples were digested at 120°C at 15–20 psi for 30–60 min with 0.026 M peroxydisulphate. High recoveries of phosphorus from lecithin, phosphorocholine and 5-adenyllic acid standards (1.7–2.5 μgP/l) and zooplankton added to distilled water were obtained (98–101%). Recoveries of phosphorus from a growing culture of Phaeodactylum tricornutum and sea water samples were also quantitative (96–100%). Incomplete recoveries of phosphorous were obtained from suspensions of bottom sediments (80%). Jenkins [76] using a similar procedure showed the ability of peroxydisulphate to cleave C–P bonds. Quantitative recoveries of phosphorus from phenylphosphoric acid and phenyl phosphorus acid standards (50 μgP) were claimed although no recoveries were reported. Cembellia et al. [24] also using a similar procedure showed that the recoveries of phosphorus from 1 and 2 aminoethyl phosphate and phosphateformate (C–P bonds) sea water standards (10 μgP/l) were not complete (81–97%). O’Connor and Syers [115] have reported the incomplete measurement of TP in waters containing particulate inorganic materials of soil origin when samples were digested with peroxydisulphate. They found that the amount of phosphorus measured in both unfiltered and filtered water samples decreased with increasing amounts of particulate material and suggested that phosphorus compounds such as phytic acid could not be degraded to orthophosphate. They suggested that the use of peroxydisulphate with autoclave heating was satisfactory only for the determination of TP in water containing particulate material largely in a readily oxidisable organic form such as sewage effluents and urban run-off.
4.3.2. Acid peroxodisulphate

Goulden and Brooksbank [54] analysed TP in lake water samples after digestion with 0.013 M potassium peroxodisulphate, 0.0006 M nitric acid and 0.001 M sulphuric acid for 30 min at 85°C. Complete recoveries of phosphorus from tripolyphosphate and adenosine 5-monophosphate standards were obtained (~100%). Jeffries et al. [75] showed that the use of autoclave digestion with 0.06 M sulphuric acid and 0.015 M potassium peroxodisulphate, for 1 h (temperature not given), was not suitable for water samples containing high concentrations of suspended solids such as raw sewage and flood waters. High recoveries of phosphorus from glucose 1-phosphoric acid, glucose 6-phosphoric acid, DNA, adenosine 5-monophosphoric acid, adenosine 5-diphosphate, adenosine triphosphate, phosphoserine, sodium-β-glycerophosphate, sodium tri phosphate, sodium meta phosphate and teta sodium pyrophosphate standards (40 μgP/l) were obtained (95–115%). Results obtained for river and lake water samples were in agreement with those obtained using the APHA-AWWA-WPCF acid peroxodisulphate digestion procedure. For raw sewage and other effluents containing a higher proportion of suspended particles, the autoclave procedure gave lower recoveries. Ridal and Moore [121] evaluated the use of autoclave digestion with 0.015–0.15 M peroxodisulphate to determine TFP in acidified sea water (pH 3). Autoclaving with peroxodisulphate gave marginally lower recoveries of phosphorus than UV-photo-oxidation with hydrogen peroxide. Best recoveries were obtained when a combination of UV-photo-oxidation followed by autoclaving with peroxodisulphate was used. Kerouel and Aminot [82] digested a range of phosphorus compounds (60 μgP/l) added to deionised water and sea water at 115°C for 30–60 min with 0.016 M peroxodisulphate and 0.133 M sulphuric acid. Complete recoveries of phosphorus from potassium dihydrogen phosphate, adenosine-5'-phosphate and β-glycerophosphate standards (0–5 μgP/l) were obtained (99–108%). Valderama [141] showed that digestion with 0.022 M potassium peroxodisulphate and 0.024 M sulphuric acid at 120°C for 1 h. Recoveries of phosphorus from adenosine 5-triphosphate disodium salt, adenosine 5-monophosphate sodium salt, tetra sodium pyrophosphate and inositol hexaphosphoric acid dodeca sodium salt standards were 89–102%. The recoveries of phosphorus from a quality assurance river water sample were 95%. Results were in agreement with those obtained using a sulphuric–nitric acid and a sulphuric acid–hydrogen peroxide digestion procedure.

4.3.3. Alkaline peroxodisulphate

Koroleff [88] introduced the use of peroxodisulphate oxidation in an alkaline medium as a method for the determination of total nitrogen in water, and D’Elia et al. [37] later modified the method. Gilbert et al. [48] adopted the alkaline peroxodisulphate method of D’Elia et al. [37] to allow simultaneous determination of total nitrogen and TP in natural water samples. Samples were digested for at least 30 min at 100–110°C with 0.015 M potassium peroxodisulphate and 0.045 M sulphuric acid. High recoveries of phosphorus from 2-aminoethylphosphonic acid, phytic acid, guanosine-5-monophosphoric acid, phosphorylcholine, riboflavin-5-monophosphoric acid, glucose-6-phosphate, adenosine-5-monophosphoric acid, ribose-5-phosphate, phosphoenolpyruvic acid and sodium B-glycerophosphate deionised water standards were obtained (99–108%). Recoveries of phosphorus from sea water standards were much lower (37–100%) especially for 2-aminoethylphosphonic acid (77%), phytic acid (70%) and phosphoryl choline chloride (37%). Ormaza-Gonzalez and Statham [116] investigated the use of acid peroxodisulphate to determine TP and TFP in river and sea water samples. Water samples were autoclaved at 120°C for 30 min with 0.014 M potassium peroxodisulphate and 0.017 M sulphuric acid. High recoveries of phosphorus from 2-aminoethyl phosphonate, tripolyphosphate, trimetaphosphate, glucose 6-phosphate, 4-nitrophenyl phosphate and adenosine 5-triphosphate distilled deionised water standards (61–93 μg/l) were obtained (>90%). Recoveries of guanosine 5-triphosphate and glycerophosphate were lower (85% and 70%, respectively). Recoveries of phosphorus compounds added to sea water were much lower (60–90%). Recovery of phosphorus from a filtered river water sample was similar to that of a high temperature oxidation procedure (98%). Recoveries from filtered sea water samples were much lower (65–98%). Rowland and Haygarth [123] determined TFP in soil solutions by digesting filtered samples with 0.026 M peroxodisulphate and 0.024 M sulphuric acid at 120°C for 1 h. Recoveries of phosphorus from adenosine 5-triphosphate disodium salt, adenosine 5-monophosphate sodium salt, tetra sodium pyrophosphate and inositol hexaphosphoric acid dodeca sodium salt standards were 89–102%. The recoveries of phosphorus from a quality assurance river water sample were 95%. Results were in agreement with those obtained using a sulphuric–nitric acid and a sulphuric acid–hydrogen peroxide digestion procedure.
An interesting finding of his work was that once samples had been digested, the oxidised samples were stable for at least 97 days. Langner and Hendrix [93] showed that the efficiency of alkaline peroxodisulphate with autoclave heating for the digestion of particulate materials was dependent on the type of sample to be analysed. They studied the recovery of phosphorus from suspensions of NIST SRM 1571 Orchard Leaves (5–15 mgP) and Aufwuchs (2 mgP) in distilled water. Phosphorus recoveries of 80–89% and 94–101%, respectively, were obtained when suspensions were digested for 1 h at 100–110°C with 0.05–0.123 M potassium peroxodisulphate and 0.15–0.37 M sodium hydroxide. The low recovery of phosphorus from Orchard Leaves suggested that phosphorus bonds resistant to peroxodisulphate were present which require more drastic digestion conditions such as sulphuric acid and nitric acid digestion to oxidise organic material and release phosphorus.

Ebina et al. [42] used alkaline peroxodisulphate digestion for the determination of total nitrogen and TP in river water samples. Samples were digested for 1 h at 120°C with 0.037 M of potassium peroxodisulphate and 0.038 M sodium hydroxide. High recoveries of phosphorus from adenosine-5'-monophosphate, adenosine-diphosphate and adenosine-5'-triphosphate disodium salt standards (up to 200 mgP/l) were obtained (96.6–100.8%). High recoveries of phosphorus were also obtained from a river water sample spiked with up to 4 mgP/l of potassium dihydrogen phosphate and up to 4 mgP/l of glucose-1-phosphate, i.e. 98.5–103.3% and 96.7–101.4%, respectively. Hosomi and Sudo [69] digested freshwater samples for 1 h at 120°C with 0.05 M potassium peroxodisulphate and 0.073 M sodium hydroxide. High recoveries of phosphorus from suspensions of NIST SRM 1571 Orchard Leaves, NIES CRM No 2 Pond Sediment, NIES CRM No 3 Chlorella and NIES CRM No 1 Pepper Bush certified reference materials in distilled water and sea water at 115°C for 30–60 min with 0.016 M peroxodisulphate and 0.035 M sodium hydroxide. High recoveries of phosphorus were also obtained (94–103%) when potassium dihydrogen phosphate, sodium tripolyphosphate, adenosine-5-monophosphate and sodium β-glycerophosphate (200 μgP/l) were added to lake water. Woo and Maher [148] further investigated the use of alkaline peroxodisulphate to digest suspensions of Chlorella and Pond sediment. Digestion of samples for 1 h at 120°C with 0.045 M potassium peroxydisulphate and 0.04 M sodium hydroxide gave high recoveries of phosphorus from Chlorella suspensions containing up to 100 μgP/l (99–103%) and Pond sediments suspensions containing up to 60 μgP/l (98–107%). Recovery of phosphorus from Pond suspensions containing 70, 80 and 90 μgP/l were 92.3±0.7%, 91±2% and 91±1%, respectively. Results obtained when TP in turbid lake waters were analysed were in agreement with those obtained using the APHA-AWWA-WPCF [5] sulphuric acid–nitric acid digestion procedure. High recoveries of phosphorus were obtained (93–117%) from potassium dihydrogen phosphate, phytic acid, 2-aminoethylphosphonic acid, DL-β-glycerophosphate, phosphoformic acid, α-phosphonyl ethanola- mine and phospho(enol)pyruvate standards and when compounds were added to a turbid lake water sample (20–100 μgP/l). Williams et al. [146] digested soil and litter extracts samples with 0.03 M potassium peroxydisulphate and 0.18 M sodium hydroxide at 110°C for 30 min to determine TFP. At low organic carbon concentrations (<100 mgC/l) complete recoveries of phosphorus from inositol hexaphosphate were obtained (99–106%). At carbon concentrations >200 mgC/l recoveries of phosphorus reduced to 10–25%. Kerouel and Aminot [82] digested a range of phosphorus compounds (60 μgP/l) added to deionised water and sea water at 115°C for 30–60 min with 0.016 M peroxodisulphate and 0.035 M sodium hydroxide.
hydroxide and 0.05 M boric acid. High recoveries of phosphorus were obtained from 2-aminoethylphosphonic acid, phytic acid, guanosine-5-monophosphate, phosphorylcholine, riboflavin-5-mono-phosphate, glucose-6-phosphate, adenosine-5-monophosphate, ribose-5-phosphate, phosphoenolpyruvic acid and sodium B-glycerophosphate were obtained from deionised water standards (98–108%) and sea water standards (88–101%). All recoveries from sea water standards were above 92% except aminoethylphosphonic acid, 88%. Ormaza-Gonzalez and Statham [116] investigated the use of alkaline peroxodisulphate to determine TP and TFP in river and sea water. Water samples were autoclaved at 120°C for 30 min with 0.02 M potassium peroxodisulphate, 0.04 M sodium hydroxide and 0.06 M boric acid. Variable recoveries of phosphorus from distilled deionised water and sea water standards (62–77 mgP/l) were obtained; 2-aminoethyl phosphonate (95%, 95%, respectively), glycerophosphate (85%, 100%, respectively), tripolyphosphate (65%, 60%, respectively), trimetaphosphate (25%, 85%, respectively), glucose 6-phosphate (90%, 100%, respectively), 4-nitrophenyl-phosphate (75%, 95%, respectively), adenosine 5’-triphosphate (70%, 55%, respectively) and guanosine 5’-diphosphate (85%, 60%, respectively). Recoveries from filtered sea water (54–90%) were lower than those obtained by a high temperature oxidation procedure.

4.3.4. Digestion vessels

Borosilicate as opposed to plastic test tubes have been widely used for the digestion of water samples because of their high resistance to chemical attack, and strength [42,48,75,91,105]. Some studies have reported the use of test tubes fitted with rigid phenolic caps having inert polytetrafluoroethylene lined discs which are resistant to repeated dry heat and steam digestion cycles [42,91]. However, it is often unclear from the literature what digestion vessels were used, how vessels have been selected or if an open or closed digestion procedure was used.

4.4. Microwave digestion

Littau and Engelhart [99] showed that microwave heating with alkaline peroxodisulphate digestion could be successfully used for TP measurements of particles (up to 80 mg). Samples were digested in two stages: (i) heating for 10 min at 961 W, 30 psig pressure, and (ii) heating for 30 min at 961 W, 135 psig with 0.166 M of potassium peroxodisulphate and 0.24 M sodium hydroxide. High recoveries of phosphorus from plant and animal tissues, dried sewage sludge, and river sediment were obtained (77–103%). They concluded that the method was effective for the oxidation of compounds with phosphorus bonds resistant to hydrolysis. Hinkamp and Schwedt [67] used on-line microwave heating coupled with flow injection analysis. Samples were digested at 650 W with 0.08 M perchloric acid and 0.05 M potassium peroxodisulphate. High recoveries of phosphorus from hexamethylenediaminetetramethylenephosphonic acid, 1-hydroxy-ethane-1,1-diphosphoric acid, adenosine-5’-monophosphoric acid, α-D-glucose-1-phosphate disodium salt tetrahydrate, disodium phenyl phosphate dihydrate, diethylphosphite and triethylphosphate standards (5 mgP/l) were obtained (87–100%). Lower recoveries were obtained for tetrasodium diphosphate decahydrate and pentasodium triposphate standards (62–80%). Johnes and Heathwaite [78] evaluated the recovery of phosphorus compounds added to river, lake and groundwater samples using microwave heating with alkaline peroxodisulphate digestion. Samples were digested for 45 min at 600 W with 0.25 M potassium peroxodisulphate and 0.038 M sodium hydroxide. High recoveries of phosphorus were obtained from potassium dihydrogen phosphate, tetrasodium pyrophosphate, glucose-1-phosphoric acid disodium salt, adenosine-5’-diphosphoric acid disodium salt and adenosine-5’-triphosphoric acid disodium dihydrogen salt deionised water standards and when these compounds were added to turbid water samples (0.2–10 mgP/l), i.e. 96–103% and 98–106%, respectively. They concluded that microwave digestion with alkaline peroxodisulphate was robust in its ability to handle a variety of natural water samples (0.01–50 mgP/l) in the pH range 5–8. Williams et al. [147] used on-line microwave heating coupled with flow injection analysis for the determination of TP in waste waters. Samples were digested at 540 W with 0.70 M of nitric acid. Pretreatment of samples containing pyrophosphate with pyrophosphate pyrophosphatase was necessary to hydrolyse condensed phosphorus compounds. High recoveries of phosphorus from potassium dihydrogen phosphate, triso-
dium metaphosphate, tetrasodium metaphosphate, and tetrasodium pyrophosphate standards (10 mgP/l) were obtained (99–101%). Good recoveries of phosphorus (99.5%) were obtained when pyrophosphate was added to waste waters (2 mgP/l). If samples or standards were not pre-treated with the enzyme only 66–68% of pyrophosphate was recovered. Benson et al. [11] used on-line microwave heating coupled with flow injection analysis and potassium peroxodisulphate to determine TP in water and wastewater samples. Samples were digested at 700 W with 0.018 M of potassium peroxodisulphate–sulphuric acid (pH = 0.6). High recoveries of phosphorus from phytic acid, phosphonoformic acid, 2-aminoethylphosphonic acid, \( \text{d-glucose-6-phosphate, } \text{DL-}\alpha\text{-glycerophosphate, O-phosphorylethanolamine and adenosine-5'-monophosphate standards (2–10 mgP/l) were obtained (88–106%). Low recoveries were obtained from adenosine 5'-di and triphosphate (41–49%) and polyphosphates standards (29–40%). They found that their procedure was suitable for the analysis of sewage effluents containing low concentrations of condensed phosphates without additional sample pretreatment. Results obtained were in agreement with those obtained when water samples were analysed by a batch procedure using the same digestion reagent.}

Woo and Maher [148] investigated the use of alkaline peroxodisulphate to digest suspensions of NIES CRM No 3 Chlorella and NIES CRM No 2 Pond sediment (20–100 \( \mu \text{gP/l})). Digestion of samples for 10 min at 450 W with 0.045 M potassium peroxodisulphate and 0.04 M sodium hydroxide gave good recoveries of phosphorus from suspensions of Chlorella (99–103%) and Pond sediments (96–107%). Results obtained were in agreement with those obtained using the APHA-AWWA-WPCF [5] sulphuric–nitric acid digestion procedure.

Benson et al. [12] used an on-line UV-thermal induced digestion system with 0.026 M ammonium peroxydisulphate and 2.1 M perchloric acid to determine TP in fresh water and waste water samples. High recoveries of phosphorus (98–108%) were obtained from phytic acid, \( \text{o-phosphorylethanolamine, phosphoformic acid, glucose 6-phosphate, 2-aminoethyl phosphonic acid, DL-}\alpha\text{-glycerophosphate, adenosine 5'}\text{-monophosphate and sodium tripolyphosphate standards (10 mg/l). Low recoveries were obtained for adenosine 5'}\text{-diphosphate, adenosine 5'}\text{-triphosphate and sodium pyrophosphate standards (85–89%). Halilweli et al. [57] used on-line heating with 0.24 M sulphuric acid and 0.016 M molybdate to convert condensed phosphates in domestic waste waters to orthophosphate after ion chromatographic separation. High recoveries of phosphorus (95–103%) were obtained when tripolyphosphate and pyrophosphate were added to filtered untreated sewage (50 and 1000 \( \mu \text{gP/l}).}

### 4.6. Discussion

#### 4.6.1. Efficiency of digestion procedures to oxidise or hydrolyse phosphorus compounds to orthophosphate

Some general trends are evident from the literature. Procedures using mineral acids alone or in combination with peroxide or peroxodisulphate are thought to be effective in converting all types of phosphorus compounds to orthophosphate [5,47,123]. Most compounds containing \( \text{C–O–P bonds are readily decomposed using oxidants such as peroxo-}

W. Maher, L. Woo / Analytica Chimica Acta 375 (1998) 5–47
disulphate (see Table 4). Some C–O–P compounds such as 4 nitrophenyl phosphate are difficult to oxidise [116]. However, in sample matrices such as sea water oxidation may be impaired [82,116]. Compounds containing P–O–P bonds can only be hydrolysed in the presence of an acid [57,149], but water samples need to be heated to temperatures of 90–120°C. Oxidants such as peroxodisulphate cannot hydrolyse P–O–P bonds [8,67,104], but are needed for the complete degradation of organic polyphosphates [52]. Compounds containing C–P bonds may or may not be oxidised depending on the structure of the compound. Compounds containing complex C–P structures such as 0,0-diethyl-0-2 [ethylthio] ethyl phosphorothioate or aminoethyl phosphonate are not oxidised while others such as phosphoformate are readily oxidised [24,74,82].

4.6.2. Use of peroxodisulphate as an oxidant

The majority of digestion procedures reported in the literature use peroxodisulphate as the oxidant (Table 4). Generally no rationale is given for the choice of oxidant concentration or the digestion temperature and time used.

The decomposition of peroxodisulphate produces oxygen and an acid on decomposition [42]:

\[
\text{K}_2\text{S}_2\text{O}_8 + \text{H}_2\text{O} \rightarrow 2\text{KHSO}_4 + \text{O}_2.
\]

With the decomposition rate given by

\[
d[S_2\text{O}_8]/dt = K_1[S_2\text{O}_8^{2-}] + K_2[S_2\text{O}_8][\text{H}^+],
\]

where \(K_1\) is the rate constant for thermal decomposition and \(K_2\) is the rate constant for acid catalysed decomposition [53,70]. Peroxydisulphate decomposes at a greater rate as the temperature increases and pH decreases. The reaction time will be limited by the decomposition rate and as Goulden and Anthony [53] state “the success or failure of the process depends on whether organic matter can be oxidised before the peroxodisulphate decomposes”. Ebina et al. [42] have calculated that the half life of peroxodisulphate at 130°C is approximately 20 s.

Goulden and Anthony [53] have shown that organics can be completely oxidised by peroxodisulphate at 90–100°C. Williams [96] when investigating the decomposition of amino acids in sea water using peroxodisulphate noted that a temperature of <100°C was required for oxidation, but greater oxidation of amino acids occurred at 100°C than 130°C. Thus a temperature of at least 90–100°C is required for oxidation, but the use of higher temperatures may be detrimental as decomposition of peroxodisulphate may occur before organic matter is oxidised. The optimum temperature will be a compromise that results in complete oxidation of material and hydrolysis/release of phosphorus from polyphosphates and particles [148].

Canelli and Mitchell [22] question whether complete mineralisation of organic matter is required to release organically bound phosphorus. Indeed many organic compounds containing phosphorus are easily hydrolysed [19,36,39] under the acidic conditions created during the decomposition of peroxodisulphate. However, for many organic compounds, phosphorus will not be released without the use of an oxidant such as peroxodisulphate [11,67]. It is interesting to note that given the half life of peroxodisulphate and typically concentrations used for the digestion of water samples, most digests will become acidic within 2–5 min even if a mixed digestion reagent containing sodium hydroxide is used. Thus during most of the digestion time, acid hydrolysis or acid extraction will be occurring. Optimum digestion times for samples containing suspended materials may be a function of the time required for acid hydrolysis of organic and polyphosphates and extraction of occluded phosphorus from particles rather than the oxidation of organic matter.

Caution must be exercised when using alkaline peroxodisulphate as the digestion reagent that the concentration of alkali is not too high such that the final pH is not acidic or hydrolysis of polyphosphate compounds will not occur (see next section). The ratios of sodium hydroxide and peroxodisulphate normally used for the digestion of water samples result in a final pH of 2 which is sufficient for the hydrolysis of polyphosphates [69,91,148].

The sample matrix may inhibit peroxodisulphate’s ability to decompose organic phosphorus compounds. For example, recoveries of phosphorus compounds from sea water are normally lower than from freshwater [82,116]. Peroxodisulphate oxidises chloride in sea water to chlorine and this diminishes its effec-
tiveness [106]. Similarly, if water samples contain high carbon concentrations (>200 μg C/l) recoveries of phosphorus from compounds such as phytic acid can be impaired [146]. Again peroxydisulphate effectiveness is diminished as it is being used to mineralise the excessive organic carbon present in solution. This is illustrated by the results of several authors who have analysed TP in suspensions of NIST SRM 1571 Orchard Leaves. Langner and Hendrix [93] using a ratio of 5 mg of sample to 200–500 mg peroxydisulphate could recover about 90% of TP. Hosomi and Sudo [69] using a ratio of 0.5 mg of sample to 200 mg of peroxydisulphate could recover 98% of TP. Hosomi and Sudo [69] and Woo and Maher [148] have shown that concentrations of peroxydisulphate typically used for digestion of samples can be used to digest water samples containing up to 50 mg/l of organic carbon. Thus matrices with higher concentrations of oxidisable material may require a higher peroxydisulphate concentration for complete oxidation of organic carbon and release of phosphorus compounds.

An alternative approach is to use peroxydisulphate with high concentrations of strong acids to generate Caro’s acid, H₂SO₅ [87]:

\[
\text{H}_2\text{SO}_5 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2.
\]

A powerful oxidising and hydrolysing mixture is produced. This approach has been used successfully to determine TP in waste water samples by a UV thermal induced digestion procedure [12].

The use of peroxydisulphate in conjunction with UV-photolysis may also improve its oxidising power. Peroxydisulphate is thought to form reactive radicals in the presence of UV light [50,70].

\[
\begin{align*}
2\text{SO}_4^{2-} & \rightarrow \text{S}_2\text{O}_8^{2-} + 2e^-,
\text{S}_2\text{O}_8^{2-} & \rightarrow 2\text{SO}_4^{4+},
\text{SO}_4^{4+} + \text{H}_2\text{O} & \rightarrow \text{HSO}_4^- + \text{OH}^+,
\text{S}_2\text{O}_8^{2-} + \text{OH}^+ & \rightarrow \text{HSO}_4^- + \text{SO}_4^{4+} + \frac{1}{2}\text{O}_2,
\text{SO}_4^{4+} + \text{OH}^+ & \rightarrow \text{HSO}_4^- + \frac{1}{2}\text{O}_2.
\end{align*}
\]

4.6.3. Hydrolysis of polyphosphates to orthophosphate

Polyphosphates are not hydrolysed to orthophosphate in a neutral medium [7,8,64].

The main factors affecting the hydrolysis of inorganic polyphosphates are acidity, temperature and reaction time [52,57,149,150]. Heat rather than UV-photolysis results in the hydrolysis of polyphosphates [52].

It should be noted that for organic polyphosphates such as adenosine 5’ di, tri and tetra polyphosphate, that in acidic conditions with the application of heat, one, two and three phosphate groups, respectively, can be easily hydrolysed. The phosphate group attached directly to the adenosine group requires the use of an oxidant to separate it from the adenosine [52].

The importance of the effect of the interaction of acidity temperature and reaction time on phosphate recoveries from polyphosphates is illustrated by the following examples.

Low recoveries of phosphate from polyphosphates have been reported using flow systems in which samples were acidified between 0.07 and 0.08 M [11,67,146]. However, the digestion times are short, i.e. 1.2–3 min. Halliwell et al. [57] and Hirai et al. [68] have shown that in acidic solutions (0.24–20 M H⁺) complete hydrolysis using short digestion times (2–3 min) can be achieved when the digestion temperature is raised to 120–130°C. In [57], it should be noted that molybdenum was also present in the digest reagent and may have had a catalytic effect on the hydrolysis of phosphorus bonds [144]. The use of alkaline peroxydisulphate in which the final pH is approximately 2 has been used successfully to convert polyphosphates to orthophosphate [42,69,91]. Digestion temperatures of 120°C and digestion times of 60 min were used. In contrast Ormaza-Gonzalez and Statham [116] reported lower recoveries of phosphates from polyphosphates (60–90%). Again a digestion temperature of 120°C was used, but the digestion time was only 30 min. At higher solution pH, longer hydrolysis times are required. Thus, it would appear that for complete recoveries of phosphorus from polyphosphates acidity, digestion temperature and time are not independent and must be co-optimised. If organic polyphosphates are present an oxidising agent will also be required for complete degradation of these compounds.

4.6.4. Conventional heating

The use of conventional heating, i.e. hot plates, sand baths or aluminium heating blocks, mostly involves
the heating of samples with mineral acids and hydrogen peroxide or peroxodisulphate. There is a paucity of literature on the recovery of phosphorus from phosphorus compounds when digestion with mineral acids and volume reduction is used. Normally boiling with mineral acids in combination with peroxide or peroxodisulphate results in the complete oxidation or hydrolysis of phosphorus compounds [47]. Thus, there is no requirement to heat water samples to high temperatures, e.g. fuming, as boiling gives high recoveries of phosphorus. Many disadvantages of using conventional heating procedures have been reported:

- Volume reduction of sample causes the precipitation of large quantities of salt and problems with colourimetric procedures [76].
- Condensed phosphorus compounds are formed with sulphuric acid [52].
- The use of perchloric acid is dangerous [135].
- Excess peroxide must be destroyed prior to colourimetric determination [61].
- Slow, requires continuous attention [107].
- Worse precision compared to autoclave heating [107].

With the advent of microprocessor controlled aluminium heating blocks, the use of less dangerous oxidant mixtures such as 0.11 M sulphuric acid and 0.03 M peroxodisulphate [47] and the use of procedures not requiring the boiling of samples to dryness many of these problems can be eliminated. However, precision is usually worse when compared with other methods such as autoclave heating [69,75,91].

4.6.5. UV-photo-oxidation

UV-photo-oxidation without the addition of an oxidant and acid is considered to be suitable for the digestion of water samples as long as samples are not turbid and do not contain condensed phosphates [8,52,104,121]. High recoveries of phosphorus from most compounds containing C–O–P and C–P bonds are obtained when small amounts of hydrogen peroxide is used as an oxidant [52,23,122]. Although high recoveries of phosphorus are achieved for standards prepared in distilled or freshwater, lower phosphorus recoveries are reported for sea water matrices [82,116]. Thus UV-oxidation without the addition of a more powerful oxidant is probably not suitable for the digestion of sea waters because of the incomplete recovery of phosphorus from organic phosphorus compounds.

Peroxodisulphate is often added to increase the oxidation efficiency of UV-photo-oxidation procedures [54,104,122,149]. The UV-photolysis of peroxodisulphate results in oxidising radicals [50,70]. Higher recoveries of TFP are obtained for sea waters and soil solutions if peroxodisulphate is used compared to UV-photo-oxidation alone. As well the conversion of phytic acid to orthophosphate occurs which is not achieved by UV-photo-oxidation alone [12,121,122]. Until validation studies on sea waters are performed the use of UV-photo-oxidation with peroxodisulphate to decompose organic phosphorus compounds in sea water cannot be recommended.

UV-photo-oxidation cannot be used to hydrolyse condensed phosphorus compounds unless an acid is added [7,8,64,116]. Acidification of samples (0.025 M) results in the complete hydrolysis of inorganic polyphosphates at 100–120°C as long as sufficient time is given for hydrolysis to occur [7,52,57].

The inability of UV-photo-oxidation (without the addition of an acid) to hydrolyse polyphosphates has been suggested as a basis for discriminating between dissolved organic phosphorus and condensed phosphorus fractions in sea water samples [132]. Dissolved organic phosphorus is determined by UV-photolysis with hydrogen peroxide and polyphosphates are subsequently hydrolysed with hydrochloric acid at 100°C.

A drawback to using UV-photolysis is that turbid samples need to be diluted or filtered before analysis [52,104]. No information on the effect of turbidity on efficiency of UV-photo-oxidation is available. Thus, UV-photo-oxidation of acidified samples can only be confidently used for TFP measurements, not for TP measurements if samples are turbid.

4.6.6. Autoclave heating

The use of autoclave heating with peroxodisulphate (with and without acid or alkali addition) has been used widely used for the determination of TFP and TP in fresh and sea waters (Table 4). The use of peroxodisulphate eliminates neutralisation, transfer, dilution steps involved with the use of mineral acids. Thus digestion with acid or alkaline peroxodisulphate has become the preferred method for TFP and TP analyses.
because of its speed, convenience, simplicity and better precision than most other procedures.

Alkaline peroxodisulphate is often used because it allows the simultaneous determination of TP and total nitrogen. Both digestion with acidic or alkaline peroxodisulphate gives high recoveries of phosphorus from C–O–P, P–O–P and C–P compounds in distilled water and when compounds were added to turbid water samples (see Table 4). Initial alkaline conditions may be more effective in hydrolysing some alkyl phosphates than acidic conditions \[31\]. However, lower recoveries have been reported from sea water when peroxodisulphate, acid peroxodisulphate or alkaline peroxodisulphate have been used as digestion reagents \[23,82,116\], probably because peroxodisulphate is used up oxidising chloride to chlorine. Normally alkaline rather than acidic peroxodisulphate is recommended for digestion of sea waters \[82,116\].

Using alkaline peroxodisulphate to digest soil extracts may cause problems if large quantities of calcium or iron are present as precipitates will occur \[20,146\].

Many authors have reported the incomplete recoveries of phosphorus from water samples as suspended solid concentrations increase, i.e. solutions become more turbid \[75,91,93,105,115,148\]. It has been suggested that lower phosphorus recoveries from turbid samples are due to occluded phosphorus in oxides or hydrous oxides of sediment particles that cannot be extracted as well as the incomplete oxidation of phosphorus compounds such as phytic acid. From the work of Woo and Maher \[148\], the former reason is more likely. We digested suspensions of Pond sediment with an alkaline peroxodisulphate reagent using autoclave and microwave heating. The microwave procedure which had a higher temperature and pressure but the same amount of oxidant gave complete recoveries of phosphorus from all suspensions tested while the autoclave procedure gave recoveries 10% lower. It is hypothesised that the higher temperature and pressure resulted in the extraction of occluded phosphorus forms. Less problems have been encountered when suspensions have been organic in nature, e.g. Aufwuchs, Orchard leaves or Chlorella \[91,93,148\]. Lower recoveries from Orchard leaves have been attributed to adsorption of phosphorus from silica residues rather than incomplete digestion. It should be noted that there is a finite limit to the amount of organic matter than can be oxidised by a given concentration of peroxodisulphate and poor recoveries of phosphorus can be expected in the presence of very high concentrations of organic matter \[69,146\].

Recoveries of phosphorus from water samples will be a function of oxidant concentration, digestion temperature and time and the sample matrix \[148\]. Peroxodisulphate concentrations ranging from 0.015 to 0.05 M, digestion temperatures ranging from 85°C to 120°C and digestion times ranging from 30 to 90 min have been used (see Table 4).

In general, as would be expected, lower concentrations of peroxodisulphate are used to hydrolyse soluble phosphorus compounds than water samples containing high concentrations of suspended materials, i.e. turbid water and sewage samples. Temperature and times used are quite variable. The work of Woo and Maher \[148\] indicates that higher temperatures, i.e. 120°C, and longer digestion times, i.e. 1 h, are needed to recover phosphorus from samples containing high concentrations of suspended matter. This indicates that prolonged exposure to acidic conditions may be needed to effectively digest suspended material.

Many authors have compared the use of acid and alkaline peroxodisulphate and autoclaving for the digestion of water samples to more rigorous digestion procedures such as magnesium nitrate fusion, and nitric–sulphuric acid wet digestion \[61,69,91,148\]. Similar results for autoclave and the more rigorous acid digestion procedures have been obtained indicating that for the concentrations of algae, macrophyte and sediment particles normally encountered in water samples less drastic digestion procedures are adequate.

In general autoclave procedures give better precision (RSD) than conventional sulphuric–nitric acid digestion procedures, e.g. 3% vs 24% \[123\], 5.3–9.4% vs 6–13% \[91\] and 0.67–2.08 vs 1.35–2.74% \[69\].

4.6.7. Microwave heating

Relatively few studies have reported procedures using microwave heating for the digestion of water samples. The use of peroxodisulphate with microwave heating has resulted in high recoveries of phosphorus from phosphorus compounds added to distilled water and turbid water samples \[67,78,148\].
As previously mentioned the use of microwave heating appears to be of benefit when turbid samples are being analysed. Higher recoveries of phosphorus from suspensions of Pond sediments were obtained by microwave heating than autoclaving [148]. Using closed vessels results in higher temperatures and pressures being generated [83]. Recovery of phosphorus from water samples will be a function of oxidant concentration, digestion temperature and time, the sample matrix and the microwave cavity used [148]. Thus no standard or optimum conditions can be recommended and must be determined by each laboratory. Microwave cavities are relatively portable which should allow the on-site measurement of TFP and TP [12].

The use of on-line microwave heating coupled with flow injection analysis and using peroxodisulphate [11,67] should also allow the automation of TFP and TP measurements once reliable procedures for the removal of unwanted material prior to and after the digestion steps have been developed.

5. Summary/conclusions

It is evident from the literature that the effectiveness of any storage technique was dependent upon the type of water sample to be stored, i.e. whether it was surface, ground water, estuarine or sea water and if the sample was filtered before storage. The biological and physical characteristics of these water types (i.e. salinity, suspended sediment concentration and type, algal cells) are different and affect the storage of phosphorus in different ways [25,63,125]. Slow freezing of filtered and turbid water samples appear to be satisfactory for the long term storage (years) of a wide variety of sample types for FRP, TFP and TP analysis and does not suffer from contamination and interference problems from added preservatives, although some authors report a loss of FRP within one day [79,109]. The addition of acid may be needed to prevent flocculation and formation of precipitates in water samples which may occur during the freezing process [2,71], especially in samples containing calcite [79,86,113]. The storage of water samples at room temperature with preservatives appears to be satisfactory for the storage of samples for only short periods (<8 days) [49,65,84,86,112,130]. Refrigeration with the addition of preservatives extends the period of short term storage up to 60 days [45,56,76,118]. The addition of preservatives must be treated with caution as for example, the presence of chloroform or mercuric acetate may interfere in phosphate measurements [76,130].

Polyethylene bottles are the most frequently used containers for the storage of water samples for phosphorus analysis, but it is not clear in many studies if high or low density polyethylene containers were used. Glass bottles are not recommended mainly because of the ease of breakage on freezing. Acid washing of plastic containers reduces the adsorption of phosphorus [25,124]. Acid washed low density polyethylene containers appear to be universally suitable for the storage of water samples [9,91].

Adsorption of phosphorus to containers may be significant at low concentrations of phosphorus (i.e. <20 µg P/l) and low ionic strength [65,112,124]. It is uncertain from the literature what is the best storage container for samples containing low phosphorus concentrations and if pretreatment of containers to reduce phosphorus adsorption is required.

From a consideration of the forms of phosphorus in natural waters any procedure used to convert all forms of phosphorus in natural waters to orthophosphate for analysis must be able to hydrolyse inorganic and organic condensed phosphates and oxidise organic phosphorus compounds containing P–O–P, C–O–P and C–P bonds quantitatively to orthophosphate. As well the digestion procedures for TP need to be able to release orthophosphates incorporated in or adsorbed to mineral phases of particles into solution.

For reasons of ease, simplicity and precision, batch digestion of samples with alkaline or acid peroxodisulphate using autoclave or microwave heating is recommended. High recoveries of phosphorus from a range of phosphorus compounds containing P–O–P, C–O–P and C–P bonds expected in natural waters have been obtained. If turbid samples are to be analysed, caution must be exercised to ensure that the carbon or suspended solids concentration does not exceed the capacity of the digestion procedure to oxidise the carbon present and release occluded phosphorus from particulate materials. Better recoveries of phosphorus from turbid water samples are achieved using microwave heating with closed vessels, prob-
ably because of the higher temperatures and pressure generated.

The use of on-line heating (microwave, thermal induced) coupled with flow injection analysis and using peroxydisulphate or an oxidising acid mixture should also allow the automation of TFP and TP measurements once reliable procedures for the removal of unwanted particulate material prior to or after the digestion steps have been developed.

References

[51] H.L. Golterman, R.S. Clymo, M.A.M. Ohnstad, Methods for Physical and Chemical Analysis of Fresh Waters, IBP