12
Phosphates in Aquatic Systems

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12.1 Introduction

In aquatic systems, phosphorus occurs in a wide variety of inorganic and organic forms (McKelvie et al., 1995). While these may exist in the dissolved, colloidal, or particulate forms, the predominant species is orthophosphate in either the mono- or diprotonated forms $\text{HPO}_4^{2-}$, $\text{H}_2\text{PO}_4^-$. The dissolved component is operationally defined by filtration, and for this reason, the term *filterable* is used in preference to either *dissolved* or *soluble*, both of which are used extensively and interchangeably in the literature.

There may also be significant amounts of organic or condensed phosphates present. Filterable condensed phosphorus (FCP) is composed of inorganic polyphosphates, metaphosphates, and branched ring structures (Figure 12.1). The filterable organic phosphorus (FOP) fraction consists of nucleic acids, phospholipids, inositol phosphates, phosphoamides, phosphoproteins, sugar phosphates, aminophosphonic acids, phosphorus-containing pesticides, and organic condensed phosphates (McKelvie, 2005).

Phosphorus in aquatic systems may originate from natural sources such as the mineralization of algae, the dissolution of phosphate minerals, from anthropogenic point source discharges of sewage and industrial effluents, and diffuse inputs from grazing and agricultural land (Mesquita et al., 2011). Environmental interest in phosphorus stems from its critical role in the process of eutrophication. In many aquatic systems, phosphorus may be a limiting nutrient for the growth of algae.

Phosphate is an important routine parameter in water analysis, being simultaneously an essential macronutrient and a possible pollutant, when its concentration is abnormally high (Mesquita et al., 2011). Given that phosphorus may exist in a variety of dissolved and particulate forms, there has been considerable emphasis on the analytical and ecological literature on the determination of the amount of bioavailable phosphorus.

12.2 Measurements of Phosphates in Water Samples

12.2.1 Sample Preservation, Storage, and Pretreatment

The concentrations of various phosphorus fractions in unpreserved samples can be affected by different processes (physical, chemical, and biological) and it is usually recommended that their determinations are carried out immediately after sampling. Indeed, there is clear evidence that the various forms of phosphorus are altered rapidly between the time of sampling and analysis. Lambert et al. (1992) showed that very rapid decreases in both filterable reactive phosphorus (FRP) and total filterable phosphorus (TFP), and total reactive phosphorus (TRP) fractions occurred within 2 h when samples were refrigerated prior to filtration and analysis. This is supported by the work of Haygarth et al. (1995) who studied the effects of different storage regimes and container types on stability of soil water samples analyzed for MRP (molybdate reactive phosphorus = TRP) over a period of 32 days. The ideal approach might consist of *in situ* nutrient analysis, thus eliminating the need of sampling and storage (Worsfold et al., 1987; Johnson et al., 2000). However, it is not always possible to carry out *in situ* measurements and it is then a matter of primary importance that samples are efficiently preserved without altering the original concentrations of the species of interest until the analyses can be performed (Dore et al., 1996).

The storage and preservation of water samples has focused the attention of the scientific community for more than 80 years with some of the first experimental attempts reported (Orton, 1923; Murphy and Riley, 1956). Since then, many different methods and protocols have been described, all with the common goal to reduce or completely inhibit microbial activity. While alternative methods based on
heat treatments such as pasteurization and tyndallization have also been reported (Aminot and Kérouel, 1997, 1998), the conventional and most popular methods are still refrigeration or the addition of chemical preservatives. Storage of very low concentration samples in the dark at 4°C for less than 24 h resulted in no statistically significant changes in nutrient concentration (Patey et al., 2010). Despite their popularity, the question of their respective efficiency is still controversial because of inconclusive and even contradictory results. The preservation efficiency will depend on factors such as sample matrix, filtration technique, or storage container (type, size, pretreatment) leading to the conclusion that there is unfortunately no universally applicable preservation/storage regime for all aqueous samples. Finally, the storage regime used for water samples is ultimately dictated by the forms of phosphorus to be determined. Hereafter, we describe the most common procedure reported in the literature for the storage of water sample for FRP, TFP, and total phosphorus (TP) measurements as well as their related advantages and limitations. Pretreatment procedures such as filtration and digestion are also presented. Table 12.1

<table>
<thead>
<tr>
<th>Water Type</th>
<th>Main Potential Issues</th>
<th>Fraction Determination</th>
<th>Recommended Treatment</th>
<th>Advice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligotrophic</td>
<td>1. High proportional P loss by biological uptake and adsorption during storage</td>
<td>TP</td>
<td>• Acidify with 1 mL conc. HCl L⁻¹ of sample or • Freeze at ≤ −10°C or • Preserve with HgCl₂</td>
<td>• Filter in the field for FRP or TFP determination with care to minimize physical cell disruption</td>
</tr>
<tr>
<td>(FRP &lt;10 μg L⁻¹ or &lt;0.3 μM)</td>
<td>2. High potential sample contamination</td>
<td>FRP/TFP</td>
<td></td>
<td>• Use PTFE or 10% (v/v) HCl or H₂SO₄ prewash LDPE containers (especially with oligotrophic waters) with low surface area ratio</td>
</tr>
<tr>
<td>Eutrophic</td>
<td>1. Quick variation in P speciation due to biological uptake and breakdown of organic compounds 2. Lysis of microbial dead cells on death</td>
<td>FAHP/FCP</td>
<td>• Filter immediately after sampling, store at ≤4°C, analyze within 24 h or • Preserve with HgCl₂</td>
<td>• Avoid use of chemical preservatives (especially with oligotrophic water)</td>
</tr>
<tr>
<td>(FRP &gt; 200 μg L⁻¹ or &gt; 6.5 μM)</td>
<td></td>
<td></td>
<td></td>
<td>• Avoid freezing unfiltered samples because of cell rupture</td>
</tr>
<tr>
<td>Calcareous</td>
<td>1. Potential loss of FRP on CO₂ degassing and on frozen storage</td>
<td>TP</td>
<td>• Acidify with 1 mL conc. HCl L⁻¹ of sample or • Preserve with HgCl₂ or • Filter immediately after sampling, store in the dark at ≤4°C if analyses within 24 h</td>
<td>• Adjust HgCl₂ quantity to sample organic content</td>
</tr>
<tr>
<td>(Ca²⁺ &gt; 100 mg L⁻¹)</td>
<td></td>
<td>FRP/TFP</td>
<td></td>
<td>• Follow above recommendations but do not freeze for preservation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FAHP/FCP</td>
<td></td>
<td>• Store samples in air tight bottles, completely filled, to minimize degassing</td>
</tr>
</tbody>
</table>

summarizes the best available practice in this context while detailed protocols and exhaustive review for sample preservation and digestion can be found in papers by Maher and Woo (1998) and Worsfold et al. (2005).

12.2.2 Filtration

Preliminary sample treatment often involves filtration, which helps to “arbitrarily” separate the particulate and the dissolved phases. Filtration is required to obtain filterable reactive and total filterable reactive phosphorus and is highly recommended at site just after sampling. Membrane filters of 0.45 and 0.2 μm nominal pore size are commonly used for this purpose, as well as glass fiber filters (GF/F ~0.7 μm and GF/C ~1.2 μm) (Broberg and Persson, 1988). Hall recommended cellulose acetate or polycarbonate membrane for dissolved constituents in natural waters (Hall et al., 1996). However, the filtrate obtained using these membranes or filters may contain significant amounts of colloidal phosphates as well as the truly dissolved phosphates. While there is widespread acceptance of 0.45 μm membranes for filtration, to the extent that this pore size is prescribed in some standards for sampling and analysis of phosphates, for example, Australian Standards (1998), there is a strong argument that 0.2 μm should be used in preference, because of the ability of 0.2 μm membranes to exclude bacteria and femtoplankton, which may comprise a sizeable fraction of the biotic particulate phosphate and reduces possibilities of altering dissolved phosphorus concentration during storage. There are a number of potential problems inherent in the filtration process. These include transmission of larger particle sizes than nominal pore diameter (Stockner et al., 1990), rupturing of cells during filtration (which is minimized by limiting the applied pressure) (Bloesch and Gavrielli, 1984), retention of small aggregates because of van der Waal’s forces, destabilization and aggregation of colloidal material, and progressive diminution of pore size because of filter cake formation and clogging (Broberg and Persson, 1988). This last effect may be minimized by the use of a cross flow or tangential flow filtration, in which the surface of the membrane is actively cleaned by the turbulent flow of sample parallel to the surface of the membrane (Vigneswaran and Ben, 1989). Filtration may result in a decrease in soluble reactive phosphorus (SRP) concentration. Patey et al. (2010) reported a decrease of 1.7 to 2.7 nM (±0.5 nM) of SRP from surface seawater samples after filtration with 0.2 μm cellulose acetate membrane.

However, the nature of the filtrate is ultimately dependent on the nature of the membrane used, the conditions under which the filtration is performed, and the nature of the sample itself, and for these reasons, the use of the terms soluble and dissolved reactive phosphorus is misleading. A less ambiguous nomenclature would be the use of the term filterable reactive phosphorus (membrane pore size (μm)), for example, FRP (0.2), where a 0.2 μm filter was used.

12.2.3 Container Type and Pretreatment

The choice of the containers in terms of nature and size is of primary importance when phosphorus is considered. Indeed, with regard to other major nutrients, phosphorus, which is characterized by a relatively high charge density, is susceptible to sorption to surfaces of the containers. Both glass and plastic containers have positively charged ion-exchanged sites that can interact with negatively charged ions from the solution. The ionic strength of the sample will also affect the intensity of the sorption process because of the competition between phosphorus and the other anions for adsorption sites. While APHA–AWWA–WEF Standard Methods (APHA, 1998) recommend the use of glass vessels for sampling and storage of phosphates, other workers have found significant adsorption of phosphate to glass, and have recommended surface treatment to avoid these losses (Hassenteufel et al., 1963). Leaching from glass containers was also reported with rates ranging from 0.02 to 0.07 μmol L⁻¹ year⁻¹ (Aminot and Kérouel, 1995). However, when plastic containers are considered, phosphate losses appeared to be less in polytetrafluoroethylene (PTFE) sample containers than in polyethylene or polystyrene containers. Rinsing plastic bottles with dilute acid (HCl or H₂SO₄) could reduce adsorption by saturating sorption sites (Clementson and Wayte, 1992). Furthermore, Haygarth et al. (1995) found that the lowest losses of phosphorus species occurred in those samples stored in larger-volume containers (>100 mL).
12.2.4 Physical Condition of Storage

12.2.4.1 Chemical Preservation

The addition of preservatives to retard biological changes within the samples is common especially during room temperature storage. Mercuric chloride, chloroform, and sulfuric acid are the most commonly used preservatives but others such as iodine, alkali, phenylmercuric acetate, tributyl tin, and dichloromethane have also been reported (Maher and Woo, 1998). The required concentration of these preservatives is still open to debate, but it needs to be sufficiently concentrated to stop biological activity. Kirkwood (1992) stated that for samples low in organic matter, addition of 1 μg of HgCl₂ per milliliter of sample is sufficient to prevent consumption of nutrients, while high organic matter samples would require an addition of 20 μg mL⁻¹ or more. Recently, Kattner (1999) reported a suitable storage of open ocean sample by adding 105 μg of HgCl₂ per mL of sample. Furthermore in terms of storage time, room temperature storage with poisoned samples is usually limited to short periods (hours to <day). A few studies have shown that refrigeration of poisoned samples prolongs storage time (days to <week) (Klingaman and Nelson, 1976; Pichet et al., 1979; Fishman et al., 1986). However, the use of such antimicrobial agents is no longer favored given potential risks of immediate FRP release from particles and algae cells, interference with the phosphorus colorimetric determination, precipitation of bacteria and protein, and hydrolysis of organic compounds, thus altering FRP in the sample during storage (Haygarth et al., 1995; Fitzgerald and Faust, 1967; Skjemstad and Reeve, 1978). Considering the difficulties associated with disposal of these toxic materials, the use of these preservatives should be approached with extreme caution and avoided where possible (Maher and Woo 1998; Jarvie et al., 2002; Worsfold et al., 2005).

12.2.4.2 Freezing

Freezing is the main alternative to addition of preservatives. Freezing offers the advantage over poisoning in that the sample matrix is not altered. From the literature, freezing appears to be the preferred method for storage of samples for phosphorus analysis (Maher and Woo, 1998). Since freezing will rupture cells and release phosphorus into the solution, sample filtration is required for FRP and TFP determinations. Unfiltered surface seawater samples from oligotrophic ocean region were frozen and presented small but significant increase in SRP concentration from 12.0 ± 1.3 to 14.7 ± 0.6 nM (Patey et al., 2010). Still, frozen storage appears to be much more efficient than the other storage options offering sample preservation for months to years. Suitable storage of stream water samples for 4–8 years with no significant changes in FRP concentrations were reported (Avanzino and Kennedy, 1993). In contrast, Clementson and Wayte recommended that marine samples for FRP analysis should be analyzed within 4 months, but even this was dependent on the size and nature of the storage container (Clementson and Wayte, 1992). Several authors suggested that quick freezing is also preferable than slow freezing because it increases the precision of the results (Morse et al., 1982). Finally, although probably the most recommended method for long-term sample storage, freezing should be strictly avoided with hard waters due to coprecipitation of phosphate with calcite when thawing the samples (Johnson et al., 1975; House et al., 1986; Gardolinski et al., 2001).

12.2.5 Preconcentration

In oceanic and pristine freshwater systems, phosphate concentrations of <0.1 μg P L⁻¹ (3 nM) may be encountered, and some means of sample preconcentration may be employed to enable detection. Preconcentration techniques involving anion exchange resins for use in low-ionic-strength waters have been described. Camarero (1994) used batch extraction of sample through anion exchanger Sep-Paks (Millipore®) under vacuum to achieve preconcentration factors of >30-fold, leading to detection limits of ca. 10 ng L⁻¹ (0.3 nM). Freeman et al. (1990) described a rapid automated preconcentration system, which employed a small anion exchange column (Bio-Rad AG 1-X8) in the injection valve of a flow injection system; elution of phosphate preconcentration from 2.9 mL of sample resulted in a detection limit of ca. 3 nM (0.1 μg L⁻¹) and resolved from interfering silica (Freeman et al., 1990). Such methods cannot be applied to high-ionic-strength waters; preconcentration of orthophosphate by magnesium...
Phosphates in Aquatic Systems

Hydroxide-induced coprecipitation (MAGIC) has been proposed as a suitable technique for use in marine waters (Karl and Tien, 1992). In a modified form, preconcentration of oceanic water by a factor of 25, with a detection limit of 0.8 ± 0.5 nM (25 ± 15 ng P L⁻¹), has been reported (Rimmelin and Moutin, 2005). Precipitation using lanthanum nitrate was used by Stevens and Stewart to preconcentrate dissolved phosphorus species from 100 L of water to a final volume of 100 mL (Stevens and Stewart, 1982). However, a lengthy (3–4 days) filtration of the precipitated lanthanum phosphate was required to achieve this 1000-fold preconcentration, and removal of iron and other cations by the use of a cation exchange resin was also necessary.

Reverse osmosis has been used as a concentration technique for dissolved organic phosphorus in freshwaters (Stewart et al., 1991). Prefiltered water (100 L) was concentrated to 2.5 L prior to the analysis of the >300 Da material that was retained. An intermediate cation exchange step was required to prevent precipitation of Ca and Mg salts. Nanny et al. used a combination of tangential flow filtration with membranes of 30 and 1 kDa cutoff and reverse osmosis to achieve preconcentration of up to 500 to 1.8 L (Nanny et al., 1994; Nanny and Minear, 1997).

### 12.2.6 Digestion

The determination of TP, TFP, and condensed phosphorus all necessitates digestion of the water sample prior to the detection of orthophosphate (Figure 12.2). Complete conversion of particulate and filterable components requires conditions that are conducive to dissolution of phosphate mineral phases, hydrolysis of phosphate esters, and oxidation of organic phosphorus species. This is greatly favored by elevated temperature in combination with acidity and oxidizing medium. Numerous methods have been proposed; most use conventional heating, autoclave, microwave, or UV photooxidation digestion (Maher and Woo, 1998). Methods using mineral acids or in combination with peroxide or peroxodisulfate appeared to

![Operational classification of physicochemical forms of phosphorus in waters, based on filtration, digestion method, and molybdate reactivity.](image)
be the most efficient to convert all types of phosphorus compounds to orthophosphate (Rowland and Haygarth, 1997). However, it should be emphasized that whichever procedure is selected for the determination of TP or TFP, the digestion efficiency should be assessed using a range of appropriate organic or condensed phosphorus model compounds and certified reference materials. A range of suitable model compounds for this purpose has been suggested by Kérouel and Aminot (1996).

12.2.6.1 Thermal Digestion Methods

12.2.6.1.1 Thermal, Wet Chemical

Wet chemical digestion methods involving peroxydisulfate alone (Menzel and Corwin, 1965) or acidified peroxydisulfate (Gales et al. 1966) are perhaps the most widespread methods for determining TP. However, more rigorous digestion procedures developed for sediment digestion may be necessary because incomplete digestion has been reported using peroxydisulfate. Nitric–sulfuric acid or nitric–sulfuric– perchloric acid digestion may prove necessary if preliminary digestion efficiency testing reveals the peroxydisulfate digestion procedures to be inadequate (APHA, 1998). Digestion may be performed at ambient pressure, or at elevated pressure and temperature using a pressure cooker or autoclave. Lambert and Maher (1995) compared autoclave peroxydisulfate and nitric–sulfuric acid digestion methods for the determination of TP in waters with turbidities of up to 200 NTU. In the most turbid waters, recovery of TP at >100 μg P L⁻¹ was incomplete using the peroxydisulfate method, and they recommend dilution to ca. 100 μg P L⁻¹ to overcome this problem. More recently, Maher et al. (2002) evaluated the use of alkaline peroxydisulfate digestion with low-pressure microwave, autoclave, and hot water bath heating for the determination of TP in turbid lake and river waters. They concluded that the use of alkaline peroxydisulfate digestion procedures was suitable for TP determination in lake water samples when suspended matter is of an organic origin. However, when turbid water samples are to be analyzed, suspended sediment concentration should be diluted to <150 mg L⁻¹ to avoid significant underestimation of the TP determination. Zhou and Struve (2004) studied the potential effects of postpersulfate digestion procedures on TP analysis in water and emphasized their importance because of the effects of insoluble particles on TP analysis, precipitation with or adsorption of phosphorus on multivalent cations or iron and aluminum hydroxides after neutralization of the digested water sample, concluding that standardized procedures are needed.

12.2.6.1.2 High-Temperature Combustion and Fusion

As alternatives to the wet chemical methods described above, high-temperature combustion alone or with magnesium sulfate followed by acid leaching (Aspila et al., 1976; Solórzano and Sharp, 1980) or high-temperature fusion with magnesium nitrate have been proposed (Cembella et al., 1986). The latter method has been shown to decompose phosphonates, which are quite refractory due to their stable C–P bonds (Kérouel and Aminot, 1996). Nevertheless, these techniques are usually recommended for soils or sediments analysis and appear less used than those above for the determination of TP or TFP in water samples.

12.2.6.1.3 Microwave Digestion

A number of workers have reported the use of microwave ovens for thermal digestion of samples for TP analysis. The main advantage of microwave use is the significantly reduced digestion procedure times. For example, Woo and Maher reported similar digestion results when solutions were autoclaved at 120°C for 60 min or microwaved at 450 W for 5–10 min (Woo and Maher, 1995). Microwave digestion has also been reported with online flow injection mode (Hinkamp and Schwedt, 1990; Williams et al., 1993; Benson et al., 1994; Almeida et al., 2004) although for complete digestion of condensed phosphates, Williams et al. (1993) found it necessary to add a hydrolytic enzyme after digestion.

12.2.6.2 UV Photooxidation

UV photooxidation may be employed to mineralize organic phosphorus to phosphate prior to detection, and this has been the subject of a comprehensive review by Golimowski and Golimowska (1996).
UV photooxidation may be performed either in a batch mode, using a high-wattage UV source and a quartz reactor vessel (Armstrong et al., 1966; Henriksen, 1970), or in a segmented continuous flow mode, using either quartz or Teflon photoreactors with lower-power UV lamps (40–100 W) (McKelvie et al., 1989; Aminot and Kérouel, 2001). Batch UV radiation systems usually involve the use of high-wattage UV lamps (ca. 1000 W) and extended irradiation times. Under these conditions, condensed phosphates are hydrolyzed, but this is almost certainly an artifact of the elevated temperature and gradual acidification of the sample as peroxydisulfate degrades to form sulfuric acid. Solórzano and Strickland (1968) have noted that UV photooxidation alone is insufficient to convert condensed phosphates to orthophosphate, and have suggested that the use of UV photooxidation provides a basis for discrimination between the organic and condensed phosphorus fractions.

Photooxidation of organic phosphorus may be performed by UV irradiation of the untreated sample, but it is more common that hydrogen peroxide, potassium peroxydisulfate, ozone, or other oxidizing agents, which enhance the oxidation process, are added. When H₂O₂ is exposed to UV light, it forms hydroxyl radicals: H₂O₂ + hv → 2OH•.

The hydroxyl radical is among the strongest oxidizing agents found in aqueous systems, and these initiate radical chain reactions with organic substances present, resulting in the mineralization of the sample (Hoigné and Bader, 1978). Photooxidation using peroxydisulfate also produces hydroxyl radicals and oxygen.

Many organic compounds can be converted to carbon dioxide using long-wavelength UV (black-light lamp) and TiO₂ as a catalyst. Excitation of an electron from the valence band (v) into the conduction band (c) creates an electron–hole pair, which may then react with oxygen adsorbed to the TiO₂ surface to form radicals such as O₂ and OH•. This approach has been applied to the determination of dissolved organic carbon, but has also been shown to mineralize organic phosphates (Low and Matthews, 1990; Matthews et al., 1990).

### 12.2.6.3 Combined Thermal Hydrolysis and Photooxidation Digestion

To determine the TP concentration in water, the digestion process must involve both oxidative and hydrolytic processes to hydrolyze P–O–P linkages (e.g., polyphosphates), and to oxidize phosphoesters and C–P compounds to inorganic phosphate. For example, in an online TP digestion system, which involved both thermal digestion and UV photooxidation (Benson et al., 1996a), it was found necessary to use a mixture of perchloric acid and peroxydisulfate to form Caro’s acid (H₂SO₅) to obtain high recoveries of both organic and condensed phosphorus. Caro’s acid produces both sulfuric acid and hydrogen peroxide on decomposition (Kolthoff and Miller, 1951).

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

It was observed that the organic phosphorus digestion ability decreased with time (days) as the hydrogen peroxide formed underwent decomposition, but that the thermal condensed phosphate digestion efficiency did not show any decrease with time because of the stability of the sulfuric acid. Since H₂O₂ decomposes slowly, Aminot and Kérouel recommended that the stock solution be assayed about twice a year, and working solutions prepared accordingly (Aminot and Kérouel, 2001).

### 12.3 Analytical Techniques

#### 12.3.1 Colorimetry and Spectrophotometry

##### 12.3.1.1 Direct Photometry, Based on Formation of Phosphomolybdate or Phosphomolybdenum Blue

The determination of phosphate is most commonly based on the formation of the heteropoly acid, 12-molybdophosphoric acid (12-MPA) under acidic conditions (see Table 12.2) (McKelvie et al., 1995). In nitric acid, the reaction is thought to be

\[
\text{H}_3\text{PO}_4 + 6\text{Mo (VI)} \rightarrow (12\text{-MPA}) + 9\text{H}^+
\]
### TABLE 12.2
Examples of Methods for the Determination of Phosphorus Species, with Indicative Detection Limits

<table>
<thead>
<tr>
<th>Technique/Method</th>
<th>Species Detected</th>
<th>Typical Limit of Detection</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular Spectroscopic Techniques</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visible photometry</td>
<td>MRP</td>
<td>150</td>
<td>4.8</td>
<td>10 mm cell</td>
</tr>
<tr>
<td>Phosphomolybdenum blue—batch method</td>
<td>MRP</td>
<td>10</td>
<td>0.32</td>
<td>100 mm cell</td>
</tr>
<tr>
<td>Visible photometry</td>
<td>MRP</td>
<td>0.4</td>
<td>0.013</td>
<td>Lower detection limit possible; detection limit defined by extent of preconcentration used</td>
</tr>
<tr>
<td><strong>Phosphomolybdenum Blue–FIA Ion Exchange Preconcentration Technique</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visible photometry</td>
<td>MRP</td>
<td>12</td>
<td>0.39</td>
<td>In situ monitoring system, LED–photodiode detector</td>
</tr>
<tr>
<td><strong>Phosphomolybdenum Blue–FIA Reagent Injection Technique</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visible photometry</td>
<td>MRP</td>
<td>2.5</td>
<td>0.08</td>
<td>Shipboard monitoring system, LED–photodiode detector</td>
</tr>
<tr>
<td><strong>Phosphomolybdenum Blue–FIA Reagent Injection Technique</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visible photometry</td>
<td>MRP</td>
<td>38</td>
<td>1.2</td>
<td>Online sulfide interference removal using permanganate</td>
</tr>
<tr>
<td><strong>Phosphomolybdenum Blue–FIA Reagent Injection Technique</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visible photometry</td>
<td>MRP</td>
<td>0.4</td>
<td>0.013</td>
<td>50 mm path length detection cell</td>
</tr>
<tr>
<td><strong>Phosphomolybdenum Blue–Segmented Continuous Flow System</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visible photometry</td>
<td>MRP</td>
<td>0.016</td>
<td>0.0005</td>
<td>2 m quartz–Teflon AF 1600 liquid core waveguide detection system</td>
</tr>
<tr>
<td><strong>Phosphomolybdenum Blue–Segmented Continuous Flow System</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visible photometry</td>
<td>Total phosphorus</td>
<td>1</td>
<td>0.03</td>
<td>UV photoreactor and thermal digestion, detection with a multireflective flow cell and low-powder light-emitting diode</td>
</tr>
</tbody>
</table>
### Portable FIA

<table>
<thead>
<tr>
<th>Method</th>
<th>MRP</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible photometry</td>
<td>10</td>
<td>0.32</td>
</tr>
<tr>
<td>Phosphomolybdate–Malachite Green Ion Pair—FIA Method</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visible spectrophotometry</td>
<td>0.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Phosphomolybdate–Malachite Green Ion Pair—FIA Solvent Extraction Method</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphomolybdate–rhodamine 6G–SIA</td>
<td>1.7</td>
<td>0.05</td>
</tr>
<tr>
<td>Fluorescence quenching of phosphomolybdate</td>
<td>2</td>
<td>0.065</td>
</tr>
</tbody>
</table>

### Atomic Spectroscopic Techniques

<table>
<thead>
<tr>
<th>Method</th>
<th>MRP</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inductively coupled plasma–atomic emission spectrometry</td>
<td>200</td>
<td>6.5</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inductively coupled plasma–atomic emission spectrometry–Fl</td>
<td>70</td>
<td>2.3</td>
</tr>
<tr>
<td>Total phosphorus + MRP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inductively coupled plasma–mass spectrometry</td>
<td>8</td>
<td>0.26</td>
</tr>
<tr>
<td>Orthophosphate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Electrochemical Techniques

<table>
<thead>
<tr>
<th>Method</th>
<th>MRP</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potentiometry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPO$_4$$^{2-}$</td>
<td>16</td>
<td>0.5</td>
</tr>
<tr>
<td>Polysyrene–polybutadiene block polymer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>membrane with the phosphate ionophore, 3-allyl-1,5,8-triazacyclodecane-2,4-dione; operational life of 40 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potentiometry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPO$_4$$^{2-}$</td>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td>Novel PVC membrane electrode containing vanadyl salophen, high selectivity, operational life of 14 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potentiometry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRP</td>
<td>1.86</td>
<td>0.06</td>
</tr>
<tr>
<td>Monohydrogen phosphate membrane sensor based on molybdenum acetylacetonate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potentiometry–FIA</td>
<td>310</td>
<td>10</td>
</tr>
<tr>
<td>Orthophosphate + tripolyphosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indirect detection using Pb(II) electrode—better selectivity for SO$_4$$^{2-}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potentiometry–FIA</td>
<td>3100</td>
<td>100</td>
</tr>
<tr>
<td>Orthophosphate + inositol phosphates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobalt electrode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzyme electrode</td>
<td>775</td>
<td>25</td>
</tr>
<tr>
<td>Biosensor based on glucose-6′-phosphate inhibition of hydrolysis by potato acid phosphatase, high selectivity for F$^-$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

continued
### TABLE 12.2 (continued)

Examples of Methods for the Determination of Phosphorus Species, with Indicative Detection Limits

<table>
<thead>
<tr>
<th>Technique/Method</th>
<th>Species Detected</th>
<th>Typical Limit of Detection</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>μ g P L⁻¹</td>
<td>μ M</td>
<td></td>
</tr>
<tr>
<td><strong>Enzyme electrode</strong></td>
<td>Orthophosphate</td>
<td>0.31</td>
<td>0.01</td>
<td>Amperometric detection of H₂O₂ produced by interaction of phosphate with maltose phosphorylase, acid phosphatase, glucose oxidase, and mutarotase immobilized on cellulose membrane</td>
</tr>
<tr>
<td></td>
<td>Orthophosphate</td>
<td>3</td>
<td>0.1</td>
<td>Amperometric detection of H₂O₂ produced by interaction of phosphate with coimmobilized nucleoside phosphorylase and xanthine oxidase</td>
</tr>
<tr>
<td><strong>Enzyme electrode–FIA</strong></td>
<td>POD/Ο₂</td>
<td>31</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Enzyme electrode–amperometry</strong></td>
<td>POD/H₂O₂</td>
<td>108</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td><strong>Enzyme electrode–amperometry</strong></td>
<td>NP, XOD, AP</td>
<td>0.31</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td><strong>Enzyme electrode–amperometry</strong></td>
<td>MP, MR, GOD, AP</td>
<td>0.31</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td><strong>Voltammetry–FIA</strong></td>
<td>MRP</td>
<td>20</td>
<td>0.65</td>
<td>Activated barrel plated nickel electrode in alkaline media</td>
</tr>
<tr>
<td><strong>Voltammetry–FIA</strong></td>
<td>Orthophosphate</td>
<td>10</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td><strong>Voltammetry</strong></td>
<td>MRP</td>
<td>9</td>
<td>0.29</td>
<td>Differential pulse polarographic detection of catalytic reduction of perchlorate or nitrate by solvent extracted phosphomolybdate</td>
</tr>
<tr>
<td><strong>Voltammetry</strong></td>
<td>MRP</td>
<td>92</td>
<td>3</td>
<td>Gold microdisk electrodes</td>
</tr>
<tr>
<td><strong>Voltammetry–FIA</strong></td>
<td>MRP</td>
<td>3.4</td>
<td>0.11</td>
<td>Amperometric detection of phosphomolybdate species</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.18</td>
<td>0.006</td>
<td>With ion exchange preconcentration in low-ionic-strength waters</td>
</tr>
<tr>
<td><strong>Amperometry</strong></td>
<td>MRP</td>
<td>9.2</td>
<td>0.3</td>
<td>Carbon paste microelectrode</td>
</tr>
<tr>
<td><strong>Amperometry</strong></td>
<td>MRP</td>
<td>3.7</td>
<td>0.12</td>
<td>Autonomous electrochemical sensor</td>
</tr>
</tbody>
</table>

**Separation Techniques**

<table>
<thead>
<tr>
<th>Technique</th>
<th>Species Detected</th>
<th>Typical Limit of Detection</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ion chromatography</strong></td>
<td>Orthophosphate</td>
<td>14.7</td>
<td>0.47</td>
<td>Unsuppressed IC—indirect UV detection, 1 mL injections</td>
</tr>
<tr>
<td><strong>Ion chromatography</strong></td>
<td>Orthophosphate</td>
<td>2</td>
<td>0.06</td>
<td>Suppressed IC, conductivity detection, concentrator column</td>
</tr>
<tr>
<td>Method</td>
<td>Analyte</td>
<td>Flow Rate</td>
<td>Injection Volume</td>
<td>Description</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------</td>
<td>-----------</td>
<td>------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Ion chromatography</td>
<td>Inositol phosphates</td>
<td>900–4400</td>
<td>29–140</td>
<td>Dionex CarbonPac PA1 column with metanosulfonic acid and water</td>
</tr>
<tr>
<td>Ion chromatography</td>
<td>Orthophosphate</td>
<td>31</td>
<td>1</td>
<td>Suppressed IC, conductivity detection, precolumn and column switching to remove chloride from sea water</td>
</tr>
<tr>
<td>Ion chromatography</td>
<td>HPO$_4^{2-}$</td>
<td>11</td>
<td>0.35</td>
<td>KOH gradient, suppressed conductivity detection</td>
</tr>
<tr>
<td>Ion chromatography</td>
<td>HPO$_3^{2-}$</td>
<td>12</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Ion chromatography</td>
<td>HPO$_2^{2-}$</td>
<td>26</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Ion chromatography</td>
<td>TP</td>
<td>100</td>
<td>3.2</td>
<td>Off-line microwave digestion of TP to orthophosphate with SO$_4^{2-}$, separation with suppressed IC, conductivity detection; column switching system to remove sulfate peak</td>
</tr>
<tr>
<td>Ion chromatography</td>
<td>TP</td>
<td>251</td>
<td>8.1</td>
<td>Off-line microwave digestion of TP to orthophosphate with H$_2$O$_2$, separation with suppressed IC, conductivity detection</td>
</tr>
<tr>
<td>Ion chromatography</td>
<td>Orthophosphate</td>
<td>0.079</td>
<td>0.003</td>
<td>1000 μL sample injection, electrolytic eluent generator, suppressed conductivity detection</td>
</tr>
<tr>
<td>Ion chromatography</td>
<td>Orthophosphate</td>
<td>10</td>
<td>0.32</td>
<td>500 μL injection</td>
</tr>
<tr>
<td>Ion chromatography</td>
<td>Diphosphate</td>
<td>20</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Ion chromatography</td>
<td>Triphosphate</td>
<td>20</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Ion chromatography</td>
<td>Orthophosphate</td>
<td>50</td>
<td>1.6</td>
<td>Ion-exclusion column of polymethacrylate-based weakly acid cation-exchange resin, postcolumn derivatization</td>
</tr>
<tr>
<td>High-performance liquid chromatography</td>
<td>Orthophosphate</td>
<td>3</td>
<td>0.1</td>
<td>Precolumn derivatization ion-pair liquid chromatography, reversed-phase silica column</td>
</tr>
<tr>
<td>Capillary electrophoresis</td>
<td>Orthophosphate</td>
<td>0.6</td>
<td>0.02</td>
<td>Preconcentration by isotachophoresis, with conductimetric detection</td>
</tr>
<tr>
<td>Capillary electrophoresis</td>
<td>Orthophosphate</td>
<td>0.3</td>
<td>0.01</td>
<td>Electromigrative preconcentration, UV detection</td>
</tr>
<tr>
<td>Capillary electrophoresis</td>
<td>Orthophosphate</td>
<td>14</td>
<td>0.45</td>
<td>Indirect UV detection using pyromellitic acid, 240 s hydrostatic injection</td>
</tr>
<tr>
<td>Capillary electrophoresis</td>
<td>Orthophosphate</td>
<td>19</td>
<td>0.60</td>
<td>Indirect UV detection using a chromate chromophore, 30 s hydrostatic injection</td>
</tr>
<tr>
<td>LWCC–FIA</td>
<td>Orthophosphate</td>
<td>17</td>
<td>0.5</td>
<td>Vanadomolybdate method, throughput 60 h$^{-1}$</td>
</tr>
<tr>
<td>LWCC–rFIA</td>
<td>Orthophosphate</td>
<td>0.02</td>
<td>0.0005</td>
<td>Spectrophotometric detection, throughput 15 h$^{-1}$</td>
</tr>
</tbody>
</table>

while in sulfuric acid, the stoichiometry is

$$\text{H}_3\text{PO}_4 + 5\text{Mo (VI)} \rightarrow (12-\text{MPA}) + 7\text{H}^+$$

The absorbance of the 12-MPA dimer may then be measured, or it may be reduced to form the highly colored PMB using a variety of reductants. The optimum wavelength and sensitivity are a function of both molybdate and acid concentrations (Crouch and Malmstadt, 1967). Because molybdenum blue may also form through direct reduction of Mo(VI) if the pH is ≤ 0.7, even in the absence of phosphorus, the acid concentration is kept within the range of 0.3–0.5 N (Crouch and Malmstadt, 1967). The selectivity of this reaction for phosphate is also highly pH-dependent, and again the reaction acidity must be strictly controlled.

12.3.1.2 Detection as Unreduced Vanadomolybdophosphoric Acid

In the presence of ammonium metavanadate and acidic ammonium molybdate, phosphate forms vanadomolybdophosphoric acid. The absorbance of this yellow-colored product is commonly measured at 470 nm. The sensitivity is generally lesser than that for reduced PMB methods, but it is quite tolerant of interfering ions, and is suitable for monitoring FRP in waste and contaminated waters. A detection limit of 200 μg P L⁻¹ (6.5 μM) using a 1 cm cuvette has been reported for this method (APHA, 1998).

12.3.1.3 Detection as Phosphomolybdenum Blue

Better sensitivity can be achieved if 12-MPA is reduced to form PMB. A wide variety of reductants have been employed for this purpose, including:

- Tin + copper sulfate + HCl (Osmond, 1887; Denigès, 1920), Sn(II) chloride (Judy et al., 1927), Sn(II) chloride and hydrazine sulfate (Golterman, 1960), 1-amino-2-naphthol-4-sulfonic acid (APHA, 1965), ascorbic acid, and potassium antimonyl tartrate (Murphy and Riley, 1962).

While the SnCl₂ reduction method gives very sensitive results, it is susceptible to salt interference, and has only a short-lived colored product. For this reason, the ascorbic acid method is preferred to the Sn(II) method; however, longer color development time is necessary when ascorbic acid alone is used. The Murphy and Riley (1962) method introduced the use of antimonyl tartrate, which catalyzes the reduction step, suppresses the interference from silicate, and avoids problems of chloride interference. Consequently, this method is used as the basis for many batch and automated techniques in current use (see Tables 12.2 and 12.4).

12.3.1.4 Detection as a 12-MPA Ion Association Complex

PM forms strong ion association complexes with basic dyes at low pH. For example, the sensitivity of a method based on spectrophotometric determination of the 12-MPA–malachite green complex (Motozuz et al., 1983a; Motomizu and Oshima, 1987) was approximately 30 times that of a reduced PM determination. Other dyes used for this purpose include saffronin, brilliant green, fuchsine red, methylene blue, methyl violet, and rhodamine B (Broberg and Pettersson, 1988). Surfactants such as polyvinyl alcohol are frequently used to avoid precipitation of the ion association complex.

12.3.1.5 Solvent Extraction of Phosphomolybdenum Blue or Phosphomolybdate Ion Association Complexes

Enhanced sensitivity may be achieved through solvent extraction of PMB or PM ion association complexes prior to spectrophotometric measurement. For example, extraction with PMB with iso-butanol enabled a detection limit of 0.2 μg P L⁻¹ (6 nM) to be achieved (Stephens, 1963). Motomizu et al. (1984) extracted the PM–malachite green ion pair into a mixture of toluene and 2-methylpentane-2-one to obtain a detection limit of 0.1 μg P L⁻¹ (3.2 nM). The use of solid-phase extraction (SPE) of PMB has also been reported (Lacy et al., 1990).
12.3.1.6 Solvent Extraction of PM

Improved sensitivity may also be achieved by solvent extraction of PM without the formation of PMB. Sugawara and Kanamori (1961) used \( n \)-butanol/chloroform for this purpose. Molybdenum was then determined spectrophotometrically with thiocyanate after decomposition of PM to give a detection limit of 0.08 µg P L\(^{-1}\) (2.6 nM).

12.3.1.7 Interferences in Photometric Techniques Based on Formation of PM and PMB

While methods based on formation of PMB or its ion association complexes are the most commonly used methods for phosphorus determination, they may be susceptible to interference from a number of sources. Sjösten and Blomqvist (1997) have reported that the rate of formation of phosphoantimonyl blue was reduced by decreasing temperature and decreasing phosphate concentrations. At low temperatures (<5°C) and concentrations (5 µg P L\(^{-1}\)), reaction times of ca. 50 min were required to reach complete color development. The authors note that these effects may cause significant nonlinearity in the calibration of automated instruments (FIA, SCFA) at low concentrations, or underestimation in samples that have not been allowed to reach ambient temperature prior to analysis.

12.3.1.7.1 Interferences due to Hydrolysis of Labile Phosphorus Species

It has been shown that both the acid conditions used and the presence of molybdate can enhance hydrolysis of dissolved organic and condensed phosphates to give an overestimate of orthophosphate (Weil-Malherbe and Green, 1951; Tarapchak, 1983). Similarly, colloidal phosphates in the filterable fraction may be molybdate reactive, which again will lead to an overestimation of the orthophosphate concentration (Stainton, 1980). Rigler observed that this overestimation of the true orthophosphate concentration may be as much as 10–100 times the true concentration of orthophosphate (Rigler, 1968). In attempts to avoid these hydrolytic effects, a “6-sec extraction method” was developed in which PM formed was rapidly removed from the acidic environment (Chamberlain and Shapiro, 1969), or excess molybdate was complexed with a citrate–arsenite reagent (Dick and Tabatabai, 1977).

12.3.1.7.2 Interferences in the Formation of Molybdenum Blue Species

Silicate, arsenate, and germanate also form heteropoly acids, which on reduction yield molybdenum blue species with similar absorption maxima (Chalmers and Sinclair, 1965). This positive interference in the determination of phosphate is particularly pronounced for silicate because of its relatively high concentration in many waters. However, the formation of siliconomolybdate may be suppressed by the addition of tartaric or oxalic acid to the molybdate reagent (Chalmers and Sinclair, 1966). If, however, the organic acid is added after the formation of the heteropoly acid, the PM is destroyed, and this is used as the basis for the determination of silicate in the presence of phosphate. Kinetic discrimination between phosphate and silicate, and arsenate and germanate is also possible because of the faster rate of formation of PM. Thus, the widely adopted Murphy and Riley method employs a reagent mixture of acidic molybdate and antimonyl tartrate (Murphy and Riley, 1962) at concentrations that are known to enhance the kinetics of PM and suppress the formation of siliconomolybdate.

Fluoride concentrations of >100 mg L\(^{-1}\) were also shown to inhibit the formation of PM, but this effect was shown to be lessened at higher silicate concentrations (Blomqvist et al., 1993).

Negative interferences in the tin(II) chloride reduction method may also be caused by the presence of higher concentrations of iron(III), aluminum, calcium, and chloride (Benson et al., 1996b). The Fe, Al, and Ca interferences are presumably due to competitive complexation of the phosphate, while that for chloride is probably due to inhibition of the PM reduction. The chloride interference in this method is particularly problematic, especially for the determination of phosphate in marine and estuarine waters, and for this reason, the ascorbic acid reduction method of Murphy and Riley (1962) is often favored.

12.3.2 Photoluminescence Techniques

A number of authors have described the detection of orthophosphate based on the measurement of the quenching of rhodamine fluorescence by PM (Table 12.2) (Fusheng et al., 1989; Kan et al., 1991).
Detection limits of ca. 0.1 μg P L⁻¹ (3.2 nM) have been reported (Table 12.2), and while this approach offers little enhancement in selectivity, it is potentially more sensitive than spectrophotometry.

An indirect method for the detection of phosphate, which involves the fluorescence quenching of the Al–Morin complex by PO₄³⁻, has also been described for the detection of phosphorus oxyacids separated by ion chromatography (IC) (Meek and Pietrzyk, 1988). More recently, a polyvinyl chloride matrix containing Al–Morin was used to prepare a membrane sensor for the detection of phosphate by fluorescence quenching. The membrane had a life span of 90–120 days and a linear detection range of 6–15 mg L⁻¹ (0.19–0.48 mM), making it suitable only for wastewater analysis (Lin et al., 2006).

The determination of orthophosphate can also be achieved using the chemiluminescent emission that occurs when PM oxidizes luminol (Yaqoob et al., 2004). Its application has been reviewed by Morais et al. (2005), which also presents a review of different flow techniques coupled to phosphorus analysis with chemiluminescent detection, including enzymatic and nonenzymatic methods. While this approach offers improved sensitivity compared with spectrophotometry, it still suffers from the selectivity problems associated with all PM-based detection chemistries.

### 12.3.3 Atomic Spectrometry

A number of indirect flame atomic absorption spectrometry (AAS) methods have been reported for the determination of phosphate (Table 12.2). For example, phosphate was determined by measuring molybdenum after solvent extraction of PM (Zaugg and Knox, 1966). A more recent variation of this method involved flotation of the malachite green–PM ion pair at an aqueous–diethyl ether interface (Nasu and Kant, 1988). After dissolution in methanol, molybdenum was determined using flame AAS (nitrous oxide flame) at 313.26 nm. The method was successfully applied to the measurement of seawater containing ca. 40 μg P L⁻¹ (1.3 μM).

Inductively coupled plasma–atomic emission spectrometry (ICP–AES) has also been applied to the analysis of phosphorus species. Manzoori et al. (1990) demonstrated a flow injection system that enabled the colorimetric determination of TRP (using unreduced phosphovanadomolybdate, λₘₐₓ = 470 nm) prior to aspiration into an ICP–AES system where TP was measured. A detection limit of ca. 200 μg P L⁻¹ (6.4 μM) was achieved for the TP measurement using the 177.49 nm phosphorus line, and the method was applied to the analysis of wastewaters. Interference from background argon emission lines tends to limit the sensitivity, and thus the application of the ICP–AES technique to the analysis of wastewaters. However, the increasing availability of high-resolution inductively coupled plasma–mass spectrometry (ICP–MS) systems is likely to result in this technique becoming more widely used for the determination of TP and other phosphorus species separated by chromatography or capillary electrophoresis (CE). For example, Guo et al. (2005) used ICP–MS for the detection of orthophosphate and glyphosate following separation by ion chromatography. Detection limits of 0.7 μg P L⁻¹ (0.02 μM) were achieved using this approach.

### 12.3.4 Electrochemical Techniques

The development of electrochemical techniques to determine environmental compounds has emerged from the need for rapid, simple, and portable quantitative methods that could be used by nonexperts of the analytical area (Villalba et al., 2009). A general review of ion-selective electrode potentiometry can be found in De Marco et al. (2007). Potentiometric methods for the detection of phosphate based on polymer wire coated or membrane ion selective electrodes have until recently suffered from poor selectivity, sensitivity, and lifetime, and have been unsuitable for most water analysis applications (Table 12.2) (Glazier and Arnold, 1989; Liu et al., 1989). Developments in membrane formulation involving PVC containing vanadyl salophen (Ganjali et al., 2003), polystyrene–polybutadiene block polymers with the phosphate ionophore, 3-allyl-1,5,8-triazacyclodecane-2,4-dione (Le Goff et al., 2004), or molybdenum bis(2-hydroxyanil) acetylacetonate complex (MMA) (Ganjali et al., 2006) show much improved membrane lifetime, sensitivity, and selectivity for phosphate, and offer considerable promise for future water-monitoring applications.
Indirect methods using lead (Coetzee and Gardner, 1986; Harra and Kusu, 1992), calcium (Koopetngarm, 1987), cadmium (Davey et al., 1990), silver (van Staden, 1993), or cobalt (Chen et al., 1997; Parra et al., 2005) electrodes have also been reported. These methods, which have detection limits in the range of 30–300 g P L⁻¹ (1–10 μM), are generally too insensitive for all but wastewater analysis.

Unlike the direct potentiometric measurement, the detection of phosphate by voltammetry is carried out indirectly, based on the determination of the PM moiety, and has been described by Fogg and Bsebsu (1981, 1982) and Fogg et al. (1983). The PM complex is formed by the reaction of Na₂MoO₄ in acid solution (pH 1.0) to form a Keggin anion (Jonca et al., 2011). Carpenter et al. (1997) have analyzed the molybdosilicate and molybdophosphate complexes with gold microdisk electrodes in the range of 1–1000 μM. Voltammetric (Guanghan et al., 1999) and amperometric (Quintana et al., 2004) approaches for phosphate analysis were also carried out with carbon paste microelectrodes. Udnan et al. (2005) has shown reliable and sensitive FRP measurement in fresh and marine waters using amperometry with a detection limit of 3.4 μg P L⁻¹ (0.11 μM). When in-valve ion exchange preconcentration was applied to the determination of oligotrophic freshwaters, a detection limit of 0.18 μg P L⁻¹ (5.8 nM) was achieved. While this approach is convenient and rapid (Table 12.2), it suffers from the lack of selectivity for orthophosphate that applies to all PM-based detection chemistries. Matsunaga et al. (1984) used a photomicrobial sensor with *Chlorella vulgaris* and an oxygen electrode for the determination of phosphate with acceptable selectivity in the range of 8–70 mM.

Biosensors were thought as an alternative route to improve the selectivity of electrodes based on the particular molecule-substrate biological affinity. Enzymes present the advantage of catalyzing a particular reaction of a specific substrate, without the interference of other isomers of this substrate or other similar substrates present. An enzymatic electrode is a combination of any electrochemical probe with a thin layer of immobilized enzyme. Owing to the great importance of orthophosphates in biological functions, there exist a great variety of specific enzymes (Villalba et al., 2009). Pyruvate oxidase (Kwan et al., 2005, Akyilmaz and Yorganci, 2007) and alkaline phosphatase (Preechaworapun et al., 2008) are two of the enzymes usually used in phosphate biosensor studies. The combinations of two or more enzymes are also investigated (Yao et al., 1994; Watanabe et al., 1988; Mousty et al., 2001), and the results depend mainly on the synergistic interaction of the multienzyme system to generate an electrochemically active product. Usually, the enzymatic reaction progress is monitored by the formation rate of the product, or the disappearance of a reagent. If the product and the reagent are electroactive, then the reaction progress can be measured directly by the current flow in response to an applied voltage. While potentiometric enzymatic electrodes for the detection of phosphate have been developed (Schubert et al., 1984; Katsu and Kayamoto, 1992), no application has been made of these to water analysis because of the relatively poor sensitivity. However, amperometric enzyme electrodes have been reported that have high sensitivity, selectivity, and long operational life, and it is expected that the use of these for water analysis will become more widespread (see Table 12.2). For example, a sensitive enzyme electrode based on the amperometric detection of hydrogen peroxide produced by membrane coimmobilized nucleoside phosphorylase and xanthine oxidase has been reported for the detection of phosphate by d’Urso and Coulet (1990). Other similar enzyme electrode systems suitable for water analysis are listed in Table 12.2. Berchmans et al. (2012) present a consistent and updated review comparing electroanalytical techniques for inorganic phosphate analysis in terms of selectivity, simplicity, miniaturization, adaptability, and suitability for field measurements, as well as the possibility of interferences from other anions.

### 12.3.5 Separative Techniques

#### 12.3.5.1 Ion Chromatography

Ion chromatography is extensively used for the determination of phosphorus species in waste, industrial, drinking, and natural waters, and is accepted as a standard method of analysis, for example, USEPA Method 300.0 (Pfaff, 1993) and APHA Standard Methods Method 4110B (Table 12.4) (APHA, 2005). Ion chromatography is traditionally performed using ion-exchange chromatography with conductimetric
or spectrophotometric detection. Nakatani et al. (2008) have separated phosphate and silicate ions with an ion-exclusion column packed with a polymethacrylate-based weakly acidic cation-exchange resin in the H⁺-form using ultrapure water as eluent. Postcolumn derivatization with molybdate and ascorbic acid were used to determine both ions simultaneously by spectrophotometry in a linear range of 50–2000 μg P L⁻¹.

Innovations in the IC field include new stationary phases and, mainly, new suppressor systems, and the development of methods for the analysis of anions in modified reversed-phase columns (ion-interaction chromatography and electrostatic ion chromatography) (Ruiz-Calero and Galceran, 2005). IC separations for phosphorus species have been reviewed by Ruiz-Calero and Galceran (2005), with extensive tables gathering data with respect to determinations of orthophosphate, including column types, mobile phases, flow rates, detection methods, LODs, and concentration ranges, among others. The use of ion chromatography arguably provides a better estimate of the concentration of bioavailable phosphorus because it avoids the problem of acid hydrolysis of labile phosphates that occurs in those methods that rely on reactions between phosphate and molybdate.

Some ion chromatography methods for the determination of TP have also been reported. These involve the off-line microwave digestion of sample with peroxydisulfate (Pfaff, 1993; Dahllöf et al., 1997; Colombini et al., 1998; Huang et al., 2000) or hydrogen peroxide (Colina and Gardiner, 1999) followed by the separation of the orthophosphate produced by suppressed ion chromatography.

12.3.5.1.1 Orthophosphate

Phosphate is commonly determined by ion chromatography, both in suppressed and in unsuppressed modes (cf. Table 12.2). For the analysis of pristine waters, some form of preconcentration may be required for direct injection (Wetzel et al., 1979), or alternately, large-volume injections may be used (Lu et al., 2002). For marine waters, interference from the high concentration of chloride may be overcome in part by dilution or by use of small precolumns (Dahllöf et al., 1997) although such approaches necessarily decrease the sensitivity, making the detection of phosphate in open ocean waters difficult.

12.3.5.1.2 Condensed Phosphates

Anion exchange chromatography and ion exchange chromatography have been used extensively for the separation and quantitation of condensed phosphates. Because phosphate is a poor UV-chromophore, the common practice has been to use large anion exchange columns, and to collect fractions for subsequent acid hydrolysis and detection as MRP (Jolley et al., 1998). However, the use of ion chromatography with postseparation hydrolysis and detection via an FIA (Yoza et al., 1985; Halliwell et al., 1996) has advantages of both speed of analysis and sensitivity.

12.3.5.1.3 Organic Phosphates

The interest in characterizing organic phosphorus present in natural waters has prompted the development of ion chromatographic separation systems for compounds such as inositol phosphates. Online UV photooxidation has been utilized for postcolumn oxidation and subsequent detection of these organic phosphate species (Clarkin et al., 1992; Mckelvie, 2005). Some ion chromatography methodologies for organic phosphorus use HCl for extraction (Chen and Li, 2003; Chen, 2004); methanesulfonic acid was alternatively used for the measurement of inositol phosphates in diets and digesta, obtaining better baseline and not requiring the use of an HPIC inert system, because methanesulfonic acid is not as aggressive as HCl (Blaabjerg et al., 2010).

12.3.5.2 Capillary Electrophoresis

Capillary electrophoresis techniques have been applied to orthophosphate analysis (Table 12.2). While generally offering much faster separations of anions in waters than, say, ion chromatography, CE with conventional UV detection suffers from a lack of sensitivity. However, use of on-capillary preconcentration
using isotachophoresis (Bondoux et al., 1992; Kaniansky et al., 1994) has enabled sub-μg L⁻¹ detection limits to be achieved in high-ionic-strength matrices, and this approach is a promising one for water analysis.

Improved detection sensitivity may also be achieved if a UV-absorbent salt, such as sodium chromate, is included in the buffer and indirect UV absorption is performed. Using this approach, minimum detectable concentrations of ions such as phosphate ≤100 μg L⁻¹ (3.2 μM) can be achieved using sample injection times of 30 s (APHA, 2005). The use of CE for the separation of organic, inorganic, and chemical warfare agent phosphate molecules in environmental samples has recently been reviewed by Chang et al. (2005), and compared to laser-induced fluorescence, UV absorption, conductometry, amperometry, atomic spectrometry, and mass spectrometry.

12.3.5.3 Liquid Waveguide Capillary Cell

A flow injection system using vanadomolybdate detection and liquid waveguide long pathlength capillary cell (LWCC) connected to a charge coupled device (CCD) spectrophotometer for the detection of phosphate have improved rapidity (throughput of 60 h⁻¹) and sensitivity (LOD = 17 μg P L⁻¹; linear range up to 500 μg P L⁻¹) in surface and groundwater samples (Neves et al., 2008). In seawater, improved sensitivity was achieved for the determination of nitrate and phosphate with an LWCC connected to a conventional segmented-flow autoanalyzer and miniaturized spectrophotometer (Patey et al., 2008), with detection limits of 0.8 nM of PO₄³⁻. Interferences (silicate and arsenate) in the analysis of nanomolar concentrations of phosphate in oceanic waters were also reported (Patey et al., 2010).

Ma et al. (2009) presented a novel reverse flow injection analysis (rFIA) method coupled to an LWCC and detection with a miniaturized fiber-optic spectrophotometer, resulting in the detection of nanomolar SRP in seawater. They achieved lower reagent consumption together with higher sensitivity and a throughput of 15 h⁻¹, in a linear range of 0–165.0 nM, and LOD of 0.5 nM.

12.3.5.4 High-Performance Liquid Chromatography

Silicate and phosphate are usually treated together in HPLC methodologies. A highly sensitive HPLC method for the simultaneous determination of soluble phosphate and silicate in environmental waters using ion-pair liquid chromatography preceded by the formation of yellow α-heteropolytungstates is described by Yokoyama et al. (2009). When compared to other methodologies, the method also suffers from lack of sensitivity due to conventional UV detection, with values in the range up to 20 mg PO₄³⁻ L⁻¹ and LOD of 0.003 mg PO₄³⁻ L⁻¹.

12.3.6 Automated Techniques

Flow analysis techniques have changed chemical analysis since the introduction of its concepts in 1975 (Hansen and Miró, 2007). Automated methods are clearly favored where there are large numbers of samples to be analyzed, when the unit processes such as digestion or separation are slow, and where the analytical measurements must be made online and unattended.

12.3.6.1 Segmented Continuous Flow Analysis

Continuous flow techniques have been widely used for automated phosphorus analysis of waters since the introduction of the segmented continuous flow analysis systems in the 1950s. Segmented continuous flow manifolds described include those that are suitable for the determination of phosphorus in water in the presence of high silica (Chang et al., 2005), for highly sensitive detection of FRP in the presence of mercuric chloride preservative (Downes, 1978), and for the determination of TFP (Ron Vaz et al., 1992). While this approach is still widely used, there has been a tendency to use flow injection analyzers for the same applications.
12.3.6.2 Flow Injection and Sequential Injection Analysis

12.3.6.2.1 Filterable Reactive P

The analysis of FRP by FIA using the Murphy and Riley ascorbic acid reduction chemistry has been reported. Better sensitivity may be achieved using SnCl₂ because the reduction kinetics are faster but this may result in susceptibility to chloride interference (Janssen et al., 1983; Freeman et al., 1990).

FIA was used to orthophosphate analysis coupled to online SPE systems. The heteropoly molybdo-phosphoric acid (MoP) or PMB paired with cetyltrimethylammonium bromide (CTAB) are efficiently extracted by Sep-Pak C₁₈ cartridges and rapidly eluted in sulfuric acid–ethanol solution. The MoP-CTAB compound can be analyzed after reaction with alkaline luminol emitting chemiluminescence (Liang et al., 2006) at micromolar levels (LOD 0.002 μmol L⁻¹), or the PMB-CTAB compound can be colorimetrically detected at 700 nm (Liang et al., 2007) at nanomolar levels (LOD 1.57 nmol L⁻¹). Ma et al. (2008) used hydrophilic–lipophilic balanced solid-phase extraction (SPE-HLB) to enrich PMB from water samples, making the use of controlled temperature and CTAB for the SPE extraction unnecessary, resulting in shorter analysis time. The PMB extracted at room temperature on an in-line HLB cartridge was eluted by 0.15 mol L⁻¹ of NaOH solution and determined spectrophotometrically (detection range 3.4–1134 nmol PO₄³⁻ L⁻¹; LOD 1.4 nmol L⁻¹).

A recent dual analytical line system has compared spectrophotometric detections (commercial and multireflective flow cells) in SI analysis for phosphate determination applied in different types of water (Mesquita et al., 2011). The multireflective flow cell proved to be better for lower phosphate concentrations with an achievable detection limit of 0.007 μM PO₄³⁻, in a linear range of 0.024–9.5 μM. Cheng et al. (2010) developed an FIA detection system for phosphate based in the suppression current of the electrocatalytic oxidation of glucose in barrel-plated nickel electrode (Ni-BPE) in alkaline medium, forming a film of Ni(OH)₂/NiO(OH). They reported linear range up to 1 mM and LOD of 0.3 μM under optimized conditions (flow rate of 300 μL min⁻¹ and detection potential of 0.55 V versus Ag/AgCl with 25 μM glucose at 0.1 M NaOH). A paired emitter–detector diode (PEDD) was constructed from two LEDs and applied to the colorimetric determination of phosphate (O’Toole et al., 2007). A timer circuit is used to measure the time taken for the photocurrent generated by the LED source discharges the junction capacitance of the detector diode (from 5 to 1.7 V). The miniaturized LED flow detector proved to be highly sensitive and low cost.

The reader is referred to an extensive review by Estela and Cérda (2005) on the analysis of phosphate by FIA and sequential injection analysis (SIA). Other recent reviews in SI and FI techniques for the determination of phosphorus in water samples are Motomizu and Li (2005) and Mesquita and Rangel (2009).

12.3.6.2.2 Total P and Total Filterable P

Automation of digestion processes is highly desirable, and a number of flow injection digestion techniques suitable for the detection of organic and TP have been described. Online TFP measurement systems, which use strong acids and oxidants and thermal- (Aoyagi et al., 1988, 1990) or microwave-assisted digestion (Hinkamp and Schwedt, 1990; Williams et al., 1993; Benson et al., 1994), have been shown to be effective. Other methods involving the use of photooxidation have been described (Henriksen, 1970), and a combined UV–thermal system for the determination of TP has been demonstrated and employed for online monitoring (Benson et al., 1996a). Because the latter systems involve photooxidation in the presence of high concentrations of peroxydisulfate, oxygen bubbles are randomly generated and must be removed online by the use of either a membrane degasser or a hydrophobic hollow fiber membrane. A new FIA method for the rapid sequential determination of DRP and DOP was developed by Tue-Ngeun et al. (2005), involving online UV photooxidation for DOP with detection of PMB after reduction of PM with tin(II) chloride. The proposed system consumes low volume of reagents for digestion (150 μL per injection). Digestion efficiency of 97% for DOP was achieved for all but phenylphosphate, and was selective for DOP species, even in the presence of condensed phosphate species. A similar method is now recommended for TFP analysis by the APHA–AWWA–WEF Standard Methods manual (APHA, 2005).
12.3.6.3 Automated Batch Analyzers

An approach to the determination of TP has been described by Dong and Dasgupta (1990). An automated microbatch analyzer system, which is composed of a sealed digestion vessel containing a fiber optic, light-emitting diode detector system, and a number of reagent addition and waste lines, was employed for high-temperature persulfate digestion of wastewaters. TP measurements took approximately 9 min per sample and gave results comparable to results obtained using the ASTM block or autoclave digestion techniques (LOD ≤ 10 μg P L⁻¹).

In recent years, there has been the remergence of automated discrete batch analyzers for the analysis of nutrient water. The SEAL AQ2 (SEAL Analytical, Mequon, WI 53092, USA) and Aquakem 250 and 600 (Labmedics Ltd., Manchester, UK) are good examples of these high-sample-throughput, high-sensitivity laboratory robotic instruments. These instruments are capable of measuring FRP or TP after manual digestion has been performed.

12.4 Applications in Water Analysis

Much of the interest in determining phosphorus in waters stems from its crucial role in the eutrophication process, or in monitoring wastewaters, which may contribute to this process. Table 12.3 shows indicative P concentration ranges for waters of various trophic classifications (Wetzel, 1983).

Phosphates are usually determined as filterable molybdate reactive phosphate (FRP; 0.2 or 0.45 μm filter) in aquatic environments, which seems to be the most bioavailable form of phosphorus in these environments (Frank et al., 2006). What is obvious is that the analytical techniques used to determine phosphorus in even eutrophic waters must be quite sensitive. In pristine waters, very low concentrations are observed, for example, 1 μg P L⁻¹ (3.2 nM) or less of FRP (Hart et al., 1991), and it is generally only in polluted waters and wastewaters that concentrations in the mg L⁻¹ range are found. The nature and origin of the sample therefore dictates the techniques that can be used. Tables 12.4 and 12.5 provide an indicative list of standard methods recommended for the determination of FRP and similarly recommended methods for digestion of organic and particulate phosphorus prior to detection as FRP. Typical detection limits for some of the techniques are shown, and these may be used as an approximate guide for the selection of a method of analysis appropriate to the sample concentration.

12.4.1 Potable Waters

Because phosphorus, as phosphates, is not harmful to human health, guidelines, for example, WHO, do not typically list criteria for acceptable phosphorus concentrations for drinking water quality (Anon, 1993). While the presence of high phosphate concentrations in drinking water may be indicative of sewage contamination, more appropriate methods of analysis, with directly more useful results, for example, E. coli, are usually employed. However, the presence of high phosphorus concentrations in reservoir waters may lead to eutrophication and result in the occurrence of nuisance blooms of microalgae, which

<table>
<thead>
<tr>
<th>General Level of Lake Productivity</th>
<th>Total (μg L⁻¹)</th>
<th>Phosphorus (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultraoligotrophic</td>
<td>&lt;5</td>
<td>&lt;0.16</td>
</tr>
<tr>
<td>Oligomesotrophic</td>
<td>5–10</td>
<td>0.16–0.32</td>
</tr>
<tr>
<td>Mesoeutrophic</td>
<td>10–30</td>
<td>0.32–0.97</td>
</tr>
<tr>
<td>Eutrophic</td>
<td>30–100</td>
<td>0.97–3.2</td>
</tr>
<tr>
<td>Hypereutrophic</td>
<td>&gt;100</td>
<td>&gt;3.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Application</th>
<th>Mode</th>
<th>Limit of Detection</th>
<th>Specified Interference</th>
<th>Detection Chemistry</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, wastewater, and brine^a</td>
<td>FIA</td>
<td>0.7 μg P L⁻¹ (from 21 replicates of a 5.0 μg P L⁻¹ standard)</td>
<td>Silica, arsenate, and iron at high concentration (&gt;30 mg L⁻¹)</td>
<td>Formation of antimony–phosphomolybdate complex reduced by ascorbic acid</td>
<td>APHA–AWWA–WEF 2005 Method 4500-P G 2005</td>
</tr>
<tr>
<td>Estuarine and coastal water^a</td>
<td>SFA</td>
<td>0.7 μg P L⁻¹ in three parts per thousand saline water</td>
<td>Interferences caused by copper, arsenate, and silicate are minimal because of the extremely low levels normally found in estuarine or coastal waters; high iron concentrations can cause precipitation of P</td>
<td>Formation of antimony–phosphomolybdate complex reduced by ascorbic acid</td>
<td>Zimmerman and Keefe (1997) USEPA Method 365.5</td>
</tr>
<tr>
<td>Water, wastewater, and brine^a</td>
<td>Batch</td>
<td>Not reported</td>
<td>Arsenate and iron at high concentration may interfere or cause P precipitation</td>
<td>Formation of antimony–phosphomolybdate complex reduced by ascorbic acid</td>
<td>USEPA Method 365.3 (1978) Method 365.2 (1 reagent) Method 365.3 (2 reagents) (1978)</td>
</tr>
<tr>
<td>Surface water, wastewater, and drinking water</td>
<td>Ion chromatography</td>
<td>Function of sample size 14 μg P L⁻¹ for a 25 μL sample loop in reagent water</td>
<td>Any substance that has a retention time similar to the analyst of interest; detection levels in natural waters may be variable because of the presence of high levels of anions</td>
<td>Separation by ion exchanger with suppressor device and measurement by conductivity</td>
<td>APHA–AWWA–WEF 2005 Method 4110 B (2005)</td>
</tr>
<tr>
<td>Surface water, wastewater, and drinking water</td>
<td>Capillary ion electrophoresis</td>
<td>Function of sample size 0.1 mg P L⁻¹ for 30 s sampling time</td>
<td>Any anion that has a migration time similar to the analyst of interest; sample with high ionic strength may show a decrease in analyte migration time</td>
<td>Separation of anions and cations by application of an electric field and detection in an UV adsorbing electrolyte</td>
<td>APHA–AWWA–WEF 2005 Method 4140 B (2005)</td>
</tr>
</tbody>
</table>

^a Refractive index correction may be required.
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can release toxins and cause taste and odor problems, resulting in the need for expensive water treatment. Consequently, measurement of phosphorus concentrations is usually performed on waters in the catchments or reservoir waters as part of an overall nutrient management strategy. Both TP and FRP are commonly measured, and sensitive batch and automated photometric methods are most frequently used. The use of portable test kits, which are designed for wastewater monitoring, is not recommended for monitoring potable waters because of the potentially large errors that may occur.

12.4.2 Wastewaters

TP is most frequently used to monitor the compliance of wastewater discharges with license agreements because it provides a measure of the efficiency of phosphorus removal in water treatment processes, whereas FRP is more commonly used as a surrogate measure of readily bioavailable phosphorus. The ability to perform frequent or even online determination of these parameters provides the potential for improved process control. A number of flow injection (Pedersen et al., 1990; Benson et al., 1996c) and SCFA systems for measurement of FRP or TRP have been developed (APHA, 2005).

12.4.3 Brackish and Estuarine Waters

Analysis of brackish and estuarine waters, by virtue of their widely varying salinity, can be problematic, especially if tin(II) chloride reduction of PM is employed. Preparation of standards in a matrix of the same salinity as the sample matrix, sample salinity adjustment, or standard addition may be necessary to compensate for salinity errors. Conventional flow injection manifolds with sample injection for the determination of reactive phosphorus in estuarine waters are limited by the Schlieren or refractive index (RI) effect, which can also cause major errors in quantitation. A simple flow injection analysis (FIA) manifold, which obviates this RI error in reactive phosphorus measurement, has been described (McKelvie et al., 1997). It involves the injection of acidic PM reagent into a carrier stream of sodium chloride of similar RI, which is then merged with sample (the salinity of which may vary widely from sample to sample) and tin chloride reductant. This approach also has the advantage that it automatically compensates for any sample background color. Reactive phosphorus was measured in samples with salinities ranging from $S = 0$ to 34 using calibration standards prepared in deionized water, with a detection limit of $6 \mu g P L^{-1}$ (0.19 $\mu M$). Salinity interference was suppressed by the use of a high chloride carrier; an improved method based on ascorbic acid reduction has also been reported (Auflitsch et al., 1997). Other reported techniques for RI correction include the use of dual-wavelength detection with the application of a correction algorithm (Zagatto et al., 1990; Liu et al., 1994; Daniel et al., 1995), the use of large injection volumes (Yamane and Saito, 1992; Yamane, 1995), or detection using single or multireflection flow cell (Jambunathan et al., 1999; Ellis et al., 2003).

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<table>
<thead>
<tr>
<th>TABLE 12.5</th>
<th>Commonly Used Standard Methods for the Digestion TP Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment Principle</td>
<td>Method Suggested</td>
</tr>
<tr>
<td>Acid sulfuric and persulfate digestion to convert polyphosphate and organic P in orthophosphate</td>
<td>Manual digestion + FIA</td>
</tr>
<tr>
<td>Organic P is converted in-line to orthophosphate by heat, UV radiation, and persulfate digestion. Inorganic polyphosphate are converted by in-line sulfuric acid digestion</td>
<td>In line UV/persulfate digestion + FIA</td>
</tr>
<tr>
<td>Oxidation of P compounds by persulfate sodium hydroxide. The oxidation occurs under acidic conditions during the final stage of the digestion (NaOH is consumed)</td>
<td>Manual digestion + SCFA</td>
</tr>
</tbody>
</table>
12.4.4 Marine Waters

The high salinity of seawater may give rise to a number of interferences, either in sample pretreatment or detection steps. For example, in the determination of TP in estuarine and marine waters, there is the potential problem of chlorine formation during digestion with peroxydisulfate:

\[ \text{K}_2\text{S}_2\text{O}_8 + 2\text{Cl}^- \rightarrow 2 \text{K}^+ + \text{Cl}_2(g) + 2\text{SO}_4^{2-} \]

This is not problematic if the sample is digested in an open vessel, where the chlorine is boiled off. If digestion is performed in a closed vessel in a microwave oven or autoclave, the chlorine is trapped, and subsequently interferes in the detection process involving the ascorbic acid reduction (Dellien and Johansson, 1981).

Similarly, the determination of TP in samples containing large amounts of salt may be complicated by salt precipitation as the sample is evaporated during digestion. Under these circumstances, separate digestions should be performed to determine the particulate phosphorus and TFP, and TP is determined as the sum of these (cf. Figure 12.2) (APHA, 2005).

Enhanced sensitivity in the determination of reactive phosphorus in seawater has been achieved by extracting PMB from volumes of up to 1000 L onto a synthetic acrylic cation exchange medium (Acrilan) using a 2 L volume of the resin fibers. Filterable unreactive phosphorus was adsorbed to Fe(III) hydroxide-coated acrylic fibers in a similar manner (Lee et al., 1992). While the extraction efficiency of this technique was >95%, it appears not to have been exploited to gain maximum sensitivity.

The SIA is well suited for the fast determination of filterable molybdate reactive phosphate in coastal waters. It is especially suited for difficult environments with steep concentration gradients and varying salinity (Frank et al., 2006). There are advantages of SIA for the online determination of nutrients like phosphate in coastal water improving the reliability of the gained data by continuously monitoring one or more standards and on the advantages of online standard additions and offline determination of manually collected samples with the online SIA system (Frank and Schroeder, 2007).

A flow injection system for the analysis of reactive phosphate in seawater was introduced by Johnson and Petty (1982). This involved the concept of reverse or reagent injection FIA, which they showed to be inherently more sensitive than the conventional sample injection flow injection approach. A major advantage of the system was that it could be used for underway analysis.

12.4.5 Development of Portable and In Situ Analysis Systems

The ability to perform on-site analysis at high frequency is highly desirable because it obviates problems associated with loss of sample integrity due to hydrolysis, microbial action, or adsorption during transport and storage. The deployment of portable or unattended analysis systems would permit discharge monitoring to be performed with greater frequency and reliability than is possible with hand sampling and off-line laboratory analysis. Process control of wastewater treatment plants could be enhanced by improved process monitoring that these systems could provide. In response to this perceived need, a number of researchers have developed in situ or remote monitoring systems suitable for water and wastewater analysis of phosphate (Worsfold et al., 1987; Benson et al., 1996c; Motomizu et al., 1997; Lyddy-Meaney et al., 2002). While most of these have only ever been demonstrated as research prototypes, some are now being marketed commercially. The use of automated flow systems for monitoring phosphorus and other nutrients both temporally and spatially has recently been reviewed by Gray et al. (2006).

12.5 Future Trends

While most water analysis for phosphate is laboratory based, it is predicted that the emergence of robust, sensitive, and commercially available portable and online instruments for the analysis of phosphate and TP will replace a major part of this analytical load. Such a move is likely to be enhanced by the development of sensitive phosphate selective enzyme electrodes using amperometric detection, which would provide a viable and selective alternative to PMB spectrophotometry. Further advances toward miniaturized flow systems are also expected.
However, in the foreseeable future, most small- to medium-sized laboratories will continue to use either batch or automated PMB-based spectrophotometry (FIA, SCFA) techniques, with the emergence of ICP–MS as a possible alternative in larger laboratories. The flexibility of the SIA is especially advantageous for the automatic adaption of the system to different conditions like different concentration ranges and an improved quality control using additional standards. While other flow systems like the SCFA or FIA are often difficult to react after a long downtime, no such problems were observed with SIA. Future work should include the replacement of the syringe pump and the detector with significant smaller parts are necessary (Frank et al., 2006). The high speed, low reagent consumption, sensitivity, and general flexibility of this SIA instrument also make it suitable for other applications like unattended continuous monitoring or the integration into an automated water measurement system (Petersen et al., 2003; Wehde et al., 2003).

The development of phosphorus-specific or higher-sensitivity detection systems for capillary electrophoresis and liquid chromatography is seen as essential if further developments in the speciation of aquatic phosphorus using these approaches are to occur.

**ABBREVIATIONS**

| AAS | atomic absorption spectroscopy |
| AES | atomic emission spectrometry |
| AP | acid phosphatase |
| CCD | charge coupled device |
| CE | capillary electrophoresis |
| CTAB | cetyltrimethylammonium bromide |
| DOP | dissolved organic phosphorus |
| DRP | dissolved reactive phosphorus |
| FAHP | filterable acid-hydrolysable phosphorus |
| FCP | filterable condensed phosphorus |
| FI | flow injection |
| FIA | flow injection analysis |
| FOP | filterable organic phosphorus |
| FRP | filterable reactive phosphorus |
| GOD | glucose oxidase |
| HLB | hydrophilic-lipophilic balanced |
| HPLC | high-performance liquid chromatography |
| IC | ionic chromatography |
| ICP | inductively coupled plasma |
| LDPE | low-density polyethylene |
| LED | light-emitting diode |
| LOD | limit of detection |
| LWCC | liquid waveguide capillary cell |
| MMA | molybdenum bis(2-hydroxyanil) acetylacetonate complex |
| MP | maltose phosphorylase |
| MPA | molybdophosphoric acid |
| MR | mutarotase |
| MRP | molybdate reactive phosphorus |
| MS | mass spectrometer |
| Ni-BPE | nickel barrel-plated electrode |
| NP | nucleoside phosphorylase |
| NTU | nephelometric turbidity units |
| PAHP | particulate acid-hydrolysable phosphorus |
| PEDD | paired emitter-detector diode |
| PM | phosphomolybdate |
PMB  phosphomolybdenium blue
POD  peroxidase
POP  particulate organic phosphorus
PRP  particulate reactive phosphorus
PTFE polytetrafluoroethylene
PVC polyvinyl chloride
RI  refractive index
SCFA segmented continuous flow analysis
SI  sequential injection
SIA sequential injection analysis
SPE solid phase extraction
SRP soluble reactive phosphorus
TAHP total acid-hydrolysable phosphorus
TFP total filterable phosphorus
TOP total organic phosphorus
TP total phosphorus
TPP total particulate phosphorus
TRP total reactive phosphorus
USEPA United States Environmental Protection Agency
UV ultraviolet
WHO World Health Organization
XOD xanthine oxidase

REFERENCES


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