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Review

Progress and recent advances in phosphate sensors: A review

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ABSTRACT

This review covers the progress made in the development of sensors for inorganic and organic phosphates that are significant pollutants within the environmental and biological systems. Phosphate sensors in the forms of amperometric, potentiometric enzyme electrodes, plant tissue electrode and screen printed electrode are described. Instrumental probes such as fluorescence, chemiluminescence, luminescence and potentiometric ion selective electrodes are also described. Recent efforts on the use of voltammetric, potentiometric and amperometric biosensors for the determination of phosphate are highlighted.

Contents

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1. Introduction

1.1. Significance of phosphate

Phosphate is a well-known contaminant of ground and surface water, and it is one of the two substances that have been implicated in the frequent eutrophication of lakes and coastal waterways, the other being nitrate [1–5]. Owing to its nature, it can be present as inorganic and/or organic phosphorus. Phosphates are generally grouped into three main classes. They are orthophosphate, condensed phosphate (which consist of pyre-, meta-, and polyphosphate) and inorganic phosphorus [6]. The largest single source of inorganic phosphorus is synthetic detergent, while food and human wastes are the major sources of organic phosphorus [1,2,4,7]. Through biological action in the environment, all phosphorus are eventually converted to the inorganic forms. Despite the various attempts that have been made in recent years to encourage the use of phosphate-free detergent and to minimise the use of phosphate fertilisers, very high concentrations of phosphate are still often found in natural waters and sediments [3,4,8]. Processes such as wind erosion, surface runoff and leaching are the main pathways for transport of phosphorus from terrestrial to aquatic ecosystems. The discharges from these processes are further accelerated by agriculture, animal husbandry and other anthropogenic activities. These various sources result in increasing levels of phosphate and the eutrophication of lakes and coastal water [9,10]. Keup [11] observed that increasing the phosphate concentration in a body of water also caused accelerated growth of plankton, which made such water unsuitable for drinking.

Phosphorus is also an essential nutrient for all plants and it is normally absorbed via the roots. In modern agriculture, phosphorus is supplied to crops as fertiliser. Commercial phosphorus is manufactured from phosphate rock, which is mined in various locations throughout the world. However, there has been some concern that the phosphate rock reserve is being rapidly depleted as a result of its increasing use. According to a recent estimate, the current reserve is expected to last for less than 100 years [9,12].

The presence of phosphate in drinking water is another area of concern. Consequently, the determination and control of phosphate in natural water sources is a high priority for maintaining good water quality. The maximum permissible concentration of phosphate in drinking water recommended by the World Health Organisation is 1 mg L^{-1}. In Australia the maximum permissible level of phosphate in drinking water is 0.046 mg L^{-1} [13].

Phosphate measurements are also important for clinical diagnosis of various disorders. The diagnosis of hyperparathyroidism, hypertension [14], vitamin D deficiency, mineral and bone disorder [15] and Franconia syndrome [16,17] is some of the clinical conditions where the determination of phosphate concentrations in body fluids is necessary. The determination of phosphate levels in body fluids can also provide useful information about several diseases such as kidney failure [15]. The adverse effects of abnormally elevated blood level of phosphate (hyperphosphatemia) such as calcium phosphate deposition can lead to kidney damage The energetic state of the cell and bone function because phosphate salts are known to provide mechanical rigidity to bones and teeth [12,14,15,18]. Phosphate activities in the body organs include intestinal absorption of phosphate from the diet, release of phosphate through bone resorption, and renal phosphate excretion [19]. Khoshiniat et al. reviewed the data that phosphate sensing mechanism may be present in various organs and such sensor will detect changes in serum or local phosphate concentration. This suggest that phosphate is a signal regulating biological process such as bone or vascular calcification [18]. Phosphate is a well known genetic component of cells responsible for the production of proteins in living systems. Adenosine triphosphate (ATP) in cells has recently been analysed by Chen et al. [20], thus, phosphate ions and its derivatives thus play an important role in energy and signal transduction [21–23]. Shervendani and Pourbeyaran [24] monitored phosphate in blood serum for phosphate management in CKD-related mineral and bone disorder.

Millions of dollars are also spent on wastewater treatment to remove phosphate from water prior to disposal [9,25]. For these reasons the monitoring of phosphate concentration is very important for maintaining good water quality and minimising pollution of natural waters. Fast, simple and sensitive methods for measuring phosphate concentrations are required to enable rapid assessment of phosphate in various systems. This review examined the developing strategies in the various fabrication methods of sensitive and selective biosensors for rapid determination of phosphate in natural waters and to compare the merits and limitation of other recent competing techniques. This review is divided into four major parts: the first part which deals with the introduction, the second part deals with methods of phosphate determination, the third parts deals with biosensor and phosphate biosensor and the fourth part is other techniques of phosphate sensing. The aim of this review is to provide an update on work on phosphate which has been published in the period from 1970 to 2012 and targeted mainly at researchers who are active in the field of phosphate sensing.

2. Methods of phosphate determination

2.1. Classical methods

Classical analytical methods commonly employed for routine determination of phosphate are gravimetric methods (phosphate precipitated as magnesium pyrophosphate, magnesium ammonium phosphate hexahydrate), volumetric methods (by titration of ammonium phosphomolybdate with sodium hydroxide) and classical instrumental method based on spectrophotometric and chromatographic measurements [26–35], but these techniques require sample pre-treatment that can be time consuming, expensive and produce toxic wastes. Due to poor sensitivity of classical methods, most samples after dissolution are analysed by means of instrumental methods. Most commonly, phosphate determination is based on the molybdenum blue method of Fiske and Subbarow [34], which are both complicated and time consuming. Nakamura et al. compared this method with a phosphate biosensor method and found that as a result of acidification of sample in molybdenum blue method, biosensor methods are less complex and more sensitive.

The methods commonly employed for determination of phosphate in natural waters include colorimetry [37–45], ion chromatography

[46–49], flow injection analysis (FIA) [50–53] and potentiometry [54–57]. Classical instrumental methods are sensitive and mainly used for laboratory determination of phosphate and they have detection limit between 20 and 150 nmol L^{-1}. Molybdate/rodamine fluorescence method gave a detection limit of 20 nmol L^{-1} [58,59]. Unfortunately, most of these methods do not permit direct or field measurement of phosphate and they also have poor selectivity, involve tedious procedures and/or operations, and sometimes require the use of heavy metal ions and hazardous chemicals which can affect human health and the environment. Some of the colorimetric phosphate test kits are now commercially available for qualitative spot test, but they are prone to interferences. The availability of a simple and compact device that can be used in the field will enable rapid detection of phosphate and immediate assessment of its impact by non-expert field officers. The use of a biosensor for phosphate determination will provide most of these advantages, however, to our knowledge, there are still no commercially available phosphate biosensors. Further studies in this area are still necessary before such development can become a reality.

2.2. Biosensor

A biosensor is a device which has a biological sensing element either intimately connected to or integrated within a transducer that gives an electrical/digital electronic signal [60–62]. More specifically, biosensors are useful for accomplishing rapid, simple, selective and economic detection of various substances. The aim in fabricating a biosensor is to produce an electrochemical signal, which is proportional to the concentration of a specific chemical or set of chemicals. The compact analytical device usually contains a biological or biologically derived sensing element, e.g. enzymes, antibodies, micro-organisms or DNA, either integrated with or in intimate contact with a physicochemical transducer, e.g. electrochemical, optical, thermometric or piezoelectric [61–63]. These bioactive sensing elements are also referred to as "bioreceptors".

The bioreceptor typically converts or helps to accelerate the conversion of the analyte of interest into another chemical species and/or physical property that is sensed and then transformed into an electrical signal by the transducer. In this case the transducer can be an electrode. In an ideal situation, where the sample matrix is not too complex, this would be accomplished without pre-treatment or the addition of any reagents. The biosensor lifetime, stability, reproducibility and calibration requirements are influenced significantly by the chosen biological component; for example, an enzyme-based biosensor composed of an immobilised enzyme at the surface of an electrochemical sensor. Consequently, the enzyme reacts with a substrate and consumes a co-reactant as illustrated below:

$$\text{Substrate} + \text{Co-factor} \overset{\text{enzyme}}{\Longrightarrow} \text{Product} \quad (1)$$

Either step can be monitored electrochemically or by generating an electroactive product. The transduction element of a biosensor must be capable of converting a specific biological reaction (binding or catalytic) into a response, which can be processed into a useable signal. This element must be suitable for the immobilisation of the biological component at or close to its surface.

Previous studies have reported biosensors for the determination of various substances, such as glucose [64–103], urea [104–107], L and D amino acids [108], sulphite [109,110], DNA [63,111], cholesterol [112–123] and phosphate [124]. Of these, the development of biosensors based on electrochemical detection has attracted most interest in recent years. Most of these sensors are based on a redox enzyme such as glucose oxidase, where an electron is transferred from the substrate through an electron mediator or directly to the transducer (e.g. amperometric detector). Electrochemical methods provide quick and quantitative site assessment that can be performed by non-expert, they are portable, they are easily miniaturised and their operation is simple. Berchmans et al. [125] in their resent review classified sensing strategies based on the electrochemical detection techniques used viz., potentiometry, voltammetry, amperometry and unconventional electrochemical methods. The different types of electrochemical detection modes employed in this area are discussed below.

2.2.1. Amperometric enzyme electrode

An enzyme electrode usually consists of a layer of immobilised enzymes attached to an electrode material, such as gold, platinum, silver, copper or carbon. The enzyme is chosen to catalyse a reaction, which generates a product or consumes a co-reactant and can be monitored electrochemically [62,63]. In amperometric enzyme electrode a constant potential is applied and the redox current generated is measured. The electrochemical signal provides a measure of the analyte concentration. The main advantage of the electrochemical biosensor is that the analytical signal is electrical in nature and this greatly reduces the complexity of the transducing system and controlling electronics.

The electrode reaction involved in most biosensing devices is often the reduction of oxygen or the oxidation of hydrogen peroxide:

$$O_2 + 2H_2O + 2e^- \rightarrow H_2O_2 + 2OH^- \quad (2)$$

$$H_2O_2 \rightarrow 2H^+ + 2e^- + O_2 \quad (3)$$

Oxygen takes part in these reactions as a reactant, while hydrogen peroxide is a product of the reactions as in Fig. 1. These electrode reactions usually take place at the surface of a platinum electrode. The reactant or the product of the enzymatic reaction is often detected amperometrically. The distinct advantage of using enzymes as the biocomponents in sensor development is the ability to achieve greater analyte specificity. The high specificity reduces the need for pre-treatment of samples so that direct analysis may be carried out regardless of the sample matrix or complexity [60].

Ideally, enzymes with absolute specificity would be preferable for analytical use [126]. Examples of these include cholesterol oxidase [127], glucose oxidase, sulphite oxidase, urease and urate oxidase. While amperometric methods used for phosphate include that of Khonisita [128] and others [129–133]. Recently, Soukup [58] used Rodamine and its compounds for amperometric phosphate sensing while Gilbert analysed phosphate in urine [300].

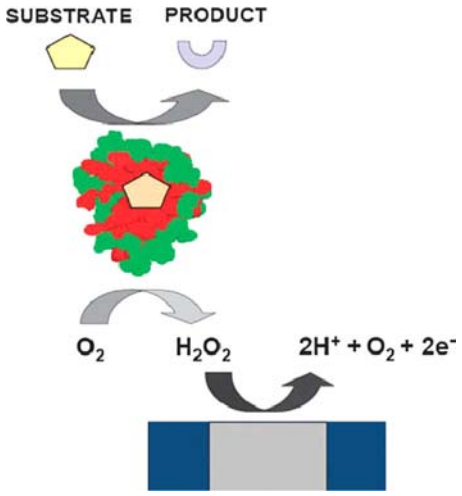

Fig. 1. Oxygen-dependent first-generation biosensor with amperometric detection [62].

2.2.2. Potentiometric enzyme electrode

A potentiometric enzyme electrode or potentiometric biosensor consists of a layer of immobilised enzymes on an electrode and the measurement of potentials at the working electrode is with respect to the reference electrode. The rate of potential change, rather than steady state potential values, is often used as the analytical signal for quantification of the substrate. Some of the reported potentiometric biosensors include the use of glucose oxidase immobilised in polypyrrole (PPy) for potentiometric detection of glucose [97,135]. Urease immobilised in an electrodeposited PPy layer was used for potentiometric detection of urea [109]. A creatinine enzyme electrode was made by co-immobilisation of creatinase, and sarcosine oxidase in a PPy matrix [136]. Menzela et al. [137] have also described a potentiometric enzyme electrode for phosphate determination which involved co-immobilisation of purine nucleoside phosphorylase (PNP) and xanthine oxidase (XOD). Adeloju and Lawal [5,139–142] also described potentiometric enzyme electrode for phosphate determination which involved co-immobilisation of purine nucleoside phosphorylase (PNP) and xanthine oxidase (XOD) into PPy.

3. Phosphate biosensors

3.1. Nanomaterial for fabrication of phosphate biosensor

Nanomaterials such as nanowires, nanorod, graphene, and nanotubes have attracted a great deal of interest in recent years for fabrication of biosensors with improved analytical performances, ruggedness and mechanical stability. These improvements are often attributed to the unique chemical and physical properties of the nanomaterials. Nanomaterial is usually used as a platform for the fabrication of biosensor. A practical approach to direct film or nanomaterial formation onto small or irregularly shaped electrodes, microelectrodes and microarray electrode is the use of electropolymerisation [143]. Electropolymerised film is self-regulating, possesses uniform thickness that covers the electrode evenly regardless of shape or size (10^{-9} m). It makes this approach attractive for microarray electrodes [143].

The electropolymerisation of pyrrole offers one of the unique approaches for direct and indirect immobilisation of enzymes and other bioactive substances in or onto conducting polymer (polypyrrole) films. Direct immobilisation by this approach involves entrapment of bioactive substances into the polymer during its electrochemical polymerisation. In contrast, the indirect immobilisation involves chemical attachment, usually by covalent bonding of the bioactive substance onto a pre-formed polypyrrole film.

Both of these approaches have gained considerable interest in the fabrication of conducting polymer biosensors [144]. Recent studies by Adeloju et al. [5,141,145,146] have demonstrated that the electrochemical immobilisation of enzymes, such as urease, sulphite oxidase, purine nucleoside phosphorylase (PNP) and xanthine oxidase (XOD) and formate dehydrogenase into conducting polypyrrole can be employed for the fabrication of biosensor for urea, sulphite, and formate determination, respectively. Gosh et al. [147] also immobilised purine nucleoside phosphorylase (PNP) and xanthine oxidase (XOD) onto electropolymerised polypyrrole. An important advantage of this electropolymerisation strategy is the ability to enable both the enzyme catalysis and analyte sensing to be performed on a single conducting polymer film. Adeloju and Lawal also used this method of immobilisation of PNP and XOD to analyse phosphate [5, 132, 139–142].

3.2. Amperometric phosphate biosensor

The first phosphate biosensor developed by Guilbault and Nanjo [204] was based on the inhibition of alkaline phosphatase by phosphate, but this lacked sensitivity and was not suitable for analysis of phosphate in natural water. The detection limit was at about 0.1 mmol L^{-1}. The sensor was also not selective for phosphate and produced signal for arsenic, borate, tungstate and molybdate. Other works on the development of phosphate biosensors focused on the use of immobilised phosphatase and in some cases, with glucose oxidase [21–26]. Guilbaut and Cserfalvi [148] suggested phosphorylase, phosphoglucomutase and glyceradehyde phosphate dehydrogenase enzyme system for use in phosphate-sensitive enzyme electrodes.

In Guilbaut and Cserfalvi system, glycogen and NADPH were employed in conjunction with three enzymes: phosphorylase, phosphoglucomutase and glyceradehyde-3 phosphate dehydrogenase or glucose-6-phosphate dehydrogenase catalysis. The catalytic reaction resulted in the formation of NADH, which was subsequently detected electrochemically. Fernandez et al. [149] also constructed a reagentless amperometric phosphate biosensor with these trienzymes and a scheme that incorporates an Os (1,10-phenanthroline-5,6-dione)$_2$Cl2 mediator for electrocatalytic recycling of NADH. These approaches were based on the electrochemical detection of NADH. However, this sensor relied upon three multiple enzymatic reactions:

$$\text{Glycogen} + \text{Orthophosphate} \overset{\text{Phophorylase -}a}{\Longrightarrow} \alpha\text{- Glucose-1-phosphate} \quad (4)$$

$$\alpha\text{-Glucose-1-phosphate} \overset{\text{Phosphoglucomutase}}{\Longrightarrow} \alpha\text{-Glucose-6-phosphate} \quad (5)$$

$$\alpha\text{-Glucose-6-phosphate} + \text{NADP} + H_2O \overset{\text{Glucose-6-phosphate-dehydrogenase}}{\Longrightarrow} 6\text{-Phosphogluconic acid} + \text{NADPH} \quad (6)$$

Addition of phosphate to a solution containing glycogen results in the production of α-glucose-1-phosphate, as indicated in Eq. (4). This product is then converted to α-glucose-6-phosphate in Eq. (5), which reacts together with NADP in Eq. (6) to produce NADPH. The resulting product (NADPH) can then be electrochemically oxidised back to NADP, thus enabling amperometric detection. This system gave a detection limit of 6 μmol L^{-1} of phosphate with an extended linear dynamic range up to 2 mmol L^{-1} The more phosphate that is added to the system, the more NADPH is produced and the greater the current will be. This system was found to work in solution, however, an immobilisation with starch or glutaraldehyde was found to inactivate some of the enzymes [89].

Phosphate biosensors based on the use of a two enzyme system, which contained purine nucleoside phosphorylase (PNP) and xanthine oxidase (XOD), were subsequently reported [150]. Several studies of this bienzyme system with PNP and XOD have been reported by several researchers such as D'Urso and Coulet [150], Watanabe and Endo [151], Male and Luong [152], Wollenberger et al. [153], Kulys et al. [130], Yao et al. [154], Konisita et al. [155], Voβ and Stuben [156] and Heammeli et al. [157], in their attempts to improve the detection limit and sensitivity of the earlier phosphate biosensors. D'Urso and Coulet [150] used the bienzymes to improve the detection limit for phosphate by a hundred-fold. In this case the enzyme catalyses the conversion of phosphate ions to hypoxanthine, as an intermediate product. They were able to increase the sensitivity of this method by using a hydrogen peroxide transducer instead of using an oxygen electrode and a detection limit of 10^{-7} mol L^{-1} was confirmed.

Adeloju and Lawal recently used this bienzyme to analyse phosphate [5,132,139–141]. The second enzyme is used to produce hydrogen peroxide and uric acid, as shown in Eqs. (7) and (8) [140,158]. Evidently, XOD plays the role of a biological amplifier,

generating 3 mol of electroactive species (2 mol of peroxide and 1 mol of uric acid) for 1 mol of phosphate.

Watanabe et al. [151] immobilised PNP and XOD on a membrane cellulose triacetateglutaraldehyde and 18 diamino-4-aminometyloctane, while D'Urso and Coulet [150] immobilised the bienzyme on polyimide membrane. Oxygen was monitored as phosphate was added to a solution containing inosine, resulting in the formation of hypoxanthine. This bienzyme is usually immobilised on oxygen or platinum electrode and the oxygen consumed or H_2O_2 produced, as indicated by reactions (7) and (8), is detected amperometrically:

$$\text{Inosine} + \text{orthophosphate} \overset{\text{PNP}}{\Longrightarrow} \text{Ribose-1-phosphate} + \text{hypoxanthine} \quad (7)$$

and

$$\text{Hypoxanthine} + 2H_2O + 2O_2 \overset{\text{XOD}}{\Longrightarrow} \text{Uric acid} + 2H_2O_2 \quad (8)$$

A reduction in current was detected as the oxygen was consumed together with hypoxhanthine, as indicated in Eq. (8). The decrease in the measured current was proportional to the phosphate concentration. A detection limit of 10^{-4} mol L^{-1} phosphate was achieved by this method. This bienzyme system appears to be very sensitive, but requires two other enzymes, urate oxidase for obtaining hydrogen peroxide from uric acid oxidation (Eq. (9)) and peroxidase for light emission in the presence of luminol:

$$\text{Uricacid} + 2H_2O + O_2 \overset{\text{Urateoxidase}}{\Longrightarrow} \text{allantoin} + H_2O_2 \quad (9)$$

The concentration of H_2O_2 produced in reaction (8) is subsequently measured colorimetrically or electrochemically [141,150–156,160]. However, the operational stability of the bienzyme system used by D'Urso and Coulet [150] was poor, losing about 30% of their initial sensitivity within 29 h. This appears to be due to the instability of the immobilised enzymes within the commercially available polyamide used in that study. It is possible, however, to overcome this problem by using other immobilisation strategies and/or other immobilisation substrates. Male and Luong [152] used the same bienzyme (PNP and XOD) system for FIA determination of phosphate, immobilising the enzymes on reactivated nylon membrane, which was attached to the tip of a platinum electrode. The operation stability was also poor, losing about 30% of its activity. An improvement of sensitivity was achieved by Wollenberger et al. [153] and Kawasaki et al. [17] when regenerated phosphate or analyte recycling was used with the bienzyme (PNP and XOD). Kulys et al. [160] also published a variation on the PNP-XOD system using a graphite electrode modified with 7,7,8,8-tetracyanoquinodimethane (TCNQ). No linear calibration was achieved and the detection limit was $2.2. \times 10^{-4}$ mol L^{-1}. Yao et al. [154] used glassy carbon electrode with glutaraldehyde, while Kinosita et al. [155] covered the electrode with a dialysis membrane for determination of inorganic phosphate in serum. In the review of phosphate sensors by Engblom [161], Vob and Stilben [156], Berchams et al. [162] and Warwick et al. [163], the enzymes PNP and XOD were immobilised on cellulose nitrate membrane pore sizes of 0.01–0.45 μmol L^{-1} and obtained a linear calibration in the range from 10^{-8} to 10^{-4} mol L^{-1}, with response times varying between 12 and 30 min. However, inosine, which is an indispensable reagent in the two-enzyme system, is degraded.

Adeloju and Lawal [132,139,140,142] immobilised PNP and XOD both in polypyrrole and BSA and GLA on a platinum electrode. Hydrogen peroxide was both amperomerically and potentiometricaly measured. The detection limits were 20 and 1 μmol L^{-1} respectively

Bienzyme system developed by Cosnier et al. [164] used alkaline phosphatase (AP) and polyphenol oxidase (PPO), which were immobilised into amphilic polypyrrole by electropolymerisation. The measurement of phosphate is based on the inhibition of the AP activity for the hydrolysis of phenylphosphate. The resulting phenol is then catalytically oxidised by PPO to *o*-quinone. The decrease in phenol formation is monitored by applying a constant potential of −0.2 V vs SCE for amperometric detection of the biocatalytically generated o-quinone. Recently, Yao et al. [116] used acid phosphatase for the development of a biosensor for determination of orthophosphate.

Another sensitive method using four enzymes for the determination of phosphate used a recycling and amplifying system consisting of maltose phosphorylase and phosphatase, glucose oxidase (GOx) and mutarotase [165]. This method is highly sensitive, achieving a detection limit of 0.01 μmol L^{-1}. However, it suffered from low reproducibility and had a narrow linear concentration range. Hence it was unsuitable for the determination of phosphate concentration in natural waters. Mousty et al. [166] used a simple method to fabricate an amperometric phosphate biosensor containing MP, MR, and GOD with a linear range of $1–50 \times 10^{-6}$ mol L^{-1}

Several systems suitable for on-site phosphate monitoring used pyruvate oxidase (PyrOx) derived from *Pendiococcus sp* or pyruvate oxidase G [167] or PyOx from *Lactobisilus* [168]. The hydrogen peroxide produced in this case was measured with an oxygen electrode [167,169–172] and the achievable detection limit was 12 μmol L^{-1}. The reaction involved was as follows:

$$\text{Pyruvate} + \text{Phosphate} + H_2O + O_2 \overset{\text{Pyruvate oxidase}}{\Longrightarrow} \text{Acetylphosphate} + H_2O_2 + CO_2 \quad (10)$$

In order to improve the sensitivity, a luminol chemiluminescence reaction was employed to detect the hydrogen peroxide produced by PyrOx reaction [167,172–177]. Although the sensor showed a linear response from 4.8 to 10 μmol L^{-1} phosphate, it was not sufficiently sensitive to detect phosphate in river water, where the ideal maximum permissible concentration is 0.32 μmol L^{-1}. Phosphate contamination of the thiamine pyrophosphate (TPP), which is a co-factor of PyrOx reaction, was a serious obstacle to the improvement of the detection limit of the biosensor. This is because the level of phosphate present in TPP limits the ability to detect lower phosphate concentration. The TPP was therefore purified by ion exchange column chromatography. Chemiluminescence detection of hydrogen peroxide has also been used with the flow injection method [178]. This system is based on the H_2O_2 generated by pyruvate oxidase that reacts with luminol catalysed by an immobilised peroxidase. The resulting chemiluminescence is detected by a photomultiplier. This system uses a flow injection analysis (FIA) system resulting in the rapid determination of phosphate. This system appears to be very sensitive; however, it requires two other enzymes: urate oxidase for obtaining hydrogen peroxide from uric acid oxidation and peroxidase for light emission in the presence of luminol, according to Eqs. (10) and (11).

Osmium bipyridyl units functionalised pyrrole monomer, copolymerized electrochemically with thiophene was used as a mediator for pyruvate oxidase electron transfer and the detection of phosphate was demonstrated successfully using this mediator. The sensitivity of detection achieved in this case is 0.2 A cm^{-2} and detection range is between 0.02 and 5 mol L^{-1} [179].

The reaction involved in this case was

$$H_2O_2 + \text{Luminol} \rightarrow \alpha\text{-Aminophthalate} + N_2 + \text{light} \quad (11)$$

Recently Jorica et al. [180] found that molybdophosphate complex formed from othphosphate in sea water is detectable by amperometry with an average precision of 2.2% for the phosphate concentration range found in the open ocean and the detection

limit was 0.12 μmol L^{-1}. Lindino et al. [181] also recently used modified chitosan electrode, a construction of an electrode modified with chitosan to analyse phosphate ions. An Amperometric technique offers portability and excellent sensitivity which make them very attractive for onsite monitoring of phosphate. Furthermore, the interference from ions like silicate and arsenate can be completely avoided as reductions of their respective species occur at different potentials. Amperometric biosensor possesses some limitations. The oxidation of peroxide is hampered by poor electrode kinetic at electrode which can lead to application of high potential before a quantifiable signal is obtained. This will also introduce a high degree of interference from oxidation of other analyte in the matrix and could lead to erroneous amplification of signal.

3.3. Amperometric electrochemical reduction of phosphomolybdates

Intensely blue colour of phosphomolybdates which allows spectrophotometric determination of trace level phosphate in an analyte is a routine analytical method. The method is not entirely and universally satisfactory. To avoid the complications arising from the use of chemical reducing agents, phosphomolybdate ions are reduced electrochemically followed by spectrophotometric analysis. This technique has successfully been employed for the determination of orthophosphates in beverages, waste waters and urine samples [182].

Several reports on the electrochemical determination of phosphate using phosphomolybdates are available in the literature [183–190]. Amperometric detection in flow injection analysis is widely reported in the literature [183,185–188]. Quintana et al. describe formation of phosphomolybdate complex in presence of nitric acid, ammonium molybdate and phosphate and its subsequent reduction at a carbon paste electrode, polarised at +0.3 V (vs Ag/AgCl) [183]. Galado et al. [191] also used molybdenum chemistry to determine phosphate with detection limits lying between 1 μmol $L^{-1}$and 1 nmol L^{-1} range

The major characteristics of this method were simplicity of the equipment, limited consumption of reagents and low limit of detection ($0.3 \times 10^{-}6$ mol $L^{-1)}$ with a linear range between 1 and 20×10^{-6} mol L^{-1} as shown in Table 1.

3.4. Potentiometric phosphate biosensor

There are few potentiometric phosphate biosensors. Katsu and Kayamotto used the reaction of *o*-caboxylphenilphosphate and alkaline phosphatase [193], i.e., the AP enzyme-induced cleavage of phosphate as shown in Fig. 2. The detection limit was 0.05 $\times 10^{-3}$ mol L^{-1}. The method was successfully applied to the determination of phosphate in blood serum [194].

Menzel et al. [195] described a potentiometric phosphate biosensor. They used fluoride-sensitive semiconductor chip and PNP and XOD system (Eqs. (7)–(9)). Lawal and Adeloju [140–142] also fabricated potentiometric phosphate biosensor by immobilising PNP/XOD into polypyrrole.

3.5. Other electrochemical phosphate sensors

3.5.1. Conductometry

Conductometry is another mode of electrochemical detection which measures resistivity. Phosphate has been determined by Zhang et al. [196] and Guedi [197]. They described the design of a conductometric biosensor to monitor aquatic environments. This biosensor is based on the measurement of the alkaline phosphatase activity of the microalgae.

The detection limit achieved was 1×10^{-6} mol L^{-1}. No interference from other anionic species was detected. The conductometric biosensor exhibited a long-term storage and operational stability as well as a good thermal stability.

3.5.2. Voltammetry

This technique involves the measurement of current as the potential of the working electrode is varied. Hence, a three-electrode system of working, reference and auxiliary is employed. The analyte concentration is proportional to the reduced or oxidised peak current of the measured species. The cyclic voltammetry (CV) mode of detection has been used to analyse cholesterol [185,198]. Phosphor–molybdenum complex has been subjected to CV to analyse phosphate by Fogg et al. [185–188,199]. They used differential-pulse voltammetric determination of phosphate as molybdo-vanadophosphate at a glassy carbon electrode and were used for assessment of phosphate in industrial effluent. This method has the disadvantage of using high voltage to get any reasonable signal which could introduce interfering signals from other analytes. Recently Norousi et al. used CV to analyse phosphate [200] while Chalk and Tyson [201] also used CV to

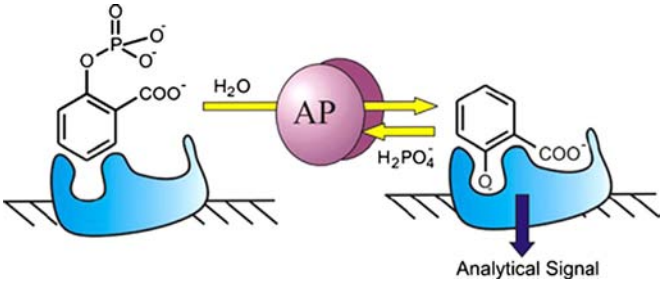

Fig. 2. Potentiometric detection of phosphate exploiting alkaline phosphatase (AP) inhibition [314].

Table 1
Summary of phosphate detection methods using amperometric analysis [163].

Method	Linear range (mmol L^{-1})	Detection limit (mmol L^{-1})	Potential issues	Reference
Enzyme based sensor, generating hydrogen peroxide		0.003	Durability in field conditions	[315]
Reduction of ferrocene based macrocyclic synthetic receptor	4.3–10.2		High linear range and unspecified detection limit. Sensitive to pH extremes	[231]
Reduction of ferrocene based macrocyclic synthetic receptor with guanidinium moiety			Detection of pyrophosphate but not orthophosphate	[231]
Reduction of phosphomolybdate complex	0.03–0.62	0.009	Requires reagents to avoid interference from silicate and to achieve signal	[183]
Reduction of phosphomolybdate complex. (with concentration step)	0.05–0.5 (0.0001–0.01)	0.03(0.0002)	Potential interference from organic phosphates. Sensitive to chloride. Phosphomolybdate complex precipitates at higher concentrations	[316]

Table 2
Summary of phosphate detection methods using voltammetric analysis [163].

Method	Linear range (mmol L^{-1})	Detection limit (mmol L^{-1})	Potential issues	Ref.
Redox of molybdo-vanadophosphate		0.03	Electrode requires daily clean. Addition of heteropolyacid required to counter intereference	[199]
Cobalt based synthetic receptors			Experiment performed in acetonitrile not water	[231]
Inhibition of redox reaction of potassium hexacyanoferrate		16	High detection limit	[289]
Zirconium dioxide nanoparticles			Only demonstrated for organo-phosphates	[317]
Inhibition of an enzyme based glucose electrode	1.2–31	0.009	Enzyme based electrode may not be robust in field conditions	[200]

monitor phosphate. Other techniques, such as piezoelectric transducers, have been used to measure small amounts of phosphate material. Electrochemical techniques have advantages over spectrophotometric methods:

1. They suffer less interference from dissolved silicon or turbidity.
2. They do not suffer from refractive index (Schlieren) effects in high-salinity samples.

However, as shown in Table 2, the LOD of these techniques is typically of the order of 0.1–5 mol L^{-1} [29], so it is necessary to combine them with analyte preconcentration.

The voltammetric and amperometric sensors suffer from a lack of selectivity, limiting their suitability and, while enzyme based amperometric sensors may perform well in biomedical applications, their suitability and robustness for onsite use is limited. Ion selective electrodes (ISE) suffer from limited lifetimes; require pretreatment in solution, or both, limiting suitability for field measurements and continuous monitoring. Similarly, the methods also suffer from poor selectivity or durability.

4. Other techniques used in phosphate sensing

4.1. Optical instrumental methods

Numerous methods for optical determination of phosphate are described as shown in Table 1. Instrumental techniques have long been used for phosphate detection with spectroscopic, coupled with flow injection analysis (FIA) routinely used for laboratory based phosphate analysis. Optical instrumental probes have been used to analyse inorganic phosphates, organo-phosphates, phosphoproteines and phosphate ions in general. The probes include fluorescence, luminescence, and chemiluminescence, spectrophotometric and UV–visible methods. Optical instrumental probes are sensitive and mainly used for laboratory determination of phosphate and they have detection limit between 20 and 150 nmol L^{-1}. Molybdate/rodamine fluorescence method gave a detection limit of 20 nmol L^{-1}, but they are prone to interference, unstable or erratic measurement and lack of selectivity due to the fact that some reagents can produce emission for more than one analyte.

4.1.1. Spectrophotometric and UV methods

Spectrophotometric methods based on the formation of the blue or yellow form of phosphomolybdate or vanadophosphomolybdate heteropolyacids are most frequently used to quantify phosphate concentrations between 1 and 20 mol L^{-1}.

In visible range, molybdenum yellow shows maximum absorption. Jorica et al. conducted continuous automatic monitoring of phosphoric ion in coastal water [180]. Sofia et al. [203] and Rodriquest et al. [204] used spectrophotometric flow system using vanadomolybdophosphate detection chemistry and a ytterbium (III) and dynamic measurements of light scattering intensity at long wavelength for the determination of phosphate. In the presence of some reducing agents, such as ascorbic acid, hydrazine and tin (II) chloride, molybdenum yellow can be reduced to form molybdenum blue, which shows stronger light absorption than molybdenum yellow and the maximum absorption wavelength are at longer wavelengths around 650–850 nm. At these wavelengths various light emitting diodes (LED) are now used to monitor phosphate and Mas et al. [205] applied this system to the on-site determination of phosphate in river water samples: LOD was 5 μmol L^{-1} of phosphorus. In spite of the relatively unstable nature of tin (II) chloride, this reductant usually gives the best overall results. Silicate, arsenate and germanate are the main interferences of these methods since these ions also form heteropolyacids, which on reduction yield molybdate blue species with similar absorption maxima. Other alternatives for the determination of phosphate are the methods based on the formation of ionic pairs of either molybdophosphate or vanadomolybophosphate with basic dye compounds such as Malachite Green, Rhodamine B, Crystal Violet, and Methylene Blue. Spectophotometric methods also include that of Mass et al., Galhado et al. and Abdallaa et al. [191,205,206] who used molybdenum blue chemistry to determine phosphate. Gimbert [207] and Nevesa et al. [208] used spectrophotometric flow system using vanadomolybdophosphate detection chemistry and a liquid waveguide capillary cell for the determination of phosphate. Di et al. [209] determined trace phosphate ion with 3,3′,5,5′-tetramethylbenzidine using a flotation-extraction preconcentration method and spectrophotometric detection. Matsunaga used photo-microbial method for selective determination of phosphate [184,208]. Jing Lu and Gui used selective determination of orthophosphate and total inorganic phosphates in detergents by flow injection photometric method (FIA) [210]. Nakatani et al. [32] used spectrophotometer to simultaneously determine phosphate and silicate ions in river water by using ion-exclusion chromatographic separation and post-column derivatisation. Partey et al. [211] used flow cell coupled with miniaturised spectrophotometers to determine phosphate in sea water. Spectrophtometric method is suitable for routine field measurements of phosphate and achieves LODs of 0.8 nmol L^{-1} phosphates but it requires complex, expensive and bulky equipment and is not amenable to field applications. This method also suffers from interference arising from arsenates, silicates, sulphides, oxidising agents and other anions. This method is labour intensive and require continued use of consumables (reagents and cuvettes), which incur direct costs and require safe disposal [163].

4.1.2. Chemiluminescence methods

Chemiluminescence (CL) can be defined as the production of electromagnetic radiation (ultraviolet, visible or infrared) as consequence of a chemical or biochemical reaction. In this procedure, the chemical reaction produces sufficient energy to induce the transition of an electron from its ground state to an excited state.

To return to its ground state the electron can either lead to the emission of a photon, a process called CL, or the excited molecule can lose energy by undergoing chemical reactions, by collisional deactivation, internal conversion or inter-system crossing. The oxidation of luminol (3-aminophthalhydrazide) results in the chemiluminescent emission of blue light (440 nm) and is the basis of several methods for phosphate analysis. The reactions involved are as in Eqs. (10) and (11).

Ikebukwo and Wakamura used pyruvate oxidase that gives rise to H_2O_2, which can be detected through its reaction with luminal that produced chemiluminescence for detection of phosphate [212], Kawazaki et al. used PNP/XOD and urate oxidase with FIA/chemiluminescence detection of phosphate [17], and Yaqoob et al. [213] determined nanomolar concentrations of phosphate in freshwaters using flow injection with luminol chemiluminescence detection. Others researchers who used chemiluminescence approach are Nakaruma [173,214–216], Nonaka [217], Saeed [218], Cardumul et al. [219]. Lin [30,217,220], Bandoline [221] and Chen [54].

Nkrumah et al. [173] later used three-enzyme system for the generation of hydrogen peroxide to produce a system capable of continuously monitoring phosphate concentrations of 1.0 μmol L^{-1}. The system consisted of maltose phosphorylase (MP, EC2.4.1.8), mutarotase (MUT, EC 5.1.3.3) and glucose oxidase (GOD, EC 1.1.3.4). In this case, no co-factors are involved in the reactions and the substrate maltose is stable and inexpensive. However, the determination relies upon three consecutive enzymatic reactions, making the optimization process difficult. Nakaruma et al. [222] also developed CL system with immobilized enzymes, and a biosensor was used for phosphate determination in drinking waters: LOD was 0.96 nM. Similarly, enzymatic flow injection CL detection methods were developed. Ines et al. [223], in a review on the flow analysis of phosphorus with chemiluminescence detection, reported that most of the developed CL flow techniques show the advantage of lower detection limits such as 0.5, 0.05 and 0.02 μmol L^{-1} respectively.

The chemiluminescence method is highly sensitive when coupled with flow system, however; they are not suitable for onsite analysis of samples. Chemiluminescence offers superior sensitivity to spectrophotometry, because the signal is determined against a low background, so it is often applied in trace analysis. The method is characterized with low limit of detection (nmol L^{-1}). However, the uses of TPP and flavin adenine dinucleotide (FAD) still affect the chemiluminescence reaction in some of chemluminescence methods. Instability of the enzyme after a month and the erratic behaviour of photomultiplier have been the bane of this method.

4.1.3. Fluorescence method

The fluorescent reactions of vanadomolybdophosphoric acid and molybdophosphoric acid with thiamine were used for the determination of phosphate by FIA. The reactions were based on the oxidation of thiamine with heteropolyacids: and the determinable range was 0.015–0.6 mg L^{-1} and 0.02–20 mg L^{-1} of phosphorus, respectively [224]. The thiamine method is very sensitive; however, the reaction is less selective to phosphorus, and the presence of oxidising agents may interfere with the determination and may cause positive errors.

Synthetic receptors (SR) are mainly used to generate fluorescent signals in the presence of phosphate (31–310 μg L^{-1}) as well as phosphorylated peptides (2.5–25 μmol L^{-1}), under aqueous conditions. No signal is generated in the presence of other anions, including: carbonate, sulphate, nitrate or chloride [225,226].

Fluorescent signals were generated when inorganic phosphate sensing proteins, comprising a phosphate binding protein and two fluorescent reporter proteins were embedded within polyacrylamide nanoparticles [227].

SR that have been used for generation of fluorescent signal includes penta-pyrrolic macrocycles [220]), octamethyl calixpyrrole structures [228], two quinoline SR with a cerium chloride complex [192,229], anthracene receptors incorporating dipicolyamine and zinc complexes [225,226], ruthenium (II) SR with two pyridine rings and boronic side chains or multiple amine groups [230,231], a dinuclear zinc based complex combined with terpyridine and tetraazacyclododecane groups [232] and macrocyclic SR comprising two copper ions each with three amine groups [233].

Chen et al., Zhou, Zhao and Schanze assembled a ratiometric fluorescence method which could be used for pyrophosphate detection [234,235]. Others who have investigated fluorescence method include Gao et al. [134], Gosh et al. [236], Guo [237], Huang et al. [238], Khatua et al. [44], Kim et al. [239], Lee et al. [240], Lin et al. [30], Nonaka et al. [217], Saeed et al. [218], Tang et al. [241], Vazques [29], Wang et al. [242], Wen et al. [243], Wygladacz [244], Kim et al. [239], Chen et al. [245], Kyung [246], Mitra et al. [247], Khatu et al. [44], Shonji et al. [248] and Wei et al. [249].

Abdallah et al. [206] use fluorescence technique with limit of detection in the range of 10 μ mol L^{-1} and 80 nmol L^{-1}. Villamil-Ramos et al. [250], Gu et al. [251], Jin and Zhang [252] and Dwevide et al. [253] also used fluorescence method while Spanagler et al. [59] reviewed the progress made in the fluorescence probes. Detailed information on fluorescent techniques in general has been presented in books by Lakowicz [254] and Valeur [255].

The major characteristics of fluorescence method were simplicity of the equipment, limited consumption of reagents and low limit of detection (nmol L^{-1}).

4.1.4. Luminescence methods

Luminescence methods are based on the measurement of the emission spectrum obtained when previously excited atoms or molecules decay to their ground state. The emission of light is always a response to an input of energy of some kind, giving rise to different types of luminescence.

Luminescence method was used to analyse phosphate. Leach [256] used radio-luminescence and bioluminescence, later followed by Cadermil [219]. Stanmiruv et al. [257] used Europium 111 complex to produce luminescence signal. Others who have used luminescence to probe phosphate ion include Andolina and marrow [221], Dollards and Billards [258], Nakaruma et al. [259] and Wang et al. [242]. As shown in Table 3, various luminescence methods used have analytical characteristic, i.e., LODs between μmol L^{-1} and nmol L^{-1} range.

Luminescent/fluorescent methods have low detection limits and could potentially measure phosphate concentrations at nanomolar level. However, none of them are both specific for phosphate and have been tested in the field with environmental samples. Furthermore they both produce their signal in solution, which requires the consumption and disposal of reagents.

4.2. Potentiometric ion selective electrode (PISE)

Potentiometric ion selective electrode is the earliest direct electrochemical phosphate detection that includes the following division: Metal/metal phosphate electrode, solid state membrane electrode, liquid membrane electrode, coated wire electrode, heterogeneous membrane electrode and redox exchange electrode. It is the earliest method in phosphate sensing and has the advantage of being highly portable, and inexpensive to produce. It is not sensitive or selective enough to be able to operate at the concentration below 1 μ molar. ISE method suffers from severe chloride interference [37]. Cobalt metal electrode was the earliest introduced by Xiao et al. [260] and phosphate detection limit was 5×10^{-5} mol L^{-1}. Bay et al. [261] used cobalt nanostructure-based

Table 3
Some of phosphate detection methods using luminescent approaches [163].

Method	Linear range (μ mol L^{-1})	Detection limit (μ mol L^{-1})	Potential issues	Ref.
Fluorescent signal produced with synthetic receptor with two pyridine rings, boronic acid and a ruthenium (II) core		3.1×10^6	No data on performance at lower concentrations. Untested against sulphate and nitrate	[230]
Fluorescent signal produced with ruthenium (II) based synthetic receptors, two pyridine rings and amine groups.	62–6200		Untested against sulphate. No data on suitability for use with environmental water samples	[231]
Quenching of fluorescent signal from calixpyrrole synthetic receptor	9–155		Sensor only used in ACN, with up to 20% water. Interference from fluoride. Untested against sulphate and nitrate	[228]
Copper based macrocyclic synthetic receptor		124	Only allows selective detection of pyrophosphates	[233]
Sapphyrin synthetic receptor yields fluorescent signal in presence of phosphate	78 000–5.58×10^6		No data on performance at lower detection limits and in presence of other anions	[220]
Flow injection method, chemiluminescence using luminol	0.03–3.26	0.03	No data on durability or suitability use	[213]

Table 4
Others conventional methods for determination of inorganic and organic phosphate.

Methodology	Reagent	LOD (μmol $L^{-}1$)	Reference
ISE	Molybdate complex	0.6	[192]
Ion chromatography		0.1	[27]
Amperometry	Molybdate complex	3	[104]
Amperometry	NPN, XOD,AP	2	[206]
Fluorescent probe	PVC matrix	5	[30]
Florescent PVC matrix	NP,XOD,HRP Almorine	3	[29]
Potentiometric	NPN/XOD	20	[139,142]
Flourescent	Molybdate and thamine		[224]
Amperometric	MP, MR, GODx, AP	0.01	[166]
Capacitance	Malachite green	0.2	[312,313]
screen-printed electrode	Immobilising pyruvate oxidase (PyOD)	3.6	[172]
Amperometric	Poly(carbamoylsulphonate) (PCS) hydrogel immobilised pyruvate oxidase	50	[159]
Plant tissue electrode	Inhibition by phosphate of potato acid phosphatase catalysed glucose and phosphate	25	[318]
Amperometric	Maltose phosphorylase, acid phosphatase, glucose oxidase and mutarotase were coimmobilised on a regenerated cellulose	0.01	[319]
Amperometric	Immobilisation of pyruvate oxidase (PyOx) on a polyioncomplex membrane	0.2	[179]
Conductometry	AP/BSA/GLA	0.4	[197]

microelectrodes for phosphate detection. Cobalt thin films with different planes, nano-scale structures were obtained on gold microelectrodes. While development and selectivity have increased in this field, there is still significant interference from chloride, bromide, sulphide and iodide in phosphate sensing, however, cobalt/metal electrode has capability of detecting phosphate to μmol L^{-1} level and high selectivity.

The cobalt wire has also been used as a stable sensitive phosphate sensor by Chen et al. in flow injection analysis [245]. Others who have used cobalt electrode PISE phosphate sensor include Morava [261], Chen [245,262], de Marco [52], Wu et al. [38], Kim [263], Lee et al. [25,264,265], Zoo et al. [266], Wang [267], Goblin [268] and Xiao [260]. Cobalt was used as electrode material by most researchers; they found selective response towards phosphate ion [52,97,245,260–268].

Tin and its compounds have recently been used in development of PISE. Tin PISE includes that of Sasaki [269], Glazier [24,269–271], Anatomise [26], Chaniostaki [272] and Glazier and Arnold [270,271] used PVC and introduced dibenzyltin into it to analyse phosphate. Organotin ISE sensors have short life due to hydrolysis in their membranes. Nickel and its compounds PISE include that of Cheng et al. [200,262] and Cheng [200], Choi [273] used multidentate needle while Beg and Ashrad used a nickelphosphate/parchment membrane to construct phosphate membrane electrode [274]. Another membrane electrode ISE was constructed by Tafesse and Enemchukwu [202], their sensor exhibited linear potential response in the concentration range of 1.0×10^{-1} to 1.0×10^{-6} mol L^{-1}. Other metal/element used is lead [275], ammonium [276], zinc [277,278], cadmium [279], vanadium [208], silver [280,281], zirconium [24,276], hydrogen [282], platinum [127], aluminium [202], molybdenum [192], zeolite [57], copper [97] and iron [283].

Phosphate binding protein that have been used in phosphate sensing include that of Gupta et al. [284], Zhang et al. [285], Kivelhan et al. [286], Salin et al. [287], Kumar et al. [288] and Aioki [289]. Azath et al. demonstrated the use of per-6-ammonium-B-cyclodextrin/nitrophenol complex as a colorimetric sensor for phosphate and pyrophosphate ions in water [39]. Zeolite was used by Ejihew [57] to probe phosphate in water. As shown in Table 4, analytical characteristic of the above systems, i.e., LODs are between μmol L^{-1} and nmol L^{-1} range.

4.3. Organophosphorous/phosphate

Organophosphorous compounds are important neuroactive molecules found mostly in pesticides. Tavakoli and Ghourchian [291] fabricated a mono-enzyme layer containing choline oxidase which was immobilized along with nafion and bovine serum albumin, by cross-linking with glutaraldehyde. This was used for the measurement of the level of two organophosphorous compounds, paraoxon (POX) and ethyl parathion (EPA) [291].

Numerous kinds of organic phosphates are essential for a functional metabolism. They include inositol, lecithin [292,293], NAD^+ [294], NADH [294], FAD, vitamin B12, thiamine, pyridoxal, choline, kephaline, farnesol, phospholipids, phosphopeptides [226], phosphoproteins [225,226,295,296] and pentenyl alcohol.

Crew et al. [297] developed a screen-printed, amperometric biosensor array incorporated into a novel automated system for the simultaneous determination of organophosphate pesticides. Tanimoto et al. used a tyrosinate screen printed electrode for determination of carbamates and organophosphorus pesticides [133], While Tavakoli and Chourichian used a new mono-enzyme for the measurement of the level of two organophosphorous compounds, paraoxon (POX) and ethyl parathion (EPA), in phosphate buffer [291], Sun and Wang developed a double layer biosensor for detecting organo-phosphorous pesticides [298]. Raghu et al. [97] used amperometric biosensor which was developed by silica sol-gel film immobilization of the enzyme onto the carbon paste electrode to monitor organophosphate. The mono-enzyme biosensor was used for the determination of two organophosphorous compounds such as methyl parathion (MP) and acephate.

4.4. Screen printed electrode (SPE)

The adaptation of biosensors to electrochemical methods of detection has increased the interest in transferring the technology to screen printed strips. SPE is ideal for electrochemical detection [133,172,273,299–301]. The development of field-deployable instruments requires that the entire system, including all necessary peripheral components, be miniaturized and packaged in a portable device AP enzyme system [302–304] for electrochemical detection has created an increased interest in SPE or Laboratory on chips A carbon paste enzyme electrode is usually amenable to screen printing. Screen printed electrode serves as a useful tool for rapid and accurate phosphate measurements in on site screening. The ease of mass production of SPE makes it economically viable for onsite field screening and compare favourably with current portable metres used in environmental and biomedical application. Miniaturization of analytical devices through the advent of microfluidics and micro total analysis systems is an important step forward for applications such as medical diagnostics and environmental monitoring. The single dip disposability of SPE because of its mass production makes it ideal for onsite monitoring of phosphate and biomedical application. Mcgraw et al. [305] developed a sensor for long-term monitoring of phosphate levels that incorporates sampling, reagent and waste storage, detection, and wireless communication into a complete, miniaturized system. The device employs a low-power detection and communication system, so the entire instrument can operate autonomously for 7 days on a single rechargeable, 12 V battery. Roger et al. [172] fabricated a screen-printed biosensor using pyruvate oxidase for rapid determination of phosphate in synthetic waste water [301]. Gilbert et al. [300] developed an amperometric assay for phosphate ions in urine based on a chemically modified screen-printed carbon electrode. Khaleed [299] compares screen-printed, carbon paste and the conventional PVC membrane electrodes and found that the screen-printed electrodes show fast response time of about 2.2 s and exhibit adequate shelf-life (4 months). Tanimoto [133] fabricated a composite electrode prepared by screen-printed tyrosinase-modified electrodes. The SPE biosensor was prepared by immobilization of Tyrosine enzyme on the composite electrode surface by cross-linking with glutaraldehyde and bovine serum albumin. Crew [297] constructed a screen-printed, amperometric biosensor array incorporated into a novel automated system for the simultaneous determination of organophosphate pesticides. Kwan et al. [131,172] also developed an SPE biosensor using pyruvate oxidase for amperometric biosensor for determining human salivary phosphate. Some of the analytical characteristics of this method, i.e., limit of detection of these systems are as shown in Table 4.

4.5. Plant tissue electrode

Biocatalytic sensors using plant tissue materials in conjunction with electrochemical setup provided an alternative to biosensors based on isolated enzymes. Sidwel and Rechiniz reviewed progress and challenges for biosensors using plant tissue materials [306].

The plant tissue electrode differs from the enzyme electrode in that the layer of immobilised enzyme is replaced with a slice of plant tissue. One reason for using plant tissue is that enzymes in their natural surroundings are generally more stable than enzymes that have been isolated and then put through an immobilisation procedure. Schubert et al. [307] used a slice of potato (*Solanum tuberosum*) as a source of acid phosphatase for detection of phosphate and the biosensor has lower detection limit $2.5 \times 10^{-}5\ \mathrm{mol\ L^{-1}}$; Sensor is stable for 28 days or 300 assays. Linder et al. [308] also used a slice of potato and then covered with cellulose acetate to detect hydrogen peroxide. Matsunnanga et al. [309] used immobilised green algae *chlorella vulgaris* to produce a phosphate sensor. This involved dropping an algal suspension onto a polycarbonate membrane, resulting in the production of oxygen under light illumination. The evolution of oxygen is enhanced in the presence of phosphate. Campanella et al. [310] determined inorganic phosphate in drug formulations and biological fluids using a plant tissue electrode. Campanella et al. [311] also determined phosphate in foodstuffs using a plant tissue electrode.

4.6. Capacitance measurement

Basheer et al. [312] developed a membrane protein based biosensor. They used capacitance of a phosphate – H^+ symporter membrane protein (Pho84) in the sensing of phosphate ions. O′ Toole et al. [313] also used capacitance measurement to determine phosphate ion.

5. Conclusions

The purpose of this has been to highlight the progress made in range of analytical methods used in phosphate sensing. In the years under review (1970–2012), many analytical protocols have been developed for the sensing of inorganic phosphate ions. Each method has its own limitations. Efforts have been made to further optimise the methodologies to achieve much lower detection limit. The basic approach of incorporating biological catalysts into an electrochemical setup targeting phosphate analysis has been highlighted. Other summary of various techniques that have been used in recent years for the analysis of phosphate is provided in Table 4. Although optical instrumental methods gave very low limit of detection, commercially available and that it is possible to analyse phosphate in $\mathrm{nmol\ L^{-1}}$ range, but interference from arsenate may be significant. There is a pressing need for a new method for the elimination of arsenate interference that is compatible with flow analysis and this method is labour intensive and not amenable to automation. PISE offer the simple requirement of instrumentation that is commercially available, low production costs and are suitable for field based analysis, environmental monitoring and clinical analysis but frequent calibration and temperature effects are some of PISE setbacks. Screen printed electrodes is preferable for onsite measurements and biomedical research when the stability and life of the biosensor becomes questionable. Screen printed electrode has at present, met the drive to automate and to miniaturise analytical system. This is with a view to make them cheap to produce, amenable to automation and mass production. Such in-situ analysers could potentially be used for on-site analysis. But there is the problem of high LOD which limits their suitability for ultra trace

($\leq$30 nmol L^{-1}) analysis. The analysis of phosphate, based on the electrochemical reduction of molybdophosphate and PISE metal/metal oxide systems are considered to be successful for field measurements. But possible interferences from other metal ions which are closely similar in potential limit PISE selectivity, durability and commercialization.

Phosphate biosensor methods can become more popular analytical techniques for the determination of phosphate when the number of enzymes participating in the detection scheme is less. Its commercialization is limited because of the shelf life of the enzymes and efforts to produce more rugged enzymes that can withstand adverse condition such as high temperature are currently in progress. Phosphate electrochemical biosensors systems have improved LOD, are generally rugged, simple, low-cost, portable, automated and capable of high sample throughput. Their minimised risk of sample contamination makes them ideally suited for on-site and ultra trace ($\leq$ 30 nmol L^{-1}) analysis.

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