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Review

Bioelectroanalytical determination of phosphate: A review

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ABSTRACT

The analysis of orthophosphate within environmental samples has risen to considerable prominence in recent years as concerns over the eutrophification of rivers and lakes mount. The portability of electrochemical sensing devices is well established but their application to phosphate detection has been less successful. The present review exposes the problems encountered in attempting to apply conventional approaches to the problem and highlights the recent efforts that are being made to extrapolate voltammetric, amperometric and potentiometric biosensor technologies to the determination of this increasingly important analyte.

Contents

1. Introduction

The detection of phosphate in surface waters is important for the assessment of nutrient transformations from biogeochemical and ecological viewpoints and ever increasingly, from a regulatory and legislative standpoint [1,2]. The latter reflects increasing concerns of eutrophification of inland waterways by agricultural run off and other anthropogenic inputs [3–12]. Recent figures from UK government sources (Dept of Environment Farming and Rural Affairs) point to over 60% of river lengths in England possessing phosphate concentrations greater than 0.1 ppm and it is estimated that 40% of phosphate in inland water arises principally from agricultural sources. Such figures will obviously vary markedly depending on regional and seasonal factors [9,10]. Nevertheless, there is a clear need to monitor the concentration of such species in order to aid the balanced and sustainable management of water resources. Detecting the diffuse agricultural sources from which excessive amounts of phosphate are released to water courses is however a considerable challenge and it could be argued that field-based measurements could be used to provide a versatile and indeed potentially invaluable screening option.

The interest in the use of field-based measurements stems from a need to provide quick on-site assessments that could cover a greater geographical spread while obviating much of the costs, time delays and issues of sample integrity associated with traditional laboratory-based analysis [1,2]. Methods that allow for rapid detection are clearly of benefit when considering field measurements but there are numerous other practical considerations that must be weighed. While a variety of colorimetric spot test kits are commercially available and possess supreme portability, they can be prone to interference and provide, at best, qualitative results. The need for quick and quantitative site evaluations that can be conducted by non-expert investigators could be addressed by the use of electrochemical detection methods. Issues of miniaturization, portability and operational simplicity have been addressed by various detectors commonly employed within decentralised sens-

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ing [12–14]. The extrapolation of such technologies to yield a viable technology platform for field testing of phosphate would appear feasible but issues of selectivity and sensitivity must be clarified [14,15].

Various detection strategies for phosphate have been proffered and include phosphate ion selective electrodes based on potentiometric techniques [16–30], indirect voltammetric detection based on the reaction of phosphate with various metals and associated complexes [31–40], and the development of sensors exploiting enzymatic reactions [41–67]. The aim of the present review is to place these competing technologies within a critical framework that compares the merits and limitations of each methodology highlights the progress that has been made in recent years.

Phosphates can exist in several forms depending on the source/nature of the discharge but are generally grouped within three broad classes: orthophosphates, condensed phosphates (pyro-, meta- and poly-) and organic phosphorus [1,2]. The present review concentrates primarily on the determination of orthophosphate—the principal component within agricultural run off and the target to which most field-based measurement are/will be designed. Instrumental techniques have long dominated phosphate detection with spectroscopic and flow injection/ion chromatographic methodologies routinely used for laboratory-based analysis [68–79]. These are not considered in great detail here but, rather, are used for comparative purposes where appropriate. The reader is directed to recent reviews that critically assess the specific developments within those fields [80–83]. The use of biological agents in the determination of phosphate, nitrate and sulfate has been briefly reviewed [84] however, the material contained within the following discussion covers the application of electromolecular technologies in the broadest sense—outlining the evolution of the various detection methodologies and critically appraising these in light of the recent evolution of the evolution of bio-electrochemical interfaces that are increasingly being examined for environmental phosphate determination. The remit of the discussion to those reports solely based on environmental contexts has been avoided. A more general approach has been taken where the evolution of technological features that could, irrespective of proposed application, subsequently be adapted and harnessed within an environmental context are presented and critically appraised.

2. Potentiometric detection

Potentiometric detection is earliest direct electrochemical approach taken to the detection of phosphate and has the attraction of possessing numerous advantages when considering the development of sensing technologies, The recording instrumentation is inexpensive to produce, highly portable and it could be envisaged that the operational qualities would mirror those adopted within other ion selective electrode (ISE) sensors. While these features would appear to offer a mature foundation from which a sensor could be readily fabricated, the acquisition of sufficient selectivity and sensitivity to operate at the concentrations reported within the DEFRA analysis (typically below 0.1 ppm) remains problematic. Various approaches have been investigated and typically fall within the following divisions:

1) Metal/metal phosphate.
2) Solid state membrane electrodes.
3) Heterogeneous metal membrane electrodes (metal phosphates covered with polymeric membranes).
4) Liquid ion exchanger.
5) Redox electrodes.

The merits and limitations of these have been reviewed [16] but there have been a number of subsequent developments that have sought to overcome the poor selectivity and advance the attractiveness of PISE systems. The measurement of phosphate concentration in a sample by a PISE depends on the change in potential as phosphate sample is added. Selectivity has to be the major criterion applied to any proposed PISE because related ions could also affect the potential and thereby interfere with the analytical signal. The design of a phosphate selective membrane however has proven to be difficult. In the first instance, the phosphate molecule is of a size that makes it difficult to devise a macrocyclic host-guest interaction that possesses sufficient specificity for that particular moiety. Also, the molecule is very hydrophilic due to the four oxygen atoms attached to the phosphorous atom. This demotes phosphate to the bottom of the Hoffmeister selectivity series, i.e.:

$$ClO_4^- > IO_4^- > SCN^- > I^- > NO_3^- > Br^- > Cl^- > NO_2^- > HCO_3^- > SO_4^{2-} > HPO_4^{2-}$$

This means that the selective element of a potential PISE has firstly to reject the more lipophilic ions, then select for the least available ion. Many of the early PISE's failed by responding to ions other than phosphate. Some progress has been made with the use of metal/metal phosphate class of PISE in recent years with bismuth phosphate [17] and silver orthophosphate [18] as the selective components. While selectivity has increased with the developments in this field, there is still significant interference from chloride, bromide, iodide and sulphide.

A distinct group of PISE that has demonstrated some success for phosphate determination is based on a cobalt/cobalt oxide electrode [19–21]. The response mechanism is subject to some debate, being either a host–guest relationship [20] or a mixed potential response [21]. Nevertheless, it has been shown to be capable of detecting phosphate to 0.1 ppm (1 μM) whilst retaining a high degree of selectivity. Another promising development of PISE has been found with the use of organic complexes of tin. Electrodes made from PVC impregnated with different forms of organotin were found to be selective for phosphate and other oxyanions [22–25]. The tin (IV) centres facilitate binding of the oxygen atoms of the phosphate molecule and the organic complex increases this binding by withdrawing electrons from the tin. This electron withdrawing property, and consequent phosphate selectivity, could be further increased by replacing alkyltin compounds with benzyltin [23]. The more powerful electron withdrawing aromatic rings lower the Hammett constant [24] and provide superior selectivity to phosphate when considering the other Hoffmeister series anions. The main drawback of using the organotin membrane includes a short lifetime due to hydrolysis in the membrane.

Indirect detection through the formation of secondary components has also been investigated and typically exploits the interaction of metal ion with phosphate. This approach is based around the detection of the metal ion whose removal through the formation of an insoluble phosphate complex can be inversely related to phosphate concentration. Chief examples are molybdate [26], lead [27], cadmium [28] and silver ion selective electrodes [29]. A similar approach was taken with fluoride where the corresponding ISE was used to detect the release of the halide ion from its aluminum complex through the preferential binding of the phosphate [30]. While in principle these methods are relatively sensitive–selectivity within complex media is questionable where other, similarly competitive, complexation reactions are liable to impact on the analytical performance.

3. Voltammetric studies

Orthophosphate is not directly accessible to voltammetric studies and strategies adopting this methodology almost invariably rely upon the indirect association with molybdate complexes.

The protocols are normally derived from the spectroscopic assay developed in the 1920s and are based on the blue coloured phospho-molybdate complex formed from the reaction of ammonium molybdate and orthophosphate [1,2]:

$$PO_4^{3-} + 12(NH_4)_2MoO_4 + 24H^+ > (NH_4)_3{\cdot}12MoO_3 + 21NH_4^+ + 12H_2O$$

The voltammetric signal can be detected using a bare glassy carbon electrode at a potential of +1.3 V and has been reported to offer detection limits in the micromolar range (typically 0.5 ppm, 5 μM). These compare favourably with many of the conventional spectroscopic procedures. However, the reaction is slow and can be prone to interference from a variety of sources (heavy metals, proteins). Nevertheless, several electrochemical methods have been successfully based on this reaction [31–39]. The main drawbacks relate to the very large operating potential—essentially at the limits of the electrode window and which could elicit responses form other matrix constituents.

An alternative approach employing host–guest interactions has also been the subject of voltammetric studies. Based on the cyclic oligosaccharide β-amino-cyclodextrin [22], phosphate was detected indirectly through the preferential displacement of ferrocene derivatives from the cavity of the macrocycle. The initial addition of the cyclodextrin derivative reduces the amperometric peak current response of the ferrocene. This is due to the complexation of the ferrocene by the CD host. Adding the phosphate ion to this mixture produces a cyclic voltammogram which corresponds to free ferrocene. There have been attempts to attach cyclodextrins to gold [40] for attachment to electrodes. The voltammetric studies though technologically promising have yet to be submitted to a more rigorous analytical appraisal. Nevertheless, the voltammetric method does appear to offer a viable route than but, as yet, there is no phosphate selective device based on a carrier made from a cyclodextrin.

4. Phosphate biosensors

The principal problem in the development of a phosphate sensor clearly centres on the acquisition of selectivity. Thus far, electrochemical methodologies appear unable to provide sufficient answers when confronted with real samples and there has arguably been an impasse in the evolution of traditional technologies. Potentiometric techniques lack a suitably specific ionophore while voltammetric approaches suffer from the redox inaccessibility of the phosphorus centre ion under aqueous conditions. The increasing interest in biosensing approaches however offers an alternative route that could provide greater selectivity through exploiting the molecular recognition capabilities inherent to biological catalysts. Orthophosphate has many roles within biological systems and a multitude of enzymes are responsible for the specific transfer of the anion to and from various organic carriers. The main questions that need to be addressed relate to how such biological components could be harnessed for the detection of phosphate and how they can be integrated within conventional electrochemical architectures.

Despite the promise of high selectivity, phosphate selective enzymes are not readily amenable to direct electrochemical interrogation. Biosensing detection methodologies commonly employ the electrochemical detection of either the enzymatic consumption of molecular oxygen or the production of hydrogen peroxide byproduct. One of the few, single enzyme, designs to adopt this approach for the detection of phosphate is based on pyruvate oxidase (POD) [41–44]. The reaction involves the conversion of pyruvate to acetyl phosphate. The reaction scheme is shown in Fig. 1 with the concentration of phosphate being inferred from the amperometric

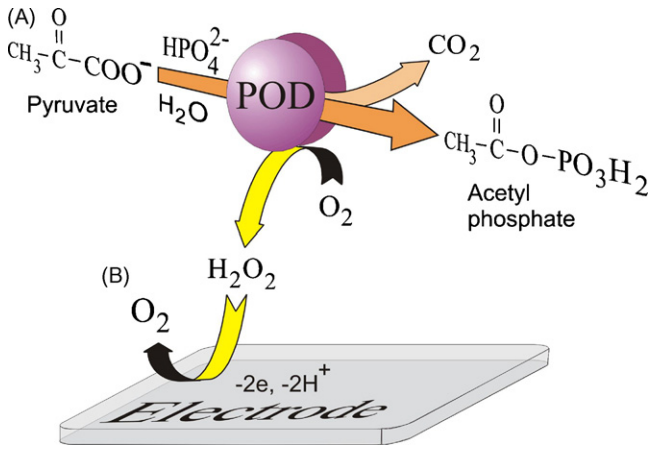

Fig. 1. Amperometric phosphate detection using pyruvate oxidase (POD) [41–44].

measurement of either oxygen depletion (**A**) [41] or the increase in concentration of hydrogen peroxide (**B**) [42].

These detection systems have the attraction of being relatively simple to implement – being built around mature electrochemical techniques – but they do possesses a number of limitations. Attaining a stable signal can make the measurement of oxygen relatively slow or ambiguous with the magnitude of the signal dependent upon the concentration of dissolved oxygen within the sample—which may be problematic for some anoxic waters. The oxidation of peroxide is hampered by poor electrode kinetics at conventional electrode materials and requires the imposition of a significant overpotential (∼+1 V vs. Ag|AgCl) before quantifiable currents can be obtained. These can induce a degree of interference through the oxidation of other matrix components and may lead to an erroneous amplification in the signal. More recent designs have taken advantage of mediated systems where oxygen is replaced by an artificial electron acceptor than can be regenerated directly at the electrode [43,44]. The current obtained from re-cycling the electron shuttle is then related to the concentration of phosphate. The main advantages being that vagaries in oxygen tension are removed and the electrode potential required for the re-oxidation of the mediator can often be set within a region where the oxidation of other matrix components is avoided.

The most commonly employed enzyme in phosphate biosensors however is alkaline phosphatase (AP). This enzyme acts upon phosphate ester functionalities and will cleave the bond releasing the inorganic orthophosphate and the corresponding alcohol derivative. The latter has traditionally been chosen to provide a distinct signal that can be easily quantified using conventional spectroscopic techniques. The classic example is highlighted in Fig. 2(A) where the hydrolysis of p-nitrophenylphosphate releases the chromogenic nitrophenolate ion. The absorbance of the anion (∼400–420 nm) can be related to enzyme action and is usually exploited as a means of quantifying AP and forms the basis of countless enzyme linked immunoassay protocols [45]. The adaptation of such systems for electrochemical detection has risen to considerable prominence with the increased interest in transferring the technology to microfluidic (lab on a chip) devices or screen printed strips—to which electrochemical detection is ideally suited [45–47]. The adaptation of the methodology for the detection of orthophosphate relies upon the inhibitory action of the latter on the hydrolysis of the ester substrate. Sample is assayed using known concentrations of enzyme/substrate with the deviation (decrease) of the signal from that expected in the absence of added phosphate being inversely related to the concentration of the latter.

Electrochemical adaptations rely upon the selective and sensitive oxidation of the hydrolysis product. The nitrophenolate

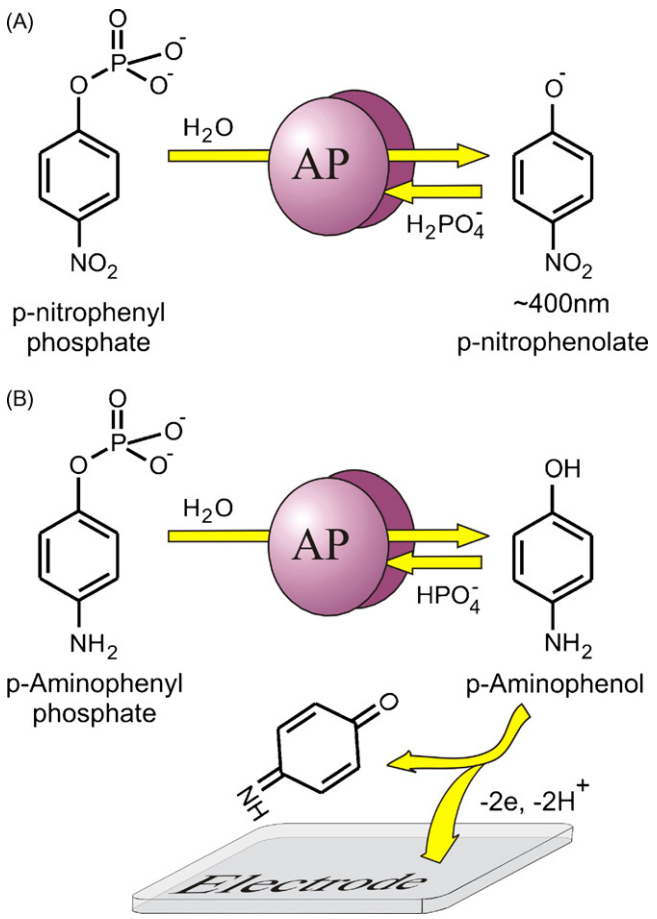

Fig. 2. Spectroscopic (A) and amperometric (B) reaction schemes based on alkaline phosphatase (AP) [45–47].

systems are predominately used for spectroscopic detection but are also electrochemically accessible. One drawback is that the potentials required to oxidise the phenol component (~+1 V vs. Ag/AgCl) are relatively large and, as with peroxide measurements, can promote interference from the oxidation of other components within the sample. A second factor that arises is where the oxidation leads to polymeric deposits on the electrode. These tend to block the electrode reducing sensitivity and compromising the reproducibility of the technique. An alternative approach is to select a label whose oxidation can be achieved at less positive potentials. Ferrocenyl [46] or aminophenol [47] derivatives (Fig. 2(B)) have been investigated as they possesses reversible behaviour (less likely to foul the electrode) and can be detected at significantly lower potentials (~+0.3 V). The concentration of orthophosphate is determined by the magnitude of the decrease in oxidation current recorded at the electrode as a consequence of the inhibition process. There are few potentiometric biosystems for phosphate but one approach has successfully harnessesed the AP enzyme-induced cleavage of phosphate. In this case the ester is based on salicylate (o-carboxyphenyl phosphate), Fig. 3, with the potentiometric detection of the latter with an ion selective membrane providing the basis of the analytical signal [48].

The majority of systems using AP tend to combine it with other enzyme catalysts—though inhibition remains the key determinant in signal processing. Such systems rely upon the synergistic interaction of the multi-enzyme assembly to yield a product (typically peroxide) that is electrochemically active and more amenable to detection than the labelled esters. The typical arrangement involves the phosphate selective enzyme (AP) producing a product which can then act as the substrate for a secondary enzyme whose purpose is to produce the electrochemical label. Quantification of the latter can therefore allow the amount of phosphate to be determined. Early implementations of bienzyme couples involved AP/Glucose Oxidase (GOX) assemblies with glucose-6-phosphate as the key substrate in the reaction, outlined in Fig. 4. Increased sample phosphate inhibits the production of glucose and hence the consumption of oxygen is decreased [49–51] as is the yield of peroxide [52]. Immobilised enzymes systems are by their nature more complex to produce but such assemblies have been proven to operate within a range of environmental matrices and include fresh and sea water samples. Interference from heavy metal ions (mercuric, cupric and zinc) can occur, but these are not likely to appear in any appreciable concentration in natural samples. The limit of detection for phosphate using the AP/GOX combination was typically 0.4 ppm (4 μM) and is comparable to those obtained using the molybdate systems.

Improvements in detection limit can be achieved through the catalytic cycling of the hydrolysed label. A number of formats can be pursued but the prime requirement is that the label possesses reversible electrochemical behaviour (capable of fast redox inter-conversion). An early approach successfully exploited for the detection of sub picomolar concentrations of AP (and hence could be adapted for orthophosphate—albeit at higher detection limits) is outlined in Fig. 5. In this case an excess of glucose is employed—and maintains the enzyme (GOX) in the reduced state. The electrochemical oxidation of the hydrolysed AP product, hydroquinone, produces benzoquinone which is chemically reduced through interaction with the flavin redox centre within GOX. A catalytic cycle builds up though which the current recorded at the electrode is effectively amplified [53]. It could be envisaged that the introduction of a sample containing phosphate would inhibit AP. Signal amplification would be retained through the HQ/BQ-GOX cycle but would be less than that experienced in the absence of the

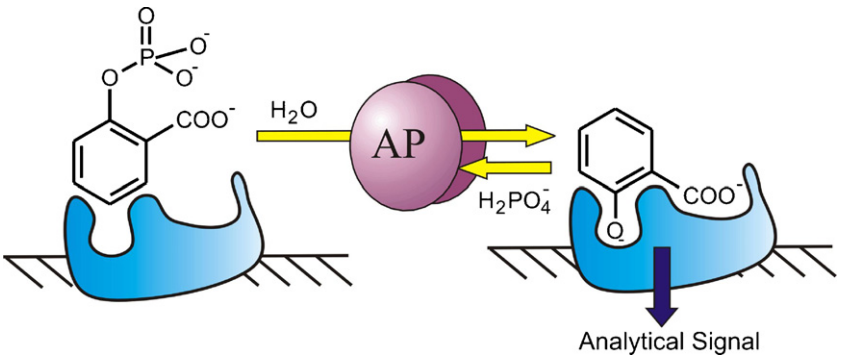

Fig. 3. Potentiometric detection of phosphate exploiting alkaline phosphatase (AP) inhibition [48].

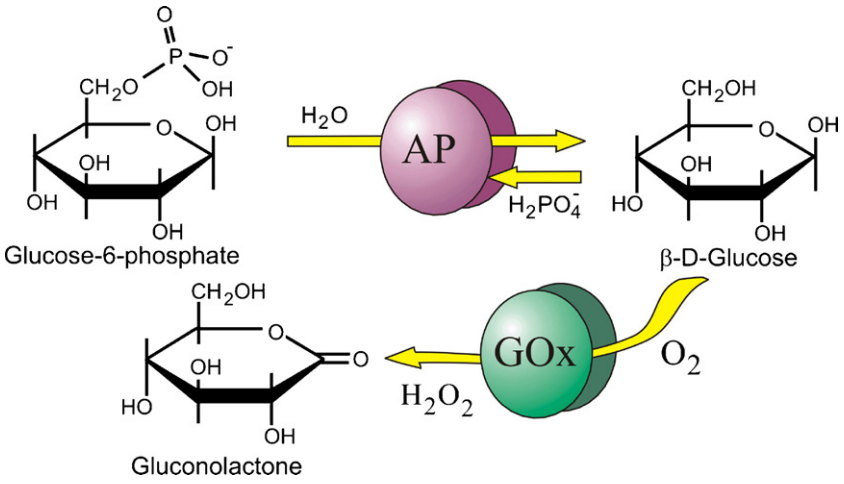

Fig. 4. Bienzyme coupling of alkaline phosphatase (AP)/glucose oxidase (GOx) for the amperometric measurement of phosphate [49–52].

added phosphate and hence could offer a means of improving the detection limits currently available to the simple AP/GOX system.

The key strength of alkaline phosphatase is that it is relatively non-specific in terms of the nature of the phosphate ester upon which it can act. This provides a significant operational advantage over some of the other enzymes in that it can be directly coupled with a wider range of secondary enzymes. The use of phenylphosphate is an important example as it provides a route through which polyphenoloxidase (PPO), an enzyme of directly facilitating substrate recycling amplification, can be incorporated within the sensing structure [54]. The basic outline is shown in Fig. 6 and involves the cleavage of the phosphate group by AP with PPO acting upon the phenolic component to yield the ortho quinone. In contrast to most redox enzymes systems, the detection strategy involves the imposition of a cathodic potential (−0.2 V) in order to reduce the quinone to the corresponding hydroquinone derivative. The current for the reduction will again be inversely related to phosphate concentration in keeping with its inhibitory action upon the AP catalysed ester hydrolysis. The major advantage in this instance is that oxidation of other matrix constituents can often be avoided. The PPO component therefore serves to aid both selectivity and sensitivity providing a detection limit of 0.2 ppm (2 μM) for phosphate [55].

More recent studies have focused on the use of nucleoside phosphorylase (NP)/xanthine oxidase (XOD) bienzyme combination as shown in Fig. 7(A). Nucleoside phosphorylase functions only in the presence of orthophosphate, producing xanthine from inosine, which is in turn oxidised to uric acid by XOD. A number of detection strategies can be employed with this assembly. The level of phosphate can be related to the decrease in concentration of oxygen [56,57], the increase in uric acid production [58–60],

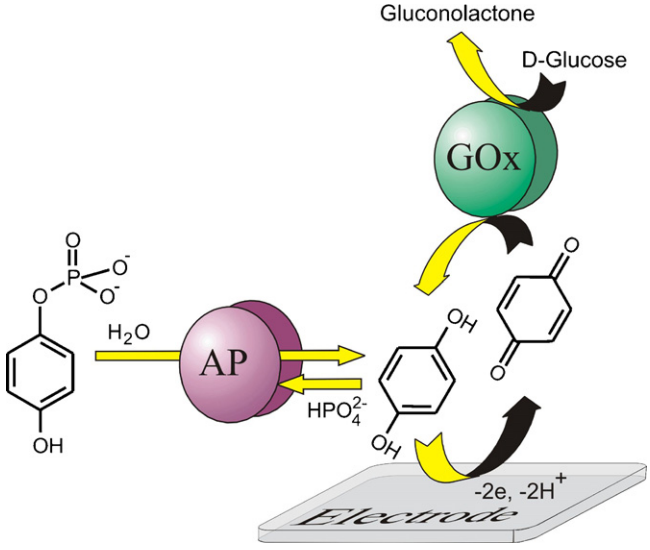

Fig. 5. Signal amplification through the glucose oxidase (GOx) promoted enzymatic recycling of the alkaline phosphatase (AP) hydroylsis product [53].

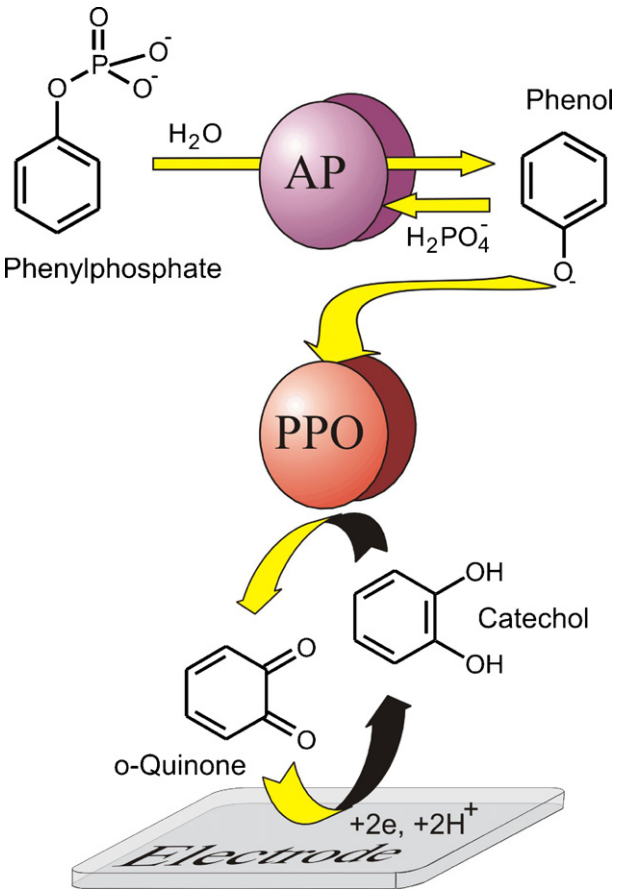

Fig. 6. Alkaline phosphatase (AP)/poly phenol oxidase (PPO) promoted detection of phosphate through the electroreduction of orthoquinone [54,55].

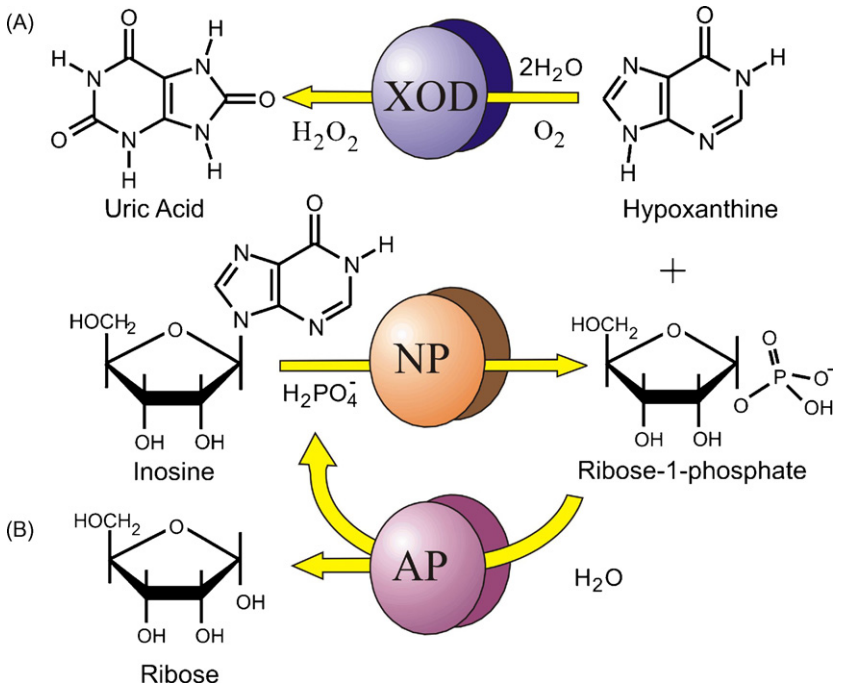

Fig. 7. (A) Synergistic interaction of nucleoside phosphorylase (NP)/xanthine oxidase (XOD) for the detection of orthophosphate [56–63]. (B) Catalytic recycling of phosphate through the addition of alkaline phosphatase (AP) to enable signal amplification [60,64].

or the increase in H_2O_2 concentration [56,60–63]. In general, the limits of detection afforded by this method are greater (ppb, submicromolar) than those with the AP, AP/GOD, or POD enzymes. The ability to exploit the uric acid signal is of considerable significance and provides an important operational advantage. Urate is endogenous to physiological systems and hence would prove to be a substantial interferent in actual analysis of clinical samples [12]. In the context of environmental analysis—few samples would be expected to contain the purine and hence the direct oxidation of the base at the electrode can be assumed to be derived solely from the enzymatic sensor assembly. The advantage of exploiting this label rather than peroxide lies in the relatively low oxidation potential of the purine (~+0.2 to +0.5 V). The oxidation of peroxide tends to be characterised by the poor electrode kinetics at conventional electrode substrates and large overpotentials (~+0.8 to +1 V vs. Ag/AgCl) are often needed to extract a quantifiable signal [12].

The system can be further refined and lower limits of detection achieved however through employing substrate recycling. In previous catalytic protocols—sample orthophosphate was implicated in the mediation of the AP enzyme activity and the hydrolysed orthophosphate was not considered in any great detail beyond contributing to the sustained enzymic inhibition. The hydrolysed alcoholic ester was the chief reagent upon which re-cycling was targeted. In contrats, the NP system, Fig. 7(B), focuses on recycling the hydrolysed orthophosphate through the addition of AP to the native NP/XOD system [60,64]. Thus, phosphate itself is recycled to act as a substrate again, and in an excess of inosine, for every phosphate molecule, a number of hypoxanthine molecules are produced to go into the xanthine oxidase catalysed reaction. This leads to amplification of the response to phosphate to provide a limit of detection in the sub ppb (nanomolar) range. The analytical signal arising from the NP system is directly proportional to the concentration of phosphate present and a increasing (positive) signal is always preferable to a decrease which could be attributed to factors other than the increase in phosphate (i.e. heavy metal ions).

5. Future directions

The search for ever more sensitive enzymatic sensing systems is one of the most important theme for biosensing in recent years. The possibility of substrate recycling lifts the biosensing approach beyond direct detection methods and offers a sensitivity in the nanomolar range. Such systems invariably require a complex interplay of enzymes—the precursor to which was discussed in the previous section (Fig. 7(B)). A more recent and elaborate system has incorporated maltose phosphorylase (MP)/mutarotase (MR)/AP/GOX enzymes [65–67] and the reaction pathway is shown in Fig. 8. In an excess of maltose, the introduction of phosphate activated MP cleaves the disaccharide whilst also phosphorylating one of the glucose moieties. As maltose is linked through an α1-4 linkage, the unphosphorylated glucose anomer will be in the alpha form. Mutarotase is added to convert between alpha and beta form as only the latter is an acceptable substrate for GOX. The inclusion of AP enables the release of a further glucose molecule but also effectively recycles the orthophosphate and effectively sustains MP activity. A catalytic cycle emerges that, although complex, has proven to be very effective for low level phosphate detection resulting in a limit of detection in the ppb (10^{-8} M) range [65–67] which is more than sufficient to probe endogenous phosphate concentrations within inland riverways.

The basic mechanistic implications of incorporating biological catalysts within an electroanalytical framework targeted at phosphate detection have been highlighted. A summary of the various approaches that have been taken in recent years to facilitate the detection of phosphate is provided in Table 1 and compares and contrast the different electrochemical assemblies with spectroscopic methods. It is clear that conventional direct electrochemical procedures are unlikely to reach the concentrations necessary for onsite testing. It must be acknowledged however that most of the laboratory-based systems themselves can only achieve the sub-ppm detection limits through employing sample separation techniques such as IC [68–70], FIA [71–73] or electrophoresis [74–76]. Very few of these approaches are at present readily trans-

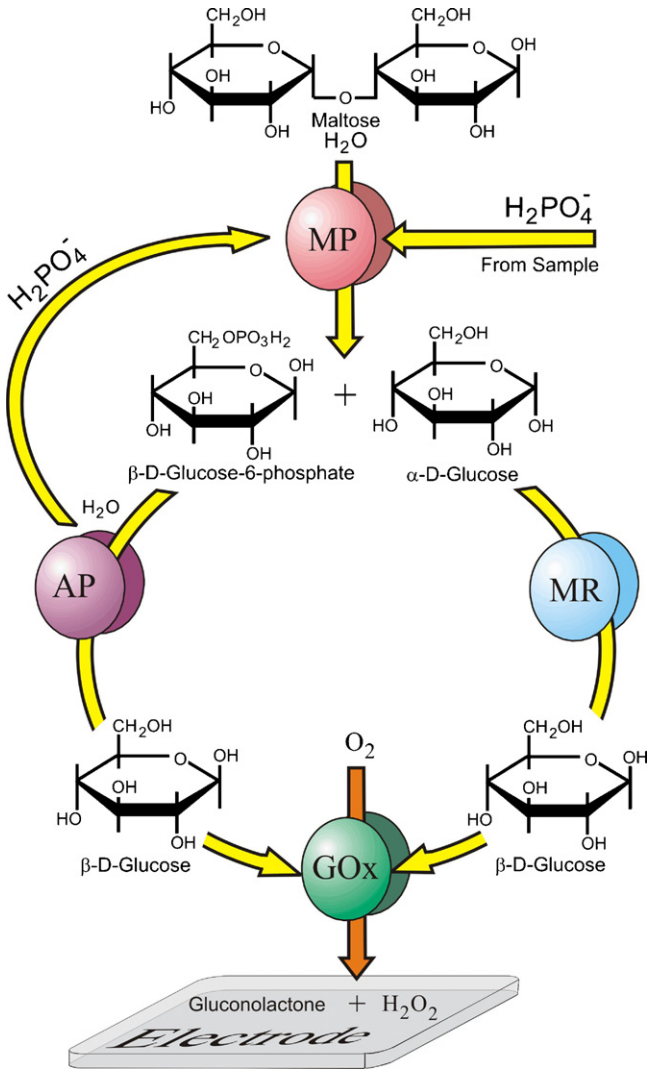

Fig. 8. Maltose phosphorylase (MP)/mutarotase (MR)/glucose oxidase (GOx)/alkaline phosphatase (AP) catalytic cycle for the trace detection of phosphate ion [65–67].

ferable to field testing nor for operation by inexpert staff. It must be acknowledged however that microfluidic devices for phosphate are beginning to emerge [84–86] which can incorporate and exploit many of the earlier electrochemical and photometric strategies (i.e. such as those based on the molybdate complexation).

While it could be argued that it is only necessary to identify gross abuses and that simpler technologies could be adopted, it is nevertheless important to provide nutrient profiles such that trends in the biogeochemical balance, and not just anthropogenic inputs, for a particular area can be established. This would require reaching the low ppb limits found within water untainted by agricultural, domestic or industrial discharge. The amplification routes available to the biological systems could comfortably operate within these trace detection limits and are comparable to the more sensitive fluorescent techniques [77–79] whilst possessing a more inherent capability for field implementation. Phosphate enzyme systems are widely exploited in biomedical research and it should be possible to transfer the technology to environmental contexts with relative ease. The matrices are, from an electrochemical sensing viewpoint, simpler as they possess fewer sources of electroactive interference. The multi-enzyme assemblies are however complex and beyond simple *ad hoc* fabrication. Given that we are concerned with the development of on-site screening technologies, it could anticipate that large numbers of identical sensors based on established screen print (SPE) manufacturing could be economically produced. The single shot disposability of SPE platforms [12,13] would appear to be ideal and while accepting some procedural variation, the operational characteristics are consistent with the current portable meters used within biomedical applications. The review has attempted to highlight a range of approaches and there are ample opportunities to further refine and optimise the systems in the pursuit of ever lower detection limits.

Table 1
Analytical characteristics of common phosphate detection methodologies.

Methodology	Reagents	LOD (μM)	Refs.
FIA-P	Cobalt electrode	100	[19]
ISE	Molybdate complex	0.06	[26]
Amperometric	Molybdate complex	0.3	[36]
Amperometric	POD/O_2	1	[41]
Amperometric	POD/H_2O_2	3.6	[42]
Amperometric	NP, XOD, AP	0.01	[64]
Amperometric	MP, MR, GOX, AP	0.01	[65–67]
Ion chromatography	N/A	0.1	[68–80]
Capillary electrophoresis	N/A	0.1	[74–76]
Luminescent plate	Europium–tetracycline	3	[77]
Fluorescent probe	NP, XOD, HRP	0.05	[78]
Fluorescent PVC matrix	Al-morin ionophore	0.2	[79]

NP: nucleoside phosphorylase; XOD: xanthine oxidase; HRP: horseradish peroxidase; POD: pyruvate oxidase; MP: maltose phosphorylase; MR: mutarotase; GOX: glucose oxidase; AP: alkaline phosphatase; FIA-P: flow injection potentiometric analysis; ISE: ion selective electrode; N/S: not specified.

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