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Polypyrrole based amperometric and potentiometric phosphate biosensors: A comparative study B

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ABSTRACT

The preparation of two electrochemical (potentiometric and amperometric) phosphate biosensors is described and compared. Purine nucleoside phosphorylase (PNP) and xanthine oxidase (XOD) were co-immobilized via entrapment into polypyrrole (PPy) films by galvanostatic polymerization. Polypyrrole entrapment was achieved with 0.5 M pyrrole by using a polymerization time of 200 s and a mole ratio of 1:8 (6.2 U/mL XOD: 49.6 U/mL PNP) in amperometric phosphate biosensor. Potentiometric bi-layer biosensor PPy–NO_3/BSA–GLA–PNP–XOD is made of an inner electropolymerized PPy–NO_3 layer and an outer layer of PNP and XOD cross-linked with a mixture of bovine serum albumen (BSA) and gultaraldehyde (GLA).The optimum conditions for potentiometric bi-layer biosensor include a polymerization time of 300 s for the inner layer at an applied current density of 0.25 mA cm^{-2}, a drying time of 30 min for the outer layer, pH 7, and 0.025 MTris–HCl. Sensitive amperometric measurements obtained from PPy–PNP–XOD–$Fe(CN)_6^{4-}$ biosensors were compared with those of potentiometric measurements obtained from PPy–NO_3/BSA/GLA–PNP–XOD bi-layer biosensor. A minimum detectable concentration of 20.0 μM phosphates and a linear concentration range of 20–200 μM were achieved with potentiometric PPy–NO_3/BSA/GLA–PNP–XOD biosensor. In comparison, a minimum detectable concentration of 10 μM and a linear concentration range of 0.1–1 mM were achieved with amperometric biosensor. The presence of uric and ascorbic acids had the least effect on the performance of the PPy–PNP–XOD–$Fe(CN)_6^{4-}$ amperometric and PPy–NO_3/BSA/GLA–PNP–XOD potentiometric bi-biosensors, therefore, they will not have any effect on phosphate measurement in both biosensors at levels normally present in water. PPy–NO_3/BSA–GLA–PNP–XOD potentiometric biosensor was used to analyse phosphate in real samples.

1. Introduction

Large quantities of phosphate have been used for detergents and for the treatment of boiler waters to prevent scale formation, therefore, it has become necessary to determine micro/trace amounts of phosphates in waters, such as drinking waters, natural waters, waste waters and polluted waters that are discharged from various sources. High phosphate concentration can pollute water resources and causes eutrophication of lakes and rivers (Nakamura et al., 2010). The eutrophication of water by phosphate can lead to over-growth of plants and toxic algae, thereby making it unsuitable for drinking or industrial use and also making water ways inaccessible (Keup, 1968). In semiconductor industries, phosphorus existing at trace/ultratrace amounts in the water can damage the quality of the semiconductors, and therefore the amounts must be lowered as much as possible. There has been a growing demand for highly sensitive, accurate and rapid determination, as well as simple and onsite analysis of phosphate (Motomisu and Li, 2005).

Phosphate determination is also important in clinical diagnosis; the determination of phosphate in body fluid provides useful informat, ion about certain diseases and about the energetic state of cells and bone function (Kivlehan et al., 2009; Shervedani and Pourbeyram, 2009).

Optical instrumental methods are sensitive and mainly used for the laboratory determination of phosphate and they have detection limit between 20 and 150 nM. Molybdate/rodamine fluorescence method gave a detection limit of 20 nM, but they are laborious and 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.

Phosphate determination based on spectroscopy (Galhardo and Masini, 2000; Mckelvie, 2000; Li et al., 2002; Nakamura et al., 2004; Yaqoob et al., 2004; Lin et al., 2006; Okoh et al., 2006; Gimbert et al., 2007; Nevesa et al., 2008; Yaqoob et al., 2008) and chromatographic techniques (Galceran et al., 1993; Bello and González, 1996;

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Colina et al., 1996; Zhu et al., 2008) were the analytical techniques commonly used for phosphate site monitoring. Ion selective electrodes were also used for phosphate determination, based on various metals and associated complexes such as Sn-complexes (Chaniotakis et al., 1993; Sasaki et al., 2004), hydroxyapatite (Petrucelli et al., 1996) and cobalt metallic wires (Chen et al., 1997, 1998; De Marco and Phan, 2003; Gimbert et al., 2007; Bai et al., 2011). Other methods used for determination of phosphate included flow injection systems based on luminescence (Kyung et al., 2009; Andolina and Morrow, 2010; Cardemil et al., 2011), chemiluminescence (Kawasaki et al., 1989; Nakamura et al., 1999a,b, 2003; Yaqoob et al., 2004; Motomisu and Li, 2005), florescence reactions (Motomisu and Li, 2005; Wang et al., 2010; Kim et al., 2011), conductometry (Zhang et al., 2008) and screen printed electrodes (Kwan et al., 2005; Tanimoto de Albuquerque and Ferreira, 2007; Zou et al., 2007; Khaled et al., 2008).

An alternative to time-consuming and laborious phosphate ion determination using classical methods (Colina et al., 1996; Bello and Gonzallez, 1999; Mckelvie, 2000) is the use of a conducting polymer biosensor, which is able to measure the substrate directly in the sample (D'Urso and Coulet, 1993; Cosnier and Gondran, 1998; Roger et al., 2003, 2005; Adeloju and Lawal, 2005, 2011; Rahman et al., 2006; Akyilmaz and Yorganci, 2007; Barsan and Bratt, 2008; Lawal and Adeloju, 2012). With the advent of enzyme-based biosensors, several approaches have been investigated for detecting phosphate ion (Haemmerli et al., 1990; Conrath et al., 1995; Menzel et al., 1995; Roger et al., 2003; Akyilmaz and Yorganci, 2007; Adeloju and Lawal, 2011). A simple alternative is the use of an enzyme sensor that is able to measure phosphate directly in the sample. Enzyme sensors have been developed based on enzymatic sequences in which a first enzyme (usually a phosphorylase) uses phosphate as a co-substrate giving a product that is the substrate for a second enzyme, usually an oxidase. Among these enzyme sensors are phosphate biosensors that use, as biorecognition elements, substances such as nucleoside phosphorylase and xanthine oxidase (D'Urso et al., 1990; Male and Luong, 1991; Wollenberger et al., 1992; D'Urso and Coulet, 1993; Su and Mascini, 1995; Chen et al., 1997; Tzanavaras and Themelis, 2002; Vazquez et al., 2003) and most commonly used alkaline phosphatase. Others are acid phosphatase (Guilbault and Nanjo, 1975; Guilbault, 1984), glucose oxidase (Su and Mascini, 1995; Zhiqiang et al., 2008), pyruvate oxidase (Mori et al., 1994; Ikebukuro et al., 1996; Mak et al., 2003; Roger et al., 2005; Rahman et al., 2006; Akyilmaz and Yorganci, 2007), sucrose phosphorylase, phosphoglucomutase and glucose 6-phosphate dehydrogenase. Combination of maltose phosphorylase (MP), mutarotase (MR) and glucose oxidase (GO*x*) have been used for fabrication of phosphate biosensors (Mousty et al., 2001; Zhiqiang et al., 2008).

Various enzyme immobilization methods, such as adsorption, covalent bonding, entrapment and cross-linking, have been used to immobilize the above enzymes. Of these, the use of cross-linking is favored by many researchers due to the simplicity it offers for direct immobilization of relevant phosphate enzymes onto different electrodes (Guilbault, 1984; Watanabe et al., 1987; Watanabe et al., 1988; D'Urso et al., 1990; D'Urso and Coulet, 1993; Konishita et al., 1995). Some specific examples of phosphate biosensors fabricated by cross-linking of enzymes include the use of gultaraldehyde (GLA) with or without bovine serum albumin (BSA) to immobilize xanthine oxidase (XOD) and purine nucleoside phosphorylase (PNP) on nylon, teflon membrane and cellulose acetate membrane (Watanabe et al., 1988; Konishita et al., 1995). Lawal and Adeloju reported a study on the use of this chemical cross-linking method with polypyrrole (PPy) films in a bi-layer arrangement for the development of a phosphate biosensor and they successfully immobilized XOD and PNP into polypyrrole films (Lawal and Adeloju, 2009a, 2009b, 2010).

Hybrid designs with two or more polymer layers (i.e. PPy or overoxidized PPy and BSA–GLA) offer a remarkable solution for interferant rejection (Patano and Kuhr, 1995; Adeloju and Lawal, 2011). When used with a BSA–GLA matrix, the permselective qualities of a multilayer configuration are coupled with the high enzyme loading and long-term stability of cross-linking with BSA and GLA (Guerrieri et al., 1998). Unlike, monolayer arrangements (Bartlett et al., 1992; Patano and Kuhr, 1995; Guerrieri et al., 1998), a hybrid arrangement can improve the long-term stability and improve the sensitivity and selectivity of the biosensor.

An amperometric biosensor requires three electrodes and application of potential before measurable current can be obtained. The use of enzyme–based amperometric biosensor has increased considerably in the past 10 years as a result of its high selectivity and the sensitivity of amperometric signal (Roger et al., 2003; Tanimoto de Albuquerque and Ferreira, 2007; Lawal and Adeloju, 2009b). There are few potentiometric biosensors that require simple construction of two electrodes and without the application of potentials for measurable potential signal to be generated (Adeloju and Lawal, 2005, 2011; Lawal and Adeloju, 2009b). Amperometric sensing can introduce interference as a result of oxidation of other matrix components which can lead to erroneous and enhanced current signal. However, in potentiometric sensing, oxidation of other matrix components is avoided Lawal and Adeloju (2012) and Villalba et al. (2009) recently reviewed the advantages and disadvantages of the electrochemical biosensors for the determination of phosphate.

The PNP–XOD bienzyme system employed recently in various studies (Cosnier and Gondran, 1998; Adeloju and Lawal, 2005, 2011; Lawal and Adeloju, 2009a, 2009b) showed that a higher amount of hypoxanthine was produced during enzymatic phosphate recycling. Enzymatic phosphate recycling also took place using MP/GOX/Ap trienzyme for low-level phosphate detection (Conrath et al., 1995; Huwel et al., 1997; Mousty et al., 2001). Wollenberger et al. (1992) employed amplification by enzymatic substrate recycling in order to lower the detection limit, involving co-immobilization of alkaline phosphatase (aP) and glucose oxidase. In the presence of phosphate ion, inosine was phosphorylated by PNP to ribose-1-phosphate. Phosphate was then liberated by aP catalysis and became available again for inosine phosphorylation. Phosphate was thus recycled between aP and PNP while a higher amount of hypoxanthine was produced and recognized by sequential oxidation by XOD.

Hypoxanthine was subsequently oxidized to H_2O_2, catalysed by XOD (Watanabe et al., 1987; D'Urso et al., 1990; Wollenberger et al., 1992; D'Urso and Coulet, 1993), as given

$$\text{Inosine}+\text{orthophosphate}\xrightarrow{\text{PNP}}\text{ribose}-1-\text{phosphate}+\text{hypoxanthine} \tag{1}$$

and

$$\text{Hypoxanthine}+2\text{H}_2\text{O}+2\text{O}_2\xrightarrow{\text{XOD}}\text{uric acid}+2\text{H}_2\text{O}_2 \tag{2}$$

In this study, biosensors were developed based on the enzymatic reaction shown in Eqs. (1) and (2). We also compare the fabrication of a novel potentiometric bi-layer biosensor for phosphate by the use of a composite bi-layer arrangement, as PPy–NO_3/BSA–GLA–PNP–XOD, consisting of an inner electropolymerized PPy–NO_3 layer and an outer layer of PNP and XOD cross-linked with a mixture of BSA and GLA with PPy–PNP–XOD–$Fe(CN)_6^{4-}$ amperometric biosensor. The PPy–NO_3/BSA–GLA–PNP–XOD biosensor combines the advantages of cross-link immobilization, such as high enzyme loading and long-term stability of the enzymes, with the excellent interferant rejection of electrosynthesized polypyrrole film. Important considerations in the development of the PPy–NO_3/BSA–GLA–PNP–XOD biosensor include influence of drying time, PNP: XOD