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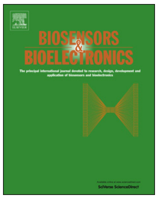

Conductance based sensing and analysis of soluble phosphates in wastewater

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ABSTRACT

The current standard method used for measuring soluble phosphate in environmental water samples is based on a colourimetric approach, developed in the early 1960s. In order to provide an alternative, label free sensing solution, a molecularly imprinted polymer (MIP) was designed to function as a phosphate receptor. A combination of functional monomer (*N*-allylthiourea), cross-linker and monomer/template ratios were optimised in order to maximise the binding capacity for phosphate. When produced in membrane format, the MIP's ability to produce a reversible change in conductance in the presence of phosphate was explored for fabrication of a sensor which was able to selectively detect the presence of phosphate compared to sulphate, nitrate and chloride. In wastewater samples the sensor had a limit of detection of 0.16 mg P/l, and a linear range between 0.66 and 8 mg P/l. This is below the minimum monitoring level (1 mg P/l) as required by current legislation for wastewater discharges, making the sensor as developed promising for direct quantification of phosphate in environmental monitoring applications.

1. Introduction

Excess concentrations of phosphate in water can result in increased algal growth, eutrophication and reduced water quality. Phosphate levels in wastewater are regulated by the Urban Waste Water Treatment Directive from the EU, which specifies maximum annual mean total phosphorus (P) concentrations of 1–2 mg/l (EU Council Directive, 1991). Furthermore, monitoring nutrient concentrations in environmental waters is becoming increasingly important, with the need to reach good ecological and chemical status by 2015, as stated in the Water Framework Directive (EU Council Directive, 2000).

The current standard method for measuring soluble phosphate in water samples is a colourimetric technique (Murphy and Riley, 1962). Handheld instruments, field based continuous monitoring analysers and off site laboratories all use a colourimetric approach. However, all of these methods require continual use of specific reagents and consumables, which incur significant costs to regulatory bodies and industries that are required, by legislation, to monitor phosphate levels on a large scale.

This legislation, specifically the Water Framework Directive (EU Council Directive, 2000), is driving the need for an alternative phosphate monitoring technique that is both direct and label free, which could, ideally, be used to provide remote, continuous monitoring, in real time.

In order to be a viable alternative to the current method, a sensing based approach would require the ability to measure concentrations at least at the minimum levels (1 mg P/l) required by the Urban Waste Water Treatment Directive (EU Council Directive, 1991). In addition, it should also demonstrate specificity for phosphate without interference from other ions, offer long term stability in field conditions and have low, infrequent maintenance requirements.

A variety of electrochemical sensing techniques have been investigated to detect orthophosphate ions in aqueous solution, summarised in reviews by Midgley (1986), Engblom (1998), Villalba et al. (2009) and Warwick et al. (2013). However, sensors capable of measuring the range of P concentrations (0.1–15 mg/l) typically found in wastewater, either suffer from limited durability, poor selectivity, or both (Ganjali et al., 2006; Kumar et al., 2008; Kumar et al., 2010; Tafesse and Enemchukwu, 2011; Modi et al., 2011). Selectivity issues and doubts regarding suitability for field use with environmental samples are also found with fluorescent based, optical sensors (Beer and Cadman, 1999; Ojida et al., 2002; Ojida et al., 2004; Hamachi, 2009).

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Obstacles to the development of a selective, sensing based solution are the presence of charged oxygen atoms, which obscure the central phosphate atom, and the size and shape of the phosphate anion compared to interfering oxyanions e.g. sulphate (Katayev et al., 2006).

In order to overcome these selectivity issues, molecularly imprinted polymer (MIP) based receptors have been developed which are able to selectively bind phosphate. For example, the ability to bind and remove phosphates from river water samples was demonstrated using a MIP (Fig. 1) which exploited the affinity and selectivity of thiourea-based functional monomers for phosphate moieties (Kugimiya and Takei, 2006, 2008). However, neither this, nor other MIP based receptors that bind phosphate groups, have been integrated with a transducer to produce a sensor capable of measuring phosphate in wastewater samples (Sasaki et al., 1998; Wulff et al., 2006; Emgenbroich et al., 2008 and Cutivet et al., 2009).

MIP design and functional development is subject to a number of variables and constraints. The interaction between the functional monomers and the template used to generate the binding site, together with their relative ratios, have a key influence on the quality and quantity of the recognition sites in the final MIP structure (Nicholls et al., 2001). Non-imprinted polymers (NIPs) are used as controls to compare non-specific binding with the template specific binding exhibited by MIPs. To achieve this, NIPs use identical functional monomers and cross-linkers as MIPs, but are synthesised without the template, thus producing no template specific cavities (Murray and Örmeci, 2012). Hence, the binding of phosphate results from the collaborative effect of at least 2 monomers, as depicted in Fig. 1. This orientation effect will be difficult to mimic in the absence of template i.e., in the NIP, due the random distribution of monomer in the final polymer.

Furthermore, in order to allow for repeated measurement cycles in a multi-use sensor format, polymer stability is an important factor. The stability of the binding cavity and that of the whole polymer network is directly influenced by the type/composition of the cross-linking agent and its proportion to porogenic solvent (Alvarez-Lorenzo and Concheiro, 2004). For example, increased flexibility of a MIP based membrane was produced by adding oligourethane acrylate (OUA) to the cross-linking monomer solution-triethylene glycol dimethacrylate (TEDMA) and produced the optimum response using OUA and TEDMA in a ratio of 15:85 (Sergeyeva et al., 1999).

MIP based sensors have been integrated with a range of electrochemical transducers (Piletsky and Turner, 2002). A number of MIP based sensors have used conductance based transduction methods, induced by conformational changes in the cavity produced when a MIP's receptor elements have bound their specific target (Piletsky et al., 1995; Sergeyeva et al., 1999; Suedee et al., 2006 and Vishnuvardhan et al., 2011). Furthermore, conductance measurements are relatively easy to perform, are already used in environmental monitoring and applicable for continuous measurement in wastewater treatment works (Levlin, 2010).

The aim of this work was to optimise the design of a MIP based on thiourea as functional monomer, and to integrate these optimised MIP receptors within a flexible membrane. Changes in membrane conductance upon phosphate binding were then exploited in order to develop a sensor, which was used to quantify various concentrations of phosphate in laboratory and wastewater samples.

2. Materials and methods

2.1. Materials

Cross-linking reagents diethyleneglycol diacrylate (DEGDA), ethylene glycol dimethacrylate (EGDMA), tetra(ethylene glycol) diacrylate (TTEGDA), N,N′-methylenebis(acrylamide), trimethylolpropane trimethacrylate (TRIM), initiator 1,1′-azobis-cyclohexanecarbonitrile, the imprinted template phenylphosphonic acid and the functional monomer N-allylthiourea (thiourea) were supplied by Sigma-Aldrich (UK). Acetonitrile (ACN), sodium sulphate and potassium chloride were obtained from Fisher Scientific (UK). Methanol, potassium dihydrogen phosphate and potassium nitrate

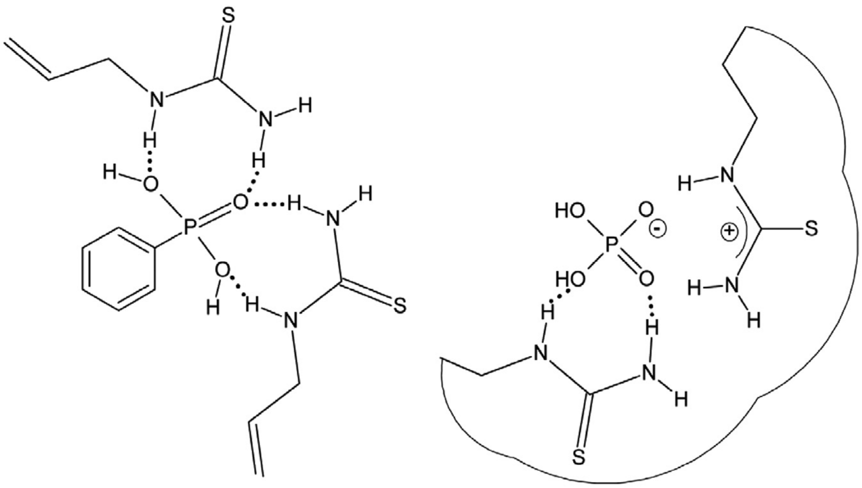

Fig. 1. Diagrammatic representation of the pre-polymerisation complex comprising thiourea based functional monomers with a phenylphosphonic acid template (on the left) used to construct a polymer cavity capable of rebinding phosphate (on the right) within a MIP (adapted from Warwick et al. (2013)). Phosphate binding occurs via a combination of hydrogen bonding (dotted lines) and ionic interactions.