

## 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 - 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.

Keywords: Phosphate, Sensor, MIP, Thiourea, Conductance, Wastewater

## **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 to 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).

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 (Figure 1) which exploited the affinity and selectivity of thiourea-based functional monomers for phosphate moieties (Kugimiya and Takei,

2006; Kugimiya and Takei, 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).

(Insert Figure 1 here)

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 synthesized 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 Figure 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 were purchased from BDH Laboratory Supplies (VWR, UK). Dimethylformamide (DMF) was supplied by Acros Organics (Belgium).

Oligourethane acrylate (OUA) was donated by the Institute of Macromolecular Chemistry (Ukraine) and synthesized according to Spirin *et al.* (1968) and German patent 1085671 (1961).

## **2.2 Equipment and Instrumentation**

The UV source used for polymerisation was a Honle UVAPRINT 100 CV1. Polymers were ground using a Retsch ZM200 grinder or mortar and pestle.

The conductance in the conductometric experiments was determined by recording the resistance (inverse of conductance) with an ohmmeter provided by Tektronix (model DMM914). Platinum electrodes were purchased from BASi (USA).

## **2.3 Synthesis and Optimisation of Phosphate Binding Receptors**

### **2.3.1 MIP Synthesis**

The MIP synthesis combined 4.00 g of cross-linker, 0.37 g phenylphosphonic acid template, *N*-allylthiourea functional monomer, (amount varied from 0.27 g to 1.08 g, depending on the molar ratio between functional monomer and template), 4.54 g of ACN solvent and 9.00 mg of 1,1'-azobis-cyclohexanecarbonitrile initiator together in a glass vial. After adding the initiator, nitrogen was bubbled into the vial for 2 minutes, thus preventing oxygen from reacting with the free radicals and inhibiting polymerisation. The vials were then exposed to UV light for 20 minutes to enable

polymerisation. In addition to preparing MIPs, non-imprinted polymer (NIPs) controls were prepared, which omitted the template.

The resulting polymers were ground and sieved through both 90  $\mu\text{m}$  and 38  $\mu\text{m}$  sieves. The polymers were purified and cleansed with methanol, using overnight Soxhlet extraction, in order to remove the template, plus any non reacted cross-linker or monomer.

### 2.3.2 Analysis of Phosphate Retention of MIPs and NIPs

Equal dry weights of each polymer (MIP and NIP) were washed with deionised water, mixed for one hour, centrifuged at 1730 x  $g$  for one minute and the supernatant removed. Phosphate retention by the MIPs (and NIPs) was then tested by adding 2 ml of 25  $\mu\text{M}$  (0.77 mg P/l) potassium dihydrogen orthophosphate (pH 7.2) for every 40 mg sample of polymer present, followed by continuous stirring and incubation for one hour at room temperature (Kugimiya and Takei, 2006).

The samples were centrifuged at 1730 x  $g$  and the concentration of phosphate in the supernatant was assessed colourimetrically using the phosphomolybdenum blue colourimetric method (Environment Agency, 1992).

### 2.3.3 Optimisation - Determining the Optimum Cross-Linking Agents

Thiourea based MIPs were prepared with a variety of alternative cross-linkers, in order to establish which cross-linking agent would provide the greatest degree of phosphate binding. The solvent used on each occasion was ACN, except when the cross-linker bisacrylamide was investigated, which required a different solvent (DMF), and for solubility reasons only 1 g was used. The additional cross-linkers

used included EGDMA, TTEGDA, TRIM and bisacrylamide. The molar ratio of functional monomer:template used was 4:1 (Kugimiya and Takei, 2006).

#### **2.3.4 Optimisation - Determining the Optimum Ratio of Monomer to Template**

Once the optimum cross-linker had been established, different ratios of the phenylphosphonic acid template and thiourea monomer were used to find the optimum ratio for maximal phosphate binding capacity. In order to obtain these different ratios between template and monomer, the concentration of template was kept constant and the concentration of monomer changed to represent molar ratios of 4:1, 2:1 and 1:1.

### **2.4 Integration of MIP Receptors with Transducer**

#### **2.4.1 Preparation of Thiourea Based MIP Receptor Membranes**

Membranes incorporating the thiourea based MIP were produced using a combination of cross-linking monomers (EGDMA and OUA) in order to provide increased membrane flexibility and mechanical stability. These cross-linkers have previously been blended in a ratio 85:15 to give the optimum conductometric response (Sergeyeva *et al.*, 1999).

Thiourea monomer (0.59 g), EGDMA (4.00 g), OUA (0.71 g) cross-linkers and the phenylphosphonic acid template (0.40 g) were combined and mixed with 2.44 g DMF solvent. 1,1'-azobis-cyclohexanecarbonitrile initiator was then added (0.07 g) to the glass vial, which was then flushed with nitrogen to remove oxygen, preventing oxygen from reacting with the free radicals and inhibiting polymerisation. Amount of DMF was optimised to reduce shrinkage and improve mechanical properties of membranes.



The polymerisation mixture was pipetted between two glass plates (8 cm x 10 cm) with a silicone spacer on the perimeter (0.7 mm thickness). The filled slides were subjected to UV irradiation for two and a half minutes on each side. The polymer membranes were cleansed with methanol, using overnight Soxhlet extraction and then dried and rehydrated in deionised water overnight.

The membrane was then inserted into a conductance cell (Figure 2), sandwiched between two sample wells, with a silicone spacer on either side, and held in position with compression. 1.5 ml potassium chloride electrolyte (1.24 g/l) was added to each well, as described previously in UK Patent Application GB1307777.1 (2013).

#### 2.4.2 Measuring the Change in Conductance across a Membrane

Platinum electrodes were placed in the electrolyte, a fixed distance apart, positioned either side of the membrane and connected to an ohmmeter (Figure 2). The resistance (inverse of conductance) across the membrane in the potassium chloride electrolyte (1.24 g/l) was recorded until equilibrium was achieved. 0.5 ml of either potassium phosphate (concentrations ranging from 0.13 to 136 mg/l), equivalent to 0.03 to 31.00 mg P/l, sodium sulphate (96.10 mg/l) or potassium nitrate (62.00 mg/l) was subsequently added to the conductivity cell and the change in conductance recorded every minute. Measurements were repeated in triplicate.

(Insert Figure 2 here)

#### 2.4.3 Measuring the Change in Conductance using Field Samples

A settled wastewater sample from a 570,000 population equivalent wastewater treatment plant in the UK (pH 6.88), with an orthophosphate concentration of 3.29 mg P/l was filtered with a 1.20 µm filter. The filtered sample was then either diluted

with deionised water, or spiked with quantities of potassium phosphate solution (310.00 mg/l) to generate phosphate standards, in a field sample matrix, equivalent to 0.16, 0.33, 0.66, 1.65, 3.29, 4.50, 6.00 and 8.00 mg P/l.

### **3. Results and Discussion**

#### **3.1 Assessment of Potential Phosphate Binding Receptors**

The MIPs were synthesised using phenylphosphonic acid as the template, rather than the actual targets (hydrogen phosphate and dihydrogen phosphate), to overcome solubility issues with the organic reagents used in MIP synthesis.

The analysis of the phosphate content of the supernatants from MIPs (and NIPs) prepared with thiourea based functional monomers and various cross-linking agents, showed that all of the MIPs retained more phosphate than their equivalent NIPs (Figure 3) confirming the findings of Kugimiya and Takei (2006). Phosphate is a small template for imprinting, consequently the MIP will have a small binding cavity, for this a short cross-linker is probably more appropriate, as it will provide enhanced rigidity to the polymeric network around the binding cavity, helping maintain its tri-dimensional structure. Accordingly, of the cross-linkers examined, the shorter ones (e.g. EGDMA and bisacrylamide) were expected to provide a more rigid structure and maintain the orientation of the monomers in the binding cavity, providing a greater contrast in phosphate retention between MIP and NIP. This was demonstrated experimentally in Figure 3. The P content of the supernatants from the MIP and NIP synthesised with the bisacrylamide cross-linker were 0.14 mg/l (0.03 mg P/g polymer) and 0.36 mg/l (0.02 mg P/g polymer) respectively, a difference of 0.22 mg/l. The P content of the supernatant from the MIP created with the EGDMA

cross-linker was 0.09 mg/l (0.03 mg P/g polymer) compared to 0.41 mg/l (0.02 mg P/g polymer) for the supernatant from the NIP, a difference of 0.32 mg/l. Consequently, of all the cross-linkers tested, EGDMA gave the best overall contrast between MIP and NIP and also the higher capacity for phosphate.

(Insert Figure 3 here)

A typical ratio of monomer to template in non-covalent methods is 4:1 (Cormack and Elorza, 2004). Experiments using variable ratios (4:1, 2:1 and 1:1) of monomer (thiourea) to template (phenylphosphonic acid) produced a wide variety of phosphate retention (Figure 3) since the presence of the template guides the orientation of the monomers during the polymerisation process. As demonstrated in Figure 3, the optimum ratio of monomer to template, yielding the greatest contrast in P retention, between MIP and NIP, was 2:1, where the supernatant from the MIP contained 0.13 mg/l and the NIP 0.46 mg/l.

This ratio of 2:1 gave a much better contrast in binding activity between MIP and NIP than the 4:1 ratio used in the original research (Kugimiya and Takei, 2006), suggesting that increased phosphate uptake from solution was due to the imprinting effect and not just to the random distribution of functional monomer in the polymer network.

### **3.2 Conductometric Analysis**

The conductance cell used platinum electrodes, kept a fixed distance apart throughout the analysis. Conductance measurements are typically performed using alternating current (AC), however direct current (DC) can also be used (Janata,

1989) and was used throughout as it allowed for a better sensor resolution, as compared with AC.

Thiourea based MIP receptors, embedded within the membrane for conductometric analysis, produced a range of responses depending on the content and concentration of the sample applied. Figures 4 to 6 depict the mean change in conductance, from just before adding the test solution (at  $t=0$ ), until a period six minutes after the addition of the test sample. The change in membrane conductivity can be attributed to conformational changes in the polymer upon template binding, which together with the presence of charged ions in the polymer matrix lead to a consequent increase in conductivity of the membrane (Piletsky *et al.*, 1995; Sergeyeva *et al.*, 1999; Suedee *et al.*, 2006 and Vishnuvardhan *et al.*, 2011).

The concentration of the potassium chloride electrolyte was kept constant (35 mM) throughout the experiments as previous research had determined that the response of a MIP based conductance sensor decreased with concentrations of sodium chloride electrolyte in excess of 35 mM (Sergeyeva *et al.*, 1999).

### 3.2.1 Phosphate Measurement using Laboratory Samples

The experiments using laboratory samples with differing phosphate content were designed to assess if there was a relationship between the change in conductance produced with different concentrations of P (3.1 to 0.03 mg/l). The responses generated by laboratory solutions containing 0.31 and 3.1 mg/l were easily reproduced (Figure 4), hence the low standard deviations achieved (maximum 0.14 nS). Samples containing 0.31 mg/l could be distinguished from both stronger and weaker concentrations of P after two or three minutes, while samples containing 0.16

mg/l could not be distinguished from the lowest concentration used (0.03 mg/l) due to the comparatively large standard deviation.

(Insert Figure 4 here)

### 3.2.2 Selectivity

The mean change in conductance, produced by the thiourea based MIP receptors, on the addition of 1 mM phosphate solution (31 mg P/l) produced a much greater response (Figure 4) than that generated by equimolar (1 mM) solutions of either nitrate (62 mg/l) or sulphate (96.1 mg/l), confirming the selectivity for phosphate, in contrast to the competing anions. For example, after one minute, the addition of phosphate gave a mean change in conductance of 6.4 nS while the presence of nitrate produced a mean change in conductance of 0.7 nS and the sulphate produced a mean change of only 0.2 nS.

Furthermore, the experiments were being performed in relatively strong concentrations of chloride (1.24 g/l), far in excess of that typically found (30-90 mg/l) in untreated wastewater (Tchobanoglous, *et al.*, 2003). It can therefore be argued that chloride would not interfere with the response using wastewater samples.

### 3.2.3 Phosphate Measurement using Wastewater Samples

Analysis using filtered wastewater samples, diluted with deionised water, or spiked with phosphate solution, produced increased changes in conductance with concentration (Figure 5). There was little variability in the signal produced for each concentration, apart from the wastewater solution spiked to 4.5 mg P/l. The magnitude of the signals produced by the wastewater samples were greater than the corresponding response exhibited by the laboratory samples. For example, the

signal generated by the wastewater sample containing 3.29 mg P/l gave a signal of 7 nS, over three times that of the 2 nS signal generated by the lab based sample containing 3.1 mg P/l.

The limit of quantification (LOQ), defined as the lowest measurement recorded that is over ten times the standard deviation of the blank used (deionised RO water) in the preparation of the standards (MacDougall *et al.*, 1980) was 0.66 mg P/l. The signals produced after two minutes were used to produce a calibration curve (Figure 5).

(Insert Figure 5 here)

The limit of detection (LOD), the minimum measured value that exceeds three standard deviations of the blank used to prepare the standards (MacDougall *et al.*, 1980), was 0.16 mg P/l (Figure 6).

(Insert Figure 6 here)

The LOQ (0.66 mg P/l) and LOD (0.16 mg P/l) exhibited by the sensor are currently a little high considering that the concentration of P typically found in wastewater ranges from 0.1 to 15 mg/l. However, the Urban Waste Water Treatment Directive from the EU, specifies maximum annual mean total phosphorus concentrations of 1 or 2 mg/l, depending on population (EU Council Directive, 1991), consequently, the linear range achieved (0.66 to 8 mg P/l) appears well suited for further development.

Wider linear ranges have been achieved with fluorescent sensors, e.g. 0.002-62 P mg/l (Sun *et al.*, 2008) and 0.03-62 mg P/l (Ojida *et al.*, 2002, 2004; Hamachi, 2009), however these results were not achieved using wastewater samples. Wastewater samples have however been used with ion selective electrodes (ISEs), which have

also produced wider linear ranges than the results achieved here e.g. 0.003-3000 mg/l (Ganjali *et al.*, 2006) and 0.002-3100 mg/l (Modi *et al.*, 2011). However, in both cases, ethylenediaminetetraacetic acid (EDTA) had been added to the samples, which would require safe disposal and potentially inhibit adoption by the water industry. In addition, both ISE methods required pretreatment in a conditioning solution for 24 hours and the sensors suffered from reduced performance after ten to fifteen weeks.

The membranes were reused up to ten times by just rinsing the conductivity cell with deionised water. However, it is interesting to note that other MIP based sensors have been used repeatedly with no loss of sensitivity. A potentiometric sensor used a MIP based membrane to measure the concentration of diethyl chlorophosphate in ground and river water and was reused over thirty times with reproducible results (Vishnuvardhan *et al.*, 2011). Additionally, a flow through conductometric sensor used a MIP based receptor for the on-line measurement of haloacetic acids, with no loss of performance after more than fifty repeat measurements (Suedee *et al.*, 2006).

The pH of untreated domestic wastewater is typically pH 6.5 to 8.5 (Humboldt State University, 2008) and should not affect the performance of the sensor, which functions well at or around neutral pH. Samples were measured from pH 6.5 to 8 with no noticeable pH effects. Previous research shows that the thiourea based MIP receptor binds phosphate at neutral pH, although the receptor had a 1.6 times greater binding affinity for phosphate at pH 3 than pH 7 (Kugimiya and Takei, 2006). Conversely, a MIP sensor, that utilised the same conductance based transduction method and crosslinker ratio as the sensor developed here, produced signals 80% lower at pH 3 compared to pH 7.5 (Sergeyeva *et al.*, 1999). Consequently, the

measurement of phosphate at neutral pH is suitable for both the receptor and transducer.

#### **4. Conclusion**

A membrane containing thiourea based MIP receptors, incorporated within a conductance based sensor, was able to measure the phosphate content of wastewater samples with a linear range close to that required by the water industry. The sensor produced a response within two minutes, and could determine phosphate in real samples without addition of any additional reagents/chelating agent. With additional optimisation, including the surface area of the electrodes, membrane thickness/surface area and the full integration of the membrane onto a probe, the development of a robust and reliable electrochemical phosphate sensor should finally be achievable.

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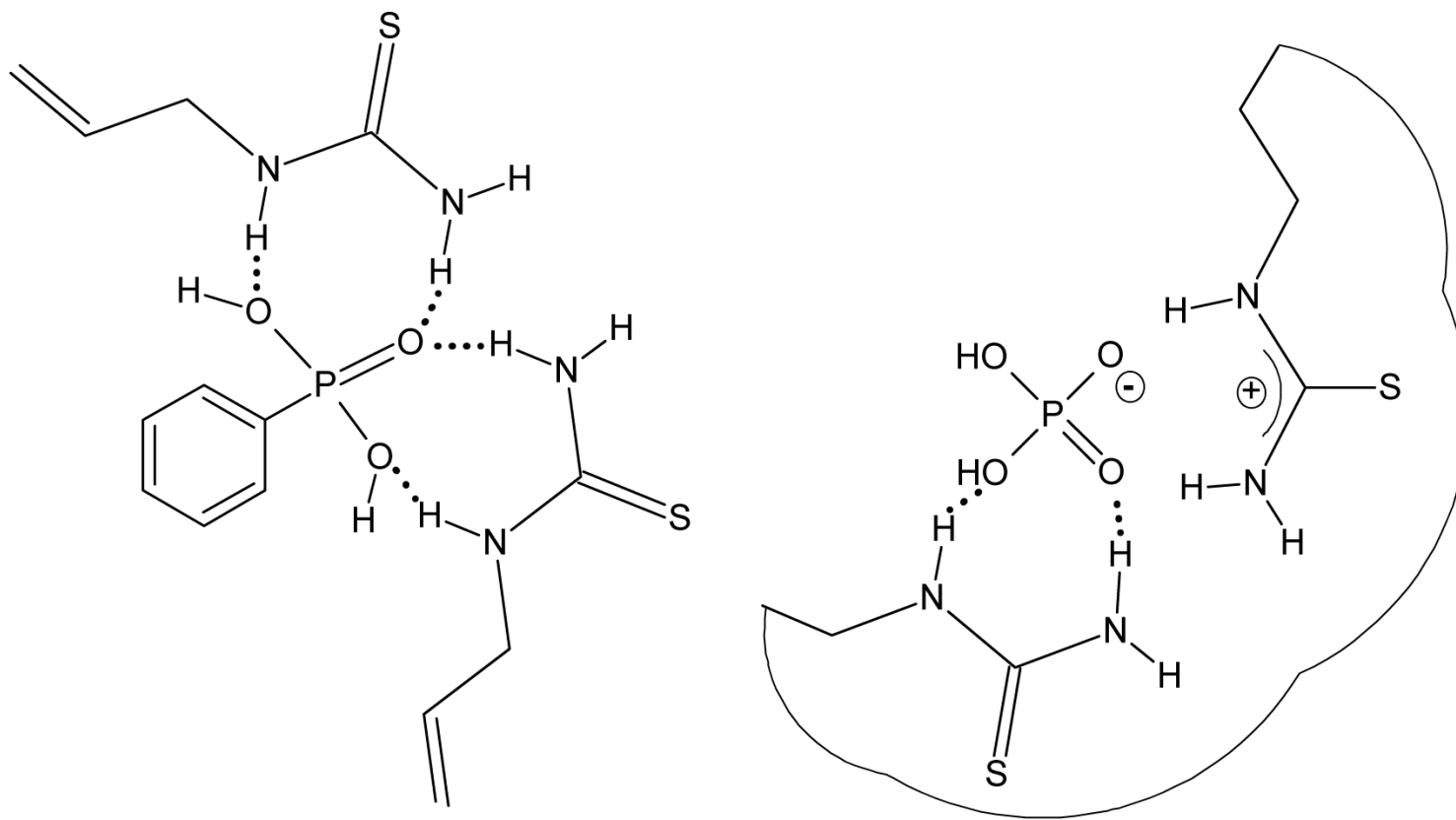


Figure 1 Diagrammatic representation of the pre-polymerisation complex comprising thiourea based functional monomers with a phenylphosphonic acid template (on the left) used to construct a polymer cavity capable of rebinding phosphate (on the right) within a MIP (adapted from Warwick *et al.*, 2013).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

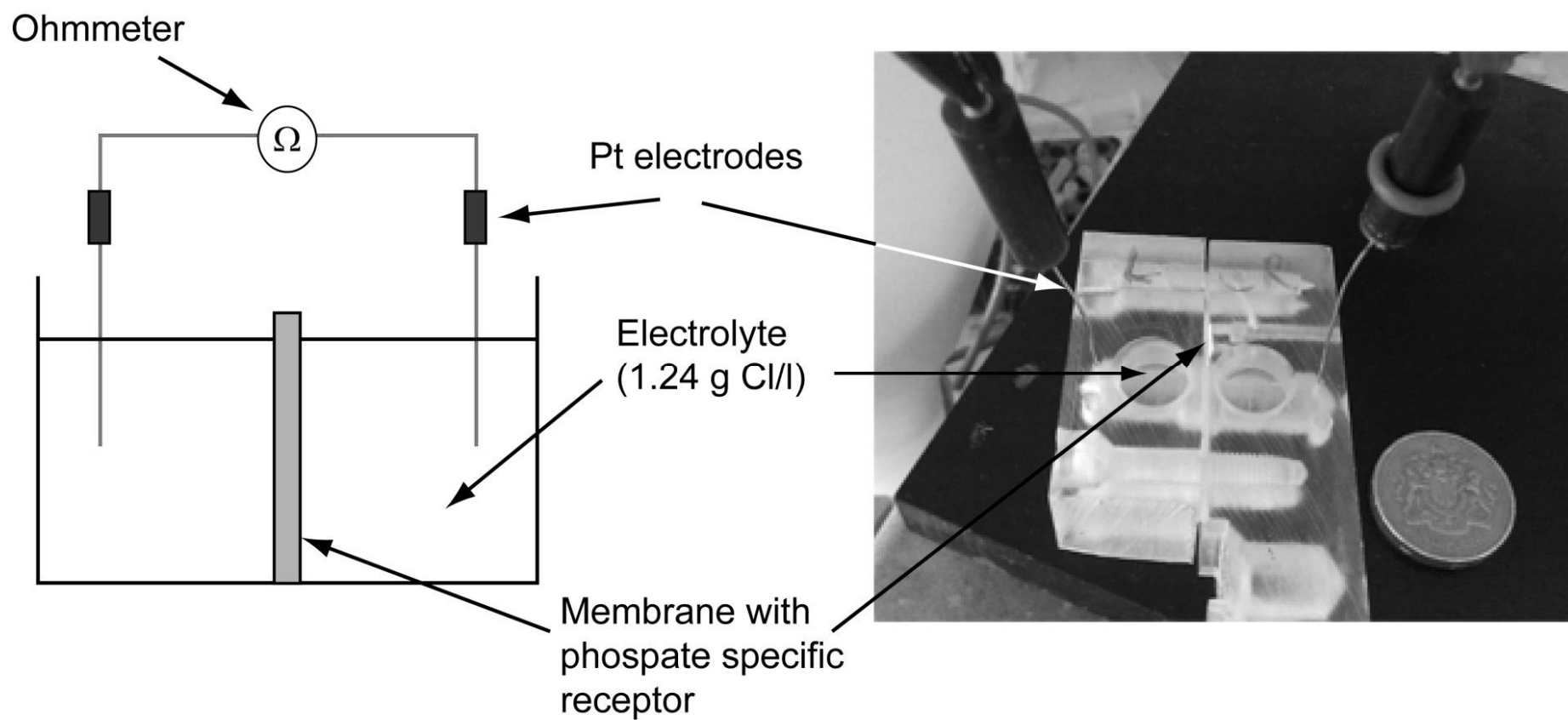


Figure 2 Diagram and photograph depicting the conductance cell and electrodes either side of the central membrane containing phosphate specific receptors. The membrane separated the two sample wells and the electrodes were used to determine conductance changes across the membrane.



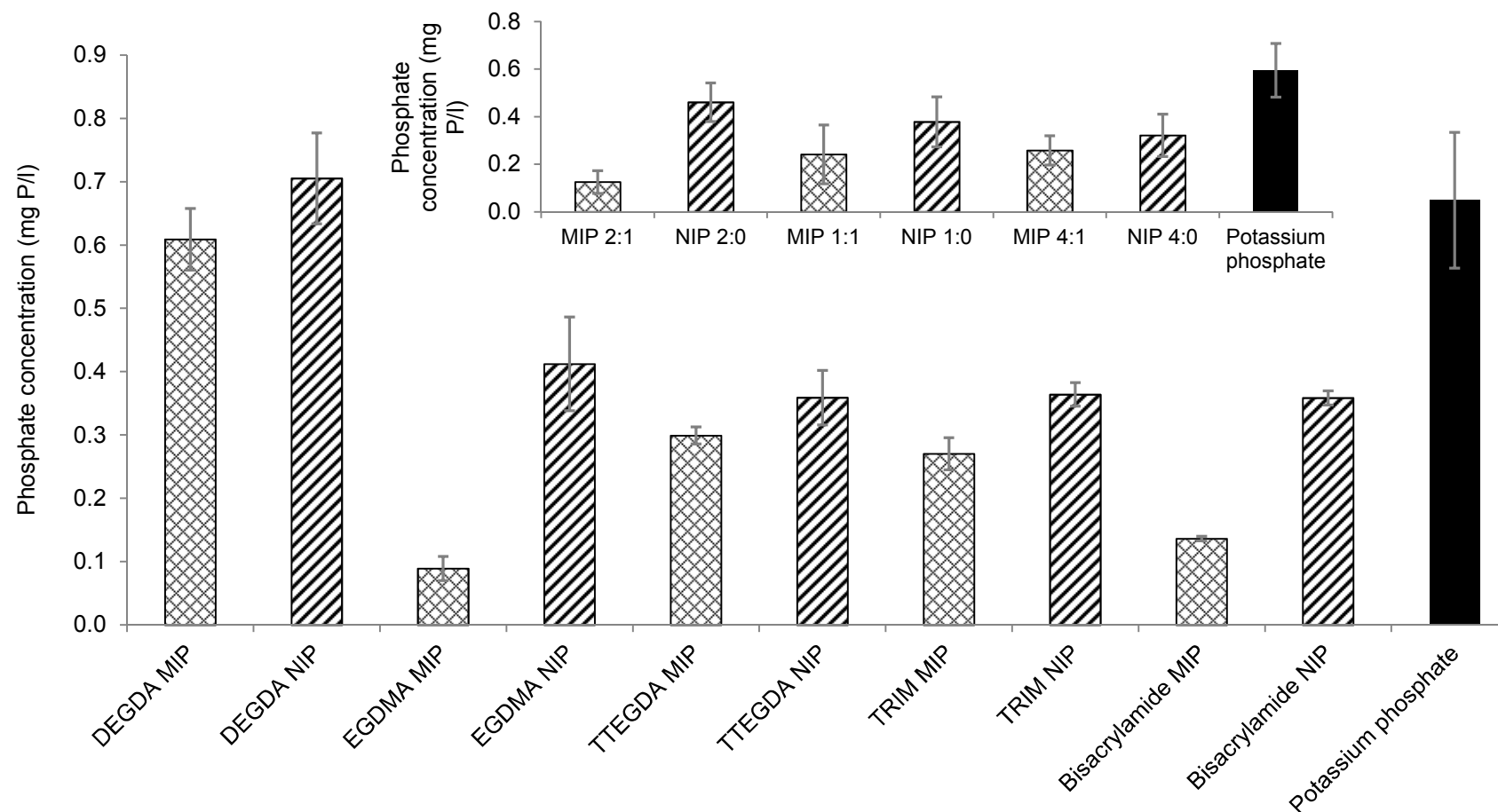


Figure 3 Main graph, the analysis of supernatants from phosphate binding experiments using different cross-linking reagents with a phenylphosphonic acid template and thiourea functional monomer. Inset, results showing the phosphate content of the supernatant from the assessment of the optimum ratio of monomer to template (using EGDMA as the cross-linking agent). Error bars show standard deviation of triplicates. The dark bar, on the right of the charts, shows the concentration of the phosphate solution originally applied to each MIP and NIP.

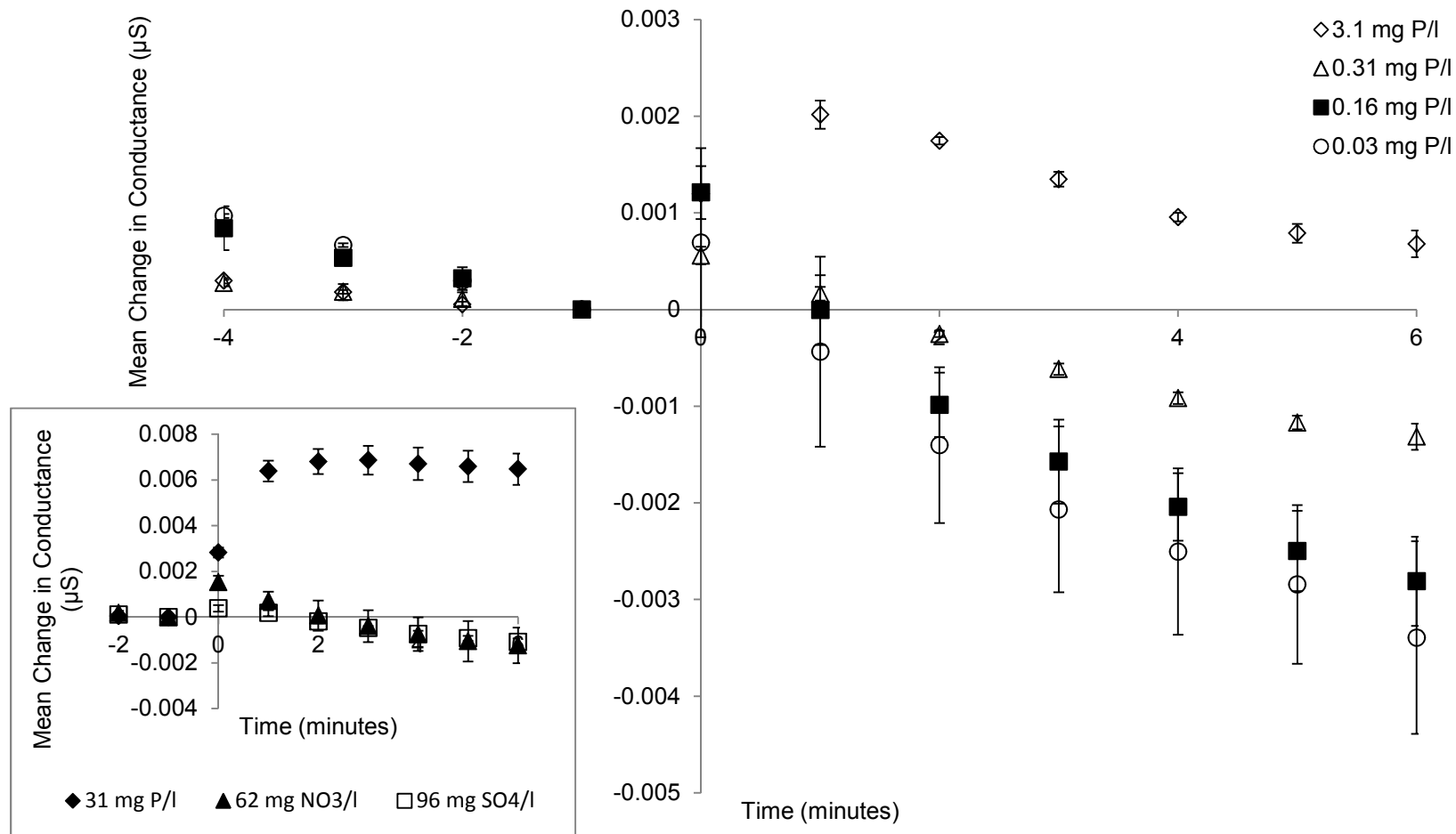


Figure 4 Main graph, the mean change in conductance using thiourea based MIP receptors on the addition (at time  $t=0$ ) of phosphate solution (3.1 - 0.03 mg/l). Error bars show standard deviation of triplicates. Inset, the mean change in conductance on the separate addition (at time  $t=0$ ) of alternative equimolar anion solutions (1 mM): phosphate solution (31 mg/l), nitrate solution (62 mg/l) and sulphate solution (96 mg/l). Error bars show standard deviation of triplicates.

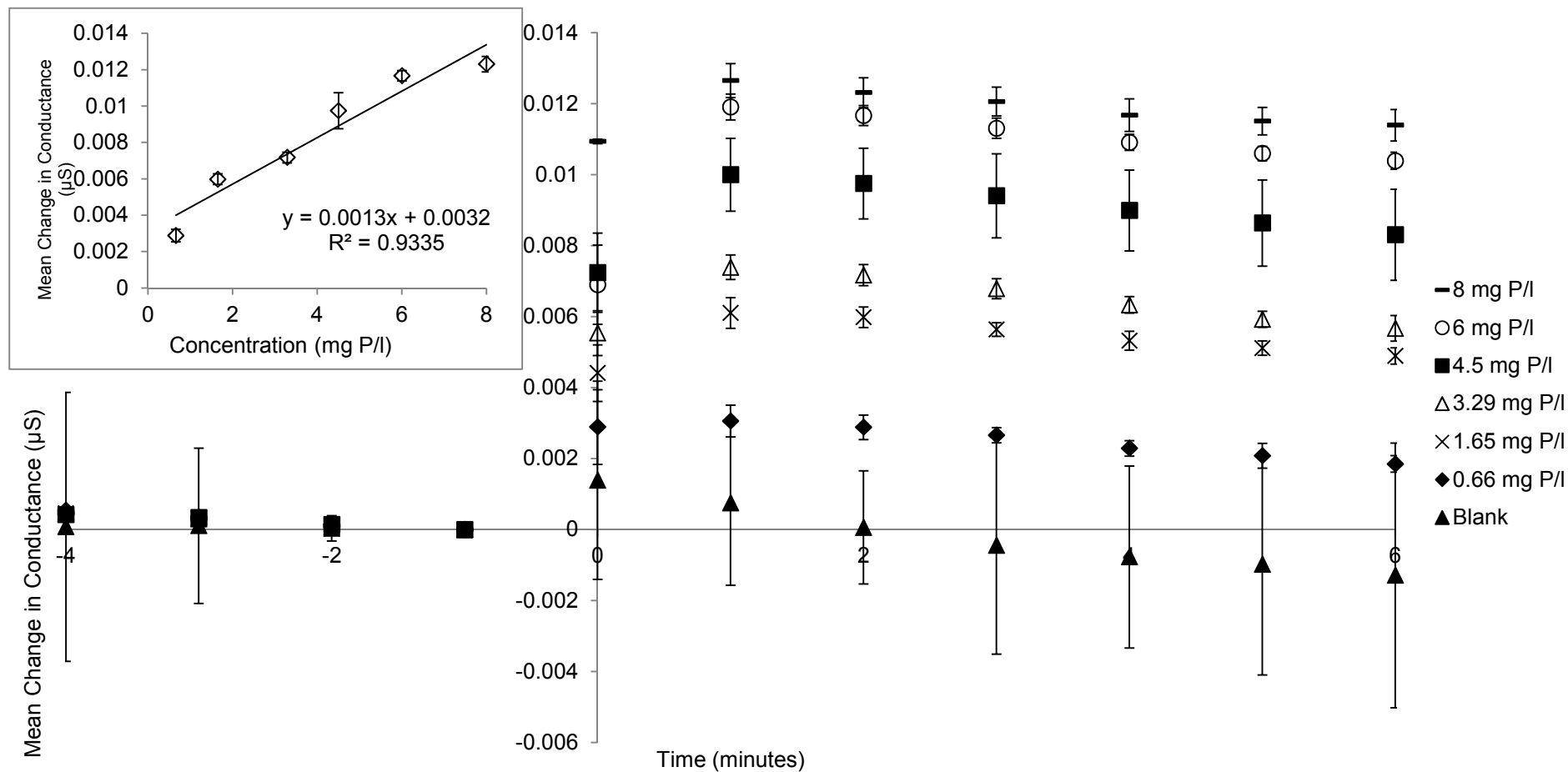


Figure 5 Main graph, the mean change in conductance and limit of quantification on the addition (at time t=0) of filtered wastewater samples (8 - 0.66 mg P/l). Error bars show standard deviation, except error bars for the blank which show LOQ (= standard deviation x10). Inset, calibration curve showing mean change in conductance after two minutes, on addition of filtered wastewater samples (8 - 0.66 mg/l). Error bars show standard deviation of triplicates.

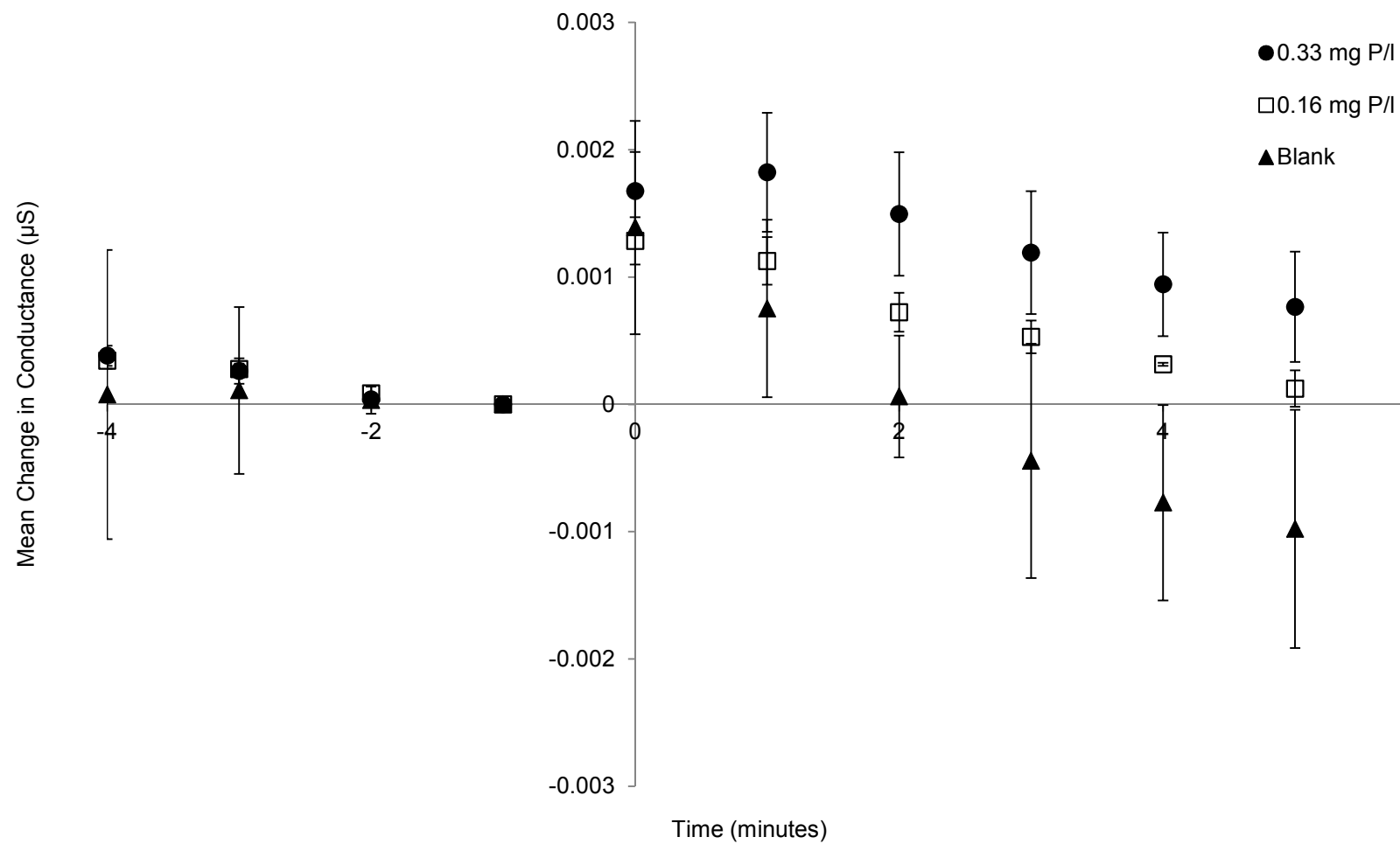


Figure 6 The limit of detection (LOD) of the phosphate sensor on the addition (at time  $t=0$ ) of filtered wastewater samples (0.33 – 0.16 mg/l). Error bars show standard deviation, except error bars for the blank, which show LOD (= standard deviation  $\times 3$ ).

# Conductance based sensing and analysis of soluble phosphates in wastewater

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