Water Science & Technology, 2014, Volume 69, Number 1, Pages 48–54

Water Science and Technology

A molecular imprinted polymer based sensor for measuring phosphate in wastewater samples

--Manuscript Draft--

Manuscript Number:	
Full Title:	A molecular imprinted polymer based sensor for measuring phosphate in wastewater samples
Short Title:	Phosphate molecular imprinted sensor
Article Type:	Research Paper
Section/Category:	Instrumentation, Monitoring, Control and Automation
Keywords:	Conductance, MIP, Phosphate, Sensor, Wastewater
Corresponding Author:	Ana Soares UNITED KINGDOM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	
Corresponding Author's Secondary Institution:	
First Author:	Christopher Warwick
First Author Secondary Information:	
Order of Authors:	Christopher Warwick
	Antonio Guerreiro
	Elizabeth Wood
	James Kitson
	James Robinson
	Ana Soares
Order of Authors Secondary Information:	
Manuscript Region of Origin:	UNITED KINGDOM
Abstract:	Phosphate detection in water samples is still completed using colourimetric standard methods that have a number of disadvantages such as being time consuming, require filtration, a number of different reagents, frequent calibration and proper disposal of waste chemicals generated. Hence, a simple cost effective analytical method and instrumentation is highly desirable aid the optimisation of treatment processes and aid the water industry in their efforts to comply with stringent regulations such as the EU's Water Framework Directive. A sensor based on molecular imprinted polymer (MIP) and a conductance transducer was developed for direct and label-free detection of phosphate in water. The sensor was able to measure the presence of phosphate in wastewater samples with good reproducibility, a linear range of 0.66 - 8 mg P L-1 and a lower detection limit of 0.16 mg P L-1. The sensor was further tested to measure phosphate concentrations in unfiltered field samples such as domestic wastewater treatment influent and river water and demonstrated a close correlation with reference measurements. The phosphate MIP sensor offers a way forward as either a handheld sensor for use in the field, or as a potential solution for remote, continuous monitoring of phosphate.
Suggested Reviewers:	Anthony Cass t.cass@imperial.ac.uk Expert on sensor development and testing

Powered by Editorial Manager® and ProduXion Manager® from Aries Systems Corporation

	Thierry Noguer noguer@univ-perp.fr Expert in molecular imprinted polymers
	Alessandra Bossi alessandramaria.bossi@univr.it expert on molecular imprinted polymers and environmental sensors

Published by IWA Publishing. This is the Author Accepted Manuscript issued with:
Creative Commons Attribution Non-Commercial License (CC:BY:NC 3.0).

Manuscript
Click here to download Manuscript: Soares P sensor paper 13jul13.docx

A molecular imprinted polymer based sensor for measuring phosphate in wastewater samples

Christopher Warwick[a], Antonio Guerreiro[b], Elizabeth Wood[c], James Kitson[c], James Robinson[c] and Ana Soares[a*]

[a]Cranfield Water Sciences Institute, School of Applied Sciences, Cranfield University, Cranfield, MK43 0AL, UK; [b]Cranfield Biotechnology Centre, Cranfield Health, Cranfield University, Cranfield, MK43 0AL, UK; [c]Yorkshire Water, Western House, Halifax Road, Bradford, BD6 2SZ, UK.
[*]Corresponding author. Tel.: +44 1234 8121; fax: +44 1234 4036; E-mail address: a.soares@cranfield.ac.uk

ABSTRACT

Phosphate detection in water samples is still completed using colourimetric standard methods that have a number of disadvantages such as being time consuming, require filtration, a number of different reagents, frequent calibration and proper disposal of waste chemicals generated. Hence, a simple cost effective analytical method and instrumentation is highly desirable aid the optimisation of treatment processes and aid the water industry in their efforts to comply with stringent regulations such as the EU's Water Framework Directive. A sensor based on molecular imprinted polymer (MIP) and a conductance transducer was developed for direct and label-free detection of phosphate in water. The sensor was able to measure the presence of phosphate in wastewater samples with good reproducibility, a linear range of 0.66 - 8 mg P L^{-1} and a lower detection limit of 0.16 mg P L^{-1}. The sensor was further tested to measure phosphate concentrations in unfiltered field samples such as domestic wastewater treatment influent and river water and demonstrated a close correlation with reference measurements. The phosphate MIP sensor offers a way forward as either a handheld sensor for use in the field, or as a potential solution for remote, continuous monitoring of phosphate.

Keywords: Conductance, MIP, Phosphate, Sensor, Wastewater

INTRODUCTION

Discharges of phosphate in treated effluents to receiving water bodies is currently regulated by the Urban Waste Water treatment Directive (98/15/EEC), the Water Frame Work Directive (2000/60/EC) and the Freshwater Fisheries Directive (2006/44/EC). Due to these regulations, and the need to reduce eutrophication incidents due to excess phosphate, the water industry must monitor phosphate concentrations at different stages of the wastewater treatment processes, effluents and river waters at an increasing number of sites. This puts great pressure on the water sector to have fast, simple and accurate methods to measure phosphates in wastewater samples. In addition, monitoring of nutrient levels in rivers, lakes, coastal waters is becoming increasingly debated due to the need of reaching good ecological and chemical status by 2015 as stated in the Water Framework Directive.

The instruments available in the market for phosphate detection are colourimetric based methods (APHA, 2005; Ingildsen and Olsson, 2002; Salter et al., 2010). Common

methods/reagents used are malachite green, molybdenum blue and yellow vanadomolybdophosphoric acid. The sample needs filtering and the reagents are added in sequence to produce a colorimetric reaction – colour formation. Hand-held and on-line monitoring instruments have been developed, but invariably, chemical reagents must be used, sample handling is necessary depending on the instrument type and products of reaction disposed of securely. Consequently, existing methods are costly, time consuming and labour intensive.

No reliable or robust sensing based alternative has emerged at commercial scale, despite numerous attempts (Warwick et al., 2013a). One of the principle reasons why there is no sensing based alternative to the colourimetric standard is due to inherent properties of the phosphate ion. Phosphate is a relatively large oxyanion, which means getting receptors to be selective for phosphate is challenging, as other anions, such as sulphate, are a similar shape and size (Katayev et al., 2006). In addition, charged oxygen atoms obscure the central phosphorous atom, resulting in a high hydration energy (Buehlmann et al., 1998). Molecular imprinted polymers (MIPs) potentially offer a means to overcome these challenges, providing a potential alternative to colorimetric based techniques. MIPs have been successfully integrated with a number of different transducers, including electrochemical and optical methods and so offer a number of potential signalling methods (Piletsky and Turner, 2002). The aim of this work was to identify a suitable functional monomer for use in MIPs that could selectively bind phosphate, optimise its binding capability and combine the monomer with a transducer to provide a quantitative signal in the presence of phosphate using wastewater samples. Furthermore the MIP sensor was validated by measuring the phosphate content of unfiltered wastewater and compared with the results from using commercial colourimetric tests.

MATERIALS AND METHODS

Cross-linking reagents diethyleneglycol diacrylate (DEGDA), ethylene glycol dimethacrylate (EGDMA), tetra(ethylene glycol) diacrylate (TTEGDA), N,N′-methylenebis(acrylamide), trimethylolpropane trimethacrylate (TRIM), the initiator 1,1'-azobis-cyclohexanecarbonitrile, the imprinted template phenylphosphonic acid and the functional monomers allylamine, 2-(diethylamino)ethylmethacrylate (DEAEM); N-allylthiourea (thiourea) and 2-methacryloyloxyethyltrimethylammonium chloride (METAC), together with L-ascorbic acid were all purchased from Sigma-Aldrich (Poole, 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). Molybdate reagent solution was supplied by Fluka (Poole, UK). One additional functional monomer was synthesised - N-phenylethylene diamine methacrylamide (NPEDMA) (Lakshmi, et al., 2009). Oligourethane acetate (OUA) was donated by the Institute of Macromolecular Chemistry (Ukraine).

Assessment of potential phosphate binding receptors

Computational modelling was performed using the molecular modelling software program CentOS linux based hardware, SYBYL, version 7.3 (Tripos International, 2006) to screen over thirty functional monomers. Four monomers allylamine, NPEDMA, DEAEM and METAC were selected for MIP synthesis MIP and phosphate binding experimentation following computational modelling. An additional monomer, thiourea, was also selected for its ability to selectively bind phosphates (Kugimiya and Takei, 2006).

MIP synthesis involved the combination of reagents (Table 1) following the addition of the initiator and nitrogen flushing to remove oxygen. A phosphate anion analogue, phenylphosphonic acid, was selected as a template to overcome potential solubility issues with using phosphate anions in organic media. The mixture was then exposed to UV light (Honle UVAPRINT 100CV1) for 20 minutes to allow for polymerisation. In addition to preparing MIPs, equivalent negative controls were prepared, non-imprinted polymers (NIPs), which followed the same method but omitted the phosphate template. The polymers were ground (Retsch ZM200 grinder), sieved and purified using Soxhlet extraction with methanol overnight. Each polymer was then dried and weighed in order to assess their phosphate binding ability.

(Table 1 should be inserted here)

Thiourea based MIPs (and NIPs) were prepared with a variety of cross-linkers. The cross-linkers used included DEGDA, together with EGDMA, TTEGDA, TRIM and bisacrylamide. The solvent was ACN, except when the cross-linker bisacrylamide was studied, which, required DMF. Three different ratios (4:1, 2:1 and 1:1) of monomer and template were examined to determine the optimum for phosphate retention.

Phosphate retention by the MIPs (and NIPs) was then tested by adding 2 ml potassium dihydrogen phosphate (0.78 mg P L^{-1}, pH 7.2) for every 40 mg sample of polymer. This was followed by continuous stirring and incubation for one hour at room temperature (Kugimiya and Takei, 2006). The samples were centrifuged at 1730×*g* for one minute and the concentration of phosphate in the supernatant quantified. All experiments were completed in triplicate.

Integration of the binding receptors with a transducer

A thiourea MIP was synthetized as a membrane using a blend of cross linking monomers, to provide greater flexibility. The cross-linking reagents used were EGDMA and OUA in a ratio of 85:15 to generate an optimum conductometric response (Sergeyeva et al., 1999). The polymer mix was inserted between two glass plates (8cm x 10cm), with a silicone perimeter and polymerised under UV light (Honle UVAPRINT 100CV1) for 2.5 minutes on each side as described previously by Soares et al., (2013). The MIP membranes were cleansed with methanol based Soxhlet extraction. Prior to use, the MIP membranes were dried and rehydrated in deionised water.

To get a signal on phosphate quantification a conductance P sensor cell was constructed. The MIP membrane was positioned between two sample wells, with a silicone spacer on both sides. Potassium chloride electrolyte solution (1.24 g L^{-1}) was applied to each well (1.5 ml). Platinum electrodes (BASi, USA), separated by the membrane, were positioned on the conductance cell containing the electrolyte solution. The electrodes were connected to an ohmmeter (DMM914, Tektronix) and the resistance (inverse of conductance) across the membrane and electrolyte was noted until equilibrium was achieved, as described previously by Soares et al., (2013). Test samples (0.5 ml) were then added to each well and the change in conductance recorded every minute.

Measuring phosphate in wastewater samples

A settled sample of wastewater from a 570,000 population equivalent wastewater treatment plant in the UK (pH 6.88) with an phosphate concentration of 3.29 mg P L^{-1} was

filtered with a 1.2 µ filter. The sample was then either diluted with deionised water or spiked with quantities of potassium phosphate solution (310 mg P L^{-1}) to generate phosphate standards, in a field sample matrix, equivalent to 0.16, 0.33, 0.66, 1.65, 3.29, 4.5, 6 and 8 mg P L^{-1}.

Additional field samples were also tested with the conductance cell. These unfiltered samples comprised screened influent and final effluent from a 2,840 population equivalent sewage treatment works (STW) in the UK, a river water sample from near Milton Keynes (UK) and two mixed solutions, to provide a range of concentrations, which combined influent from the STW (phosphate content 6.1 mg P L^{-1}) and river water (phosphate content <0.5 mg P L^{-1}). All phosphate measurements in the sensor were recorded in triplicate at room temperature (21-23°C).

Analytical methods

Phosphate concentrations in water samples were assessed using Spectroquant cell tests (Merck, West Drayton, UK) and validated externally by a commercial laboratory (Anglian Water Labs, Huntingdon, UK) that used the phosphomolybdenum blue colourimetric method (APHA, 2005). Field samples were analysed for pH and interfering ions: ammonia, nitrate, nitrite, alkalinity, chloride, fluoride, sulphate, sodium, potassium, magnesium, calcium and iron, using Standard Methods (APHA, 2005).

RESULTS AND DISCUSSION

The computational modelling results demonstrated that the highest binding energy for dihydrogen phosphate were charged versions of allylamine (-111.89 kcal/mol), NPEDMA (-87.20 kcal/mol) and DEAEM (-77.36 kcal/mol). Another monomer with a relatively high binding energy for the target was METAC (-41.55 kcal/mol). To validate the modelling results, the MIPs and respective NIPs were prepared and placed in solution with known amounts of phosphate. Polymers could then be evaluated for their ability to bind phosphate if the supernatant samples contained relatively low concentrations of phosphate, compared to the concentration originally added and the NIP (Figure 1). No phosphate was recovered from the supernatant of the MIP or NIP prepared with the METAC functional monomer even if more concentrated solution of potassium phosphate (7.75 mg P L^{-1}) was applied, suggesting that METAC could potentially offer functionality as a phosphate adsorbent. Apart from the thiourea based MIP, the phosphate content of the supernatants of the MIPs either exceeded those of the NIPs (*e.g.* NPEDMA) or there was little difference (allylamine and DEAEM), indicating that these monomers were not providing any selectivity for binding phosphate. The results from the thiourea based MIP show that the supernatant contained less phosphate (0.61 mg P L^{-1}) than the supernatant from the corresponding NIP (0.77 mg P L^{-1}), indicating that the thiourea MIP was capable of binding more (22%) of the applied phosphate (0.78 mg P L^{-1}), than the NIP, which bound only 1% of the applied phosphate. This confirms the affinity for phosphate using thiourea based MIPs by Kugimiya and Takei (2006). However, they established that the MIP bound 89% of the applied phosphate, compared to 4% for the corresponding NIP.

(Figure 1 should be inserted here)

Optimisation of the thiourea based MIP and integration into a transducer

The thiourea MIP was optimized by evaluating the impact of cross-linkers and template ratios in the polymer mixture. When varying the choice of cross-linker with thiourea based MIPs, all MIPs bound more phosphate than the corresponding NIP, i.e., the MIP

constructed using bisacrylamide cross-linker bound 82% of the applied phosphate, while the corresponding NIP bound 54%, a difference of 28%. In comparison, the EGDMA based thiourea MIP bound 89% of the phosphate compared to 47% for the NIP, a difference of 42%. Consequently EGDMA was selected for further tests.

Different ratios of thiourea to phenylphosphonic acid were tested including 4:1, 2:1 and 1:1. Of the ratios tested, a 2:1 ratio delivered the best performance, with 80% binding for the MIP and 35% for the equivalent NIP, a difference of 45% and the best contrast in phosphate retention of all configurations tested, suggesting that the improved uptake resulted from the imprinting process and not simply dispersed functional monomer within the polymer mixture. Overall, optimisation dramatically improved phosphate retention between the original configuration using DEGDA cross-linker and the optimised MIP using a 2:1 monomer to template ratio with EGDMA cross-linker.

The integration of thiourea based MIP receptors into membranes for use with ion-selective electrodes did not produce Nernstian responses (data not shown), and integration with other transducers was attempted. When the thiourea based MIP receptors were incorporated into a membrane for conductance analysis, the presence of phosphate in water samples could be quantified, using the conductance phosphate sensor cell. Experiments using various phosphate concentrations in deionised water (0.03 to 31 mg P L^{-1}) were completed to assess the relationship between the change in conductance produced with concentration and a correlation (r^2) of 0.97 was established. Furthermore the MIP sensor selectively detected the presence of phosphate compared to sulphate, nitrate and chloride and the binding was found to be reversible, enabling the re-use of the membrane for multiple measurements (Warwick et al., 2013b).

Measuring phosphate in wastewater samples

When assessing the concentration of phosphate in filtered wastewater samples, the MIP sensor produced signals proportional to the phosphate concentration, giving a correlation (r^2) of 0.93 (Figure 2).

(Figure 2 should be inserted here)

The limit of quantification (LOQ - defined as the lowest measurement that exceeds ten times the standard deviation in the blank, MacDougal *et al*., 1980) and the limit of detection (LOD – defined as the value that exceeds three standard deviations of the blank, MacDougal *et al*., 1980) were 0.16 mg P L^{-1} (LOD) and 0.66 mg P L^{-1} (LOQ). These limits of quantification were in the same order of magnitude as the phosphate concentrations found in wastewater and rivers, which vary from 10 to 0.04 mg P L^{-1} (Tchobanoglous, et al., 2003; UK TAG, 2008). Fluorescent based sensors have achieved broader linear ranges e.g. 0.03-62 mg P L^{-1} (Ojida et al., 2002, 2004; Hamachi, 2009) and 0.002-62 mg P L^{-1} (Sun et al., 2008), although these results were not produced by testing wastewater samples. Ion selective electrodes (ISEs) have also produced wider linear ranges and have been tested in wastewater samples e.g. 0.003-3000 mg P L^{-1} (Ganjali et al., 2006) and 0.002-3100 mg P L^{-1} (Modi et al., 2011). However, ethylenediaminetetraacetic acid (EDTA) had to be added to the samples in both examples, and EDTA requires safe disposal and this requirement would potentially hinder widespread use in the water industry.

Although the longevity of the MIP P sensor was not fully established, the membrane could be re-used at least ten times over a number of days (3-5 days), simply by rinsing the membranes

with deionised water. Other, membrane based, MIP sensors have also reported repeated reuse, with good reproducibility, i.e., the membrane from a MIP based, potentiometric sensor was used more than thirty times with no significant loss in sensitivity, to determine diethyl chlorophosphate concentrations in ground and river water (Vishnuvardhan et al., 2011). In addition, a MIP based receptor, utilised in a flow through conductometric sensor, was used in excess of fifty times with no reported loss in (Suedee et al., 2006).

The phosphate content of unfiltered wastewater and river samples were tested with the MIP sensor (after two minutes), using the calibration curve (Figure 2) and compared with results from colourimetric cell tests (Table 2). It was interesting to note that the two reference methods (the cell test and the commercial lab colourimetric test) did not record the same concentrations. For example, the cell test measured the phosphate content of screened influent at 6.10 mg P L^{-1} compared to the laboratory's colourimetric method, which recorded 5.35 mg P L^{-1}, a difference of 0.75 mg P L^{-1}. In contrast the MIP sensor determined a reading between these two values (6.0 mg P L^{-1}), very close to that shown by the cell test. Analysis of the blended wastewater and river sample using the sensor gave results closer to those found by the reference methods. The sensor recorded the phosphate content of the blended sample at 2.50 mg P L^{-1}, while the cell test and laboratory method showed a slightly higher phosphate content (2.80 and 2.67 mg P L^{-1} respectively). The MIP sensor measured a higher concentration of phosphate (2.20 mg P L^{-1}) for the river water, downstream from the STW, compared to other analysis (1.20 mg P L^{-1} and 1.12 mg P L^{-1}).

(Table 2 should be inserted here)

The wastewater, river water and blended sample were also analysed for concentrations of other anions, cations, pH and alkalinity (Table 2). Interference from other anions could have been the cause of the overstimation of P content (>1 mg P L^{-1}) in the river water and yet, apart from nitrate and sulphate, there were no anions present in the river water that were in significantly higher concentrations than the influent. Furthermore, it has already been demonstrated that higher concentrations of nitrate (62 mg L^{-1}), than those present in the river water sample (8 mg L^{-1}), did not produce a signal in tests to assess selectivity (Warwick et al., 2013b), which also confirmed the findings of Kugimiya and Takei, (2006). Another difference between the influent and river water was the degree of alkalinity, which was lower in the river water (268 mg $CaCO_3$) than the influent (405 mg $CaCO_3$). It is conceivable that this reduced level of alkalinity (i.e. lower concentrations of hydroxides, carbonates and bicarbonates) could have had an effect on the signal. The pH of the samples were similar (8.09 and 8.31 for influent and river water respectively). The pH of untreated domestic wastewater is typically pH 6.5 to 8.5 (Tchobanoglous, et al., 2003) and should not affect the performance of the sensor, which functions well at or around neutral pH.

CONCLUSIONS

A thiourea based MIP receptor, combined with a conductance based transducer has produced a MIP phosphate sensor with the selectivity and level of detection required by the water industry. The MIP phosphate sensor had the ability to measure phosphate concentrations in unfiltered wastewater and compares favourably with reference methods. The MIP phosphate sensor has the potential to be developed into a handheld sensor for field use or adapted to provide remote continuous monitoring. This development could ultimately replace the colourimetric based assessments of the phosphate content of wastewater.

ACKNOWLEDGEMENTS

The authors would like to thank Yorkshire Water and the Engineering Physical Sciences Research Council (EPSRC) for research funding.

BIBLIOGRAPHY

APHA. *Standard Methods for the Examination of Water and Wastewater* 2005 21th edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.

Buehlmann, P., Pretsch, E. and Bakker, E. 1998 Carrier-based ion-selective electrodes and bulk optodes. 2. ionophores for ootentiometric and optical sensors. *Chemical Reviews* **98**, 1593-1687.

EU Council Directive. (1998). Directive 98/15/EEC amending directive 91/271/EEC. Urban waste water treatment.

.EU Council Directive. (2000). Directive 2000/60/EC. Establishing a framework for Community action in the field of water policy.

EU Council Directive. (2006). Directive 2006/44/EC. The quality of fresh waters needing protection or improvement in order to support fish life.

Ganjali M., Norouzi, P., Ghomi, M. and Salavati-Niasari, M. 2006 Highly selective and sensitive monohydrogen phosphate membrane sensor based on molybdenum acetylacetonate. *Analytica Chimica Acta*, **567**, 196-201.

Hamachi, I. 2009 Patent No. 7,521,250 B2. USA.

Ingildsen, P., and Olsson G. 2002 Exploiting online in-situ ammonium, nitrate and phosphate sensors in full-scale wastewater plant operation. *Water Science and Technology*. **46**(4-5),139-147.

Katayev, E., Ustynyuk, Y. A. and Sessler, J. L. 2006 Receptors for tetrahedral oxyanions. *Coordination Chemistry Reviews*, **250**, 3004-3037.

Kugimiya, A. and Takei, H. 2006 Preparation of molecularly imprinted polymers with thiourea group for phosphate. *Analytica Chimica Acta*, **564**, 179-183.

Lakshmi, D., Whitcombe, M. J., Davis, F., Chianella, I., Piletska, E., Guerreiro, A., Subrahmanyam, S., Brito P., S., Fowler, S., A. and Piletsky, S. A. 2009 Chimeric polymers formed from a monomer capable of free radical, oxidative and electrochemical polymerisation. *Chemical Communications*, 2759-2761.

MacDougal, D. and Crummett, W. 1980 Guidelines for data acquisition and data quality evaluation in environmental chemistry. *Analytical Chemistry*, **52**(14), 2242-2249.

Modi, N., Patel, B., Patel, M. and Menon, S. 2011 Novel monohydrogenphosphate ion-selective polymeric membrane sensor based on phenyl urea substituted calix[4]arene. *Talanta*, **86**, 121-127.

Ojida, A., Mito-oka, Y., Inoue, M. and Hamachi, I. 2002 First artifical receptors and chemosensors toward phosphorylated peptide in aqueous solution. *Journal of the American Chemical Society*, **124**, 6256-6258.

Ojida, A., Mito-oka, Y., Sada, K. and Hamachi, I. 2004 Molecular recognition and fluorescence sensing of monophosphorylated peptides in aqueous solution by bis(zinc(II) dipicolylamine)-based artificial receptors. *Journal of the American Chemical Society*, 126, 2454-2463.

Piletsky, S. A. and Turner, A. P. 2002 Electrochemical sensors based on molecularly imprinted polymers. *Electroanalysis*, **14**(5), 317-323.

Slater, C., Cleary, J., Lau, K.-T., Snakenborg, D., Corcoran, B., Kutter J. P., and Diamond, D. 2010 Validation of a fully autonomous phosphate analyser based on a microfluidic lab-on-a-chip. *Water Science and Technology*. **61**(7), 1811-1818.

Sergeyeva, T., Piletsky, S., Brovko, A., Slinchenko, E., Sergeeva, L. and El'skaya, A. 1999 Selective recognition of atrazine by molecularly imprinted polymer membranes. Development of conductometric sensor for herbicides detection. *Analytica Chimica Acta*, **392**, 105-111.

Soares, A., Guerreiro. A., Warwick, C. 2013 UK Patent Application GB130777.1

Suedee, R., Intakong, W. and Dickert, F. 2006 Molecularly imprinted polymer-modified electrode for on-line conductometric monitoring of haloacetic acids in chlorinated water. *Analytica Chimica Acta*, **569**, 66-75.

Sun, H., Scharff-Poulsen A. M., Gu, H., Jakobsen, I., Kossmann J. M., Frommer W. B. and Almdal, K. 2008 Phosphate fensing by fluorescent reporter proteins embedded in polycrylamide nanoparticles. *ACS Nano*, 2(**1**), 19-24.

Tchobanoglous, G., Burton, F. and Stensel, D. 2003 *Wastewater Engineering - treatment and reuse* 4th ed. McGraw Hill. New York.

UK TAG. 2008 *UK Environmental standards and conditions - Phase 1*. UK Technical Advisory Group on the Water Framework Directive.

Vishnuvardhan, V., Kalyan, Y., Prathish, K. P., Gangadhar, B., Tharakeswar, Y., Prasada-Rao, T. and Naidu, G. R. 2011. Imprinted polymer inclusion membrane based potentiometric sensor for determination and quantification of diethyl chlorophosphate in natural waters. *American Journal of Analytical Chemistry*, **2**, 376-382.

Warwick, C., Guerreiro, A. and Soares, A. 2013a. Sensing and analysis of soluble phosphates in environmental samples: A Review. *Biosensors and Bioelectronics*, **41**, 1-11.

Warwick, C., Guerreiro, A., Gomez-Caballero, A., Wood, E., Kitson, J., Robinson, J. and Soares, A. 2013b Conductance based sensing and analysis of soluble phosphates in wastewater. Submitted to *Biosensors and Bioelectronics*.

Figure

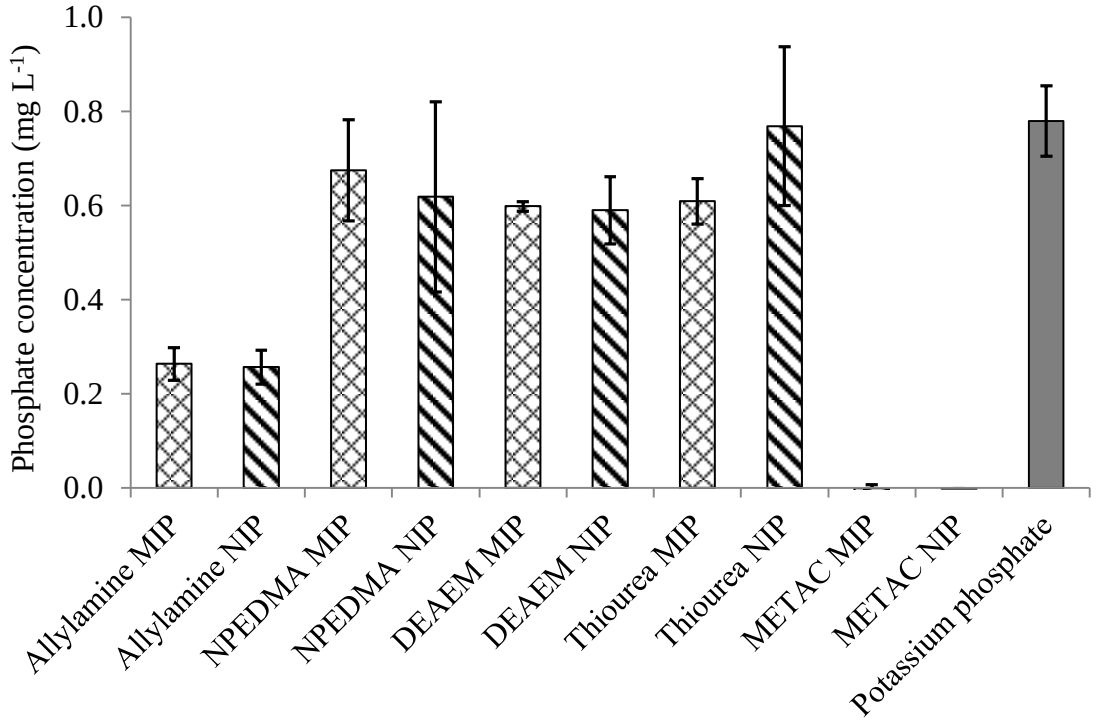


Figure 1 Results showing the phosphate content of the supernatant in an assessment of the binding ability of MIPs and NIPs for potassium phosphate (0.78 mg L^{-1}). MIPs synthesised with phenylphosphonic acid template with the functional monomers shown. NIPs omit the template altogether to act as a negative control. Error bars show standard deviation of triplicates.

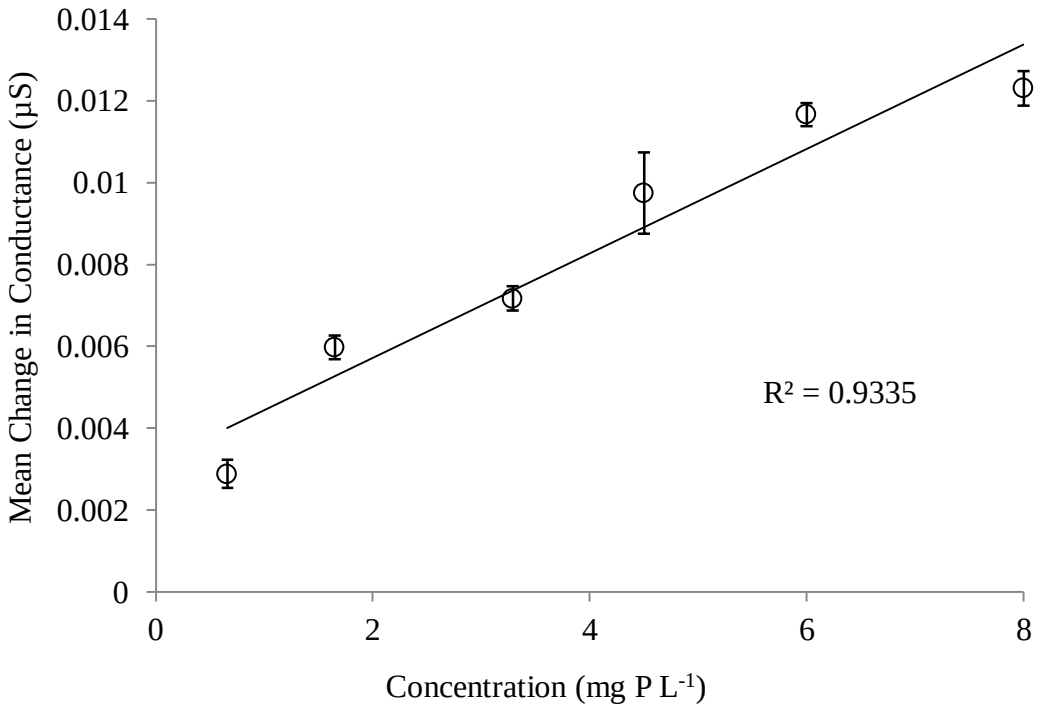


Figure 2 Calibration curve showing mean changes in conductance after two minutes following the addition of wastewater samples containing 0.66-8 mg P L^{-1}. Error bars show standard deviation of triplicates.

Table 1 Recipes for MIP preparation using different functional monomers

Monomer (mass in g)	**Template (mass in g)**	**Monomer: template molar ratio***	**Cross-Linker (mass in g)**	**Solvent (mass in g)**
NPEDMA (0.96 g)	Phenylphosphonic acid (0.37 g)	2:1	DEGDA (4.00 g)	ACN (4.96 g)
DEAEM (0.87 g)	Phenylphosphonic acid (0.18 g)	4:1	DEGDA (4.00 g)	ACN (4.87 g)
Thiourea (0.54 g)	Phenylphosphonic acid (0.37 g)	2:1	DEGDA (4.00 g)	ACN (4.54 g)
METAC (1.21 g)	Phenylphosphonic acid (0.18 g)	4:1	DEGDA (4.00 g)	ACN (5.21 g)
Allylamine (0.29 g)	Phenylphosphonic acid (0.20 g)	4:1	EGDMA (4.00 g)	DMF (4.29 g)

*The molar ratio of monomer to template was determined according to the number of amine groups present on the monomer used. A typical ratio of monomer to template in non-covalent methods is 4:1 (Cormack and Elorza, 2004). Consequently, when there was only one amine group present, the molar ratio of monomer to template used was 4:1. Where two amine groups were present, the molar ratio of monomer to template used was 2:1.

In each case, the initiator used was azobis-cyclohexanecarbonitrile (0.09 g).

Table 2 Comparison of phosphate determinations using MIP sensor readings and colorimetric methods, and ion concentrations of unfiltered wastewater and river samples.

	Influent from STW	River water, (downstream from STW)	Blend of influent and river water
Mean change in conductance (µS) (standard deviation)	0.01099 (0.00126)	0.00607 (0.00066)	0.00639 (0.00032)
Phosphate determined MIP sensor cell (mg P L^{-1})	6.0	2.2	2.5
Phosphate determined using cell tests (mg P L^{-1})	6.1	1.2	2.8
Phosphate determined by commercial lab (mg P L^{-1})	5.3	1.1	2.7
Total phosphorus (mg L^{-1})	6.3	1.2	3.1
pH	8.1	8.3	8.0
Ammonia (mg N L^{-1})	33.4	0.1	23.4
Nitrate (mg N L^{-1})	0.8	8.0	1.2
Nitrite (mg N L^{-1})	<0.004	0.16	<0.004
Alkalinity (mg $CaCO_3$)	405.0	268.0	295.0
Chloride (mg Cl L^{-1})	76.5	40.5	50.3
Fluoride (mg F L^{-1})	0.3	0.4	0.3
Sulphate (mg SO_4 L^{-1})	60.6	145.0	103.0
Sodium (mg L^{-1})	101.0	37.7	38.2
Potassium (mg L^{-1})	17.2	5.2	9.5
Magnesium (mg L^{-1})	7.5	13.3	9.9
Calcium (mg L^{-1})	60.8	147.0	97.8
Iron (mg L^{-1})	0.1	<0.02	<0.02



https://dspace.org/

A molecular imprinted polymer based sensor for measuring phosphate in wastewater samples

Warwick, Christopher; Guerreiro, Antonio R.; Wood, Elizabeth; Kitson, James; Robinson, James ...

2014-01-31
IWA Publishing
http://dx.doi.org/10.2166/wst.2013.550
Warwick, C. et al. (2014) A molecular imprinted polymer based sensor for measuring phosphate in wastewater samplesA molecular imprinted polymer based sensor for measuring phosphate in wastewater samplesA molecular imprinted polymer based sensor for measuring phosphate in wastewater samples, Vol. 69, Iss. 1, pp. 48-54

Downloaded from a DSpace repository. https://dspace.org/