

In situ monitoring of environmental water quality using an autonomous microfluidic sensor.

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Abstract—An autonomous microfluidic sensor for phosphate in environmental waters has been developed and assessed in laboratory and field trials. The sensor is based on the molybdenum yellow method for phosphate detection in which a phosphate-containing sample is mixed with a reagent containing ammonium molybdate and ammonium metavanadate in an acidic medium. The yellow-colored compound which is formed absorbs strongly below 400nm and its absorbance is proportional to the concentration of phosphate in the original sample. The sensor utilizes a microfluidic manifold where mixing, reaction and detection take place. Optical detection is performed using a LED (light emitting diode) light source and a photodiode detector. The sensor also combines pumping system, power supply, reagent and waste storage, and wireless communications into a compact and portable device. Here we report the successful use of the sensor to monitor phosphate levels in an estuarine environment.

Keywords; *sensor; microfluidic; water quality; phosphate; environmental monitoring*

I. INTRODUCTION (HEADING 1)

Eutrophication of environmental water bodies is a significant problem in many regions, occurring due to excessive inputs of phosphate and other nutrients. Anthropogenic sources of phosphate pollution include agricultural runoff (fertilizers, animal wastes), treated and untreated municipal wastewater discharges, and industrial sources [1]. Currently, monitoring for phosphate levels in natural waters is generally performed using manual sampling procedures in which a sample is collected and returned to a laboratory for analysis using standard techniques such as the molybdenum blue method [2]. While this approach yields reliable and accurate results (assuming that the correct sampling, storage and analysis protocols are adhered to), the associated high manpower costs mean that monitoring can be performed at only a limited number of locations and at a low frequency. Sampling frequencies of 4 to 12 samples per year are common, with obvious potential for significant pollution events or natural fluctuations to escape detection.

In situ monitoring of phosphate has shown that this kind of ‘snap shot’ monitoring is not adequate to assess risk or to classify a particular waterway and that high temporal resolution

monitoring is needed [3],[4]. The availability of a low cost, autonomous and deployable system for the detection of phosphate in natural waters would therefore be of significant benefit to local authorities, environmental agencies and environmental researchers by facilitating high-frequency monitoring of multiple locations at a cost which is more affordable than current techniques. In particular, such a system would help achieve compliance with the monitoring requirements prescribed by the European Union’s Water Framework Directive [5].

A sensor for phosphate in aqueous samples has been developed and assessed as previously reported [6],[7]. The system has been successfully used to monitor phosphate levels in water discharged from a wastewater treatment plant in Co. Kildare, Ireland [8]. The sensor is based on the molybdenum yellow method for phosphate detection in which a phosphate-containing sample is mixed with a reagent containing ammonium molybdate and ammonium metavanadate in an acidic medium. This results in the formation of vanadomolybdophosphoric acid, a yellow-colored compound which absorbs strongly below 400nm. The absorbance of the compound is measured using a LED (light emitting diode) light source (370nm) and a photodiode detector. Mixing, reaction and detection take place within a custom-designed and fabricated microfluidic chip. The system also contains the components required for sampling, calibration, storage and pumping of the various solutions, power supply, data storage and wireless communications.

In this paper we report the successful use of the sensor to monitor phosphate levels in an estuarine environment in Co. Dublin, Ireland during two separate deployment periods in September-October and October-November 2009.

II. SENSOR DESIGN AND OPERATION

The design of the analyzer has been described in detail elsewhere [9] and is summarized here. The system contains polyethylene bags for storing the reagent, calibration solutions and cleaner, a sample port for collecting and filtering the water sample to be analyzed, and an array of solenoid pumps for pumping the required liquids through the microfluidic chip. The sample port holds a polyethersulfone membrane filter with 0.45 μm pore size, which prevents particulate matter from

entering the microfluidic system. The microfluidic chip allows for the mixing of the reagent and sample. The chip also presents the reacted sample to a LED and photodiode for an absorbance measurement. The analyzed sample is then pumped to the waste storage. All of the fluid handling and analytical components are controlled by a microcontroller that also performs the data acquisition and stores the data in a flash memory unit. A GSM (Global System for Mobile communications) modem is used to communicate the data via the SMS protocol to a laptop computer. The complete integrated system is shown in Fig. 1.

The microcontroller used to control the system is the MSP430F449 (Texas Instruments). This was chosen for its low power consumption during operational and sleep mode. A 2 megabyte SPI flash chip mounted on the PCB with the microcontroller allows for 16,384 data points to be logged. The solenoid pumps and the power to the GSM modem are controlled via an array of field effect transistors (FETs). Power is provided by a 12 V, 7Ah lead acid battery. A photodiode (S1227-33BR, Hamamatsu Photonics UK Limited, Hertfeshire, UK) and a 370nm LED (NSHU550E, Nichia Corporation, Tokushima, Japan) are used for the absorbance measurement. A transimpedance amplifier circuit based around a TLV2772 operational amplifier (Texas Instruments) is used to condition the signal from the photodiode. This circuit is built on a separate board close to the microfluidic chip, thereby limiting the noise on the signal from the photodiode. The entire system is enclosed within a robust and portable case (1430 Case, Peli Products, Barcelona, Spain) which is water- and airtight when closed.

The function of the microfluidic chip is to mix the sample, blank or phosphate standard with the reagent and to present the resulting mixed solution to the LED and photodiode spectrophotometer. To this effect the chip contains a T-Mixer and a micro-cuvette. To ensure mixing at a 1:1 ratio, channels leading from the inlets to the T-mixer are of equal length and cross-sectional area. This ensures that the fluidic resistance is equal for all channels leading to the T-mixer. Provided that the solutions to be mixed are injected at equal pressure they will have equal flow rates when they meet at the T-Mixer and will thus mix at a 1:1 ratio.

The microfluidic chip design is shown in Fig. 2. The three layers were fabricated using a CNC micro-mill (CAT-3D-M6, DATRON, UK) from PMMA (poly methyl-methacrylate) sheets (Radionics, Ireland). The layers were then sonicated in distilled water to remove debris from the machining process. To assemble the chip the mating surfaces are irradiated with UV light at 185 and 254 nm. This process made the surface of the normally hydrophobic PMMA hydrophilic which allows them to be bonded below the glass transition temperature of PMMA [10]. The layers were aligned and assembled using 2mm steel dowel pins placed in alignment holes in the corners. The assembled chip put under pressure using G-clamps and heated to 80° C for 2 hours. 0.8 mm inner diameter PEEK tubes are inserted into the inlet holes as interconnects.



Fig. 1. The prototype phosphate analyser system. (1) Electronics board. (2) GSM modem. (3) Microfluidic chip / detector assembly. The battery, storage bottles, and solenoid pumps are contained within the lower part of the case.

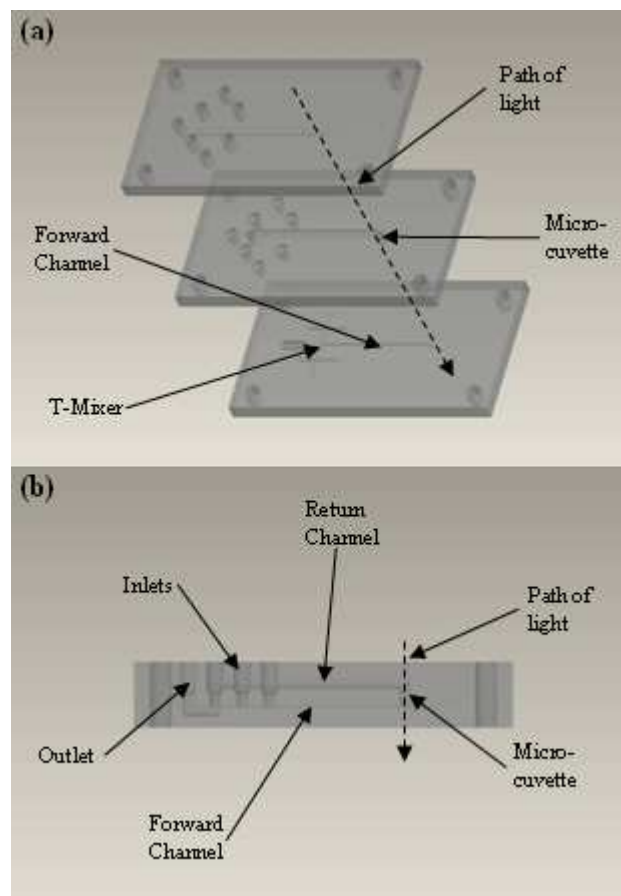


Fig. 2. (a) Exploded view of the microfluidic chip. (b) Side view of fully assembled chip showing the micro-cuvette.



Fig. 3. The phosphate sensor in situ during the trial at Broadmeadow Water, Co. Dublin, Ireland in September 2009. The sample inlet is visible below the water line.

Following a laboratory-based calibration procedure, the sensor was placed *in situ* at Broadmeadow Water in Co. Dublin, Ireland on 25 September 2009. This is an estuarine water body which is known to have significantly elevated nutrient levels due to a combination of inputs from agricultural sources and wastewater treatment plant discharges. The sensor was located at a small islet in one of the estuary channels and held in place using a steel anchoring device. As this was a tidal location, the GSM modem antennae was located outside the box and elevated above the high-water mark to ensure constant network coverage, as the sensor enclosure itself was fully immersed at high tide. The sensor operated with hourly sample frequency, and data was transmitted by the GSM modem in SMS (Short Message Service) mode to a laboratory-based laptop computer at 5 hour intervals. For validation purposes, daily manual samples were collected as close as possible to the sensor's sample inlet, and timed to coincide with the sensor's sampling time. Samples were filtered immediately, and analysed using a Hach-Lange DR890 portable colorimeter and the appropriate reagent pack (amino acid method for high-range phosphate).

III. RESULTS

Figure 4 shows the sensor's output over the trial period. The sensor performed 236 measurements during the period in question with a distinct daily pattern of variation which is ascribed to tidal influences in the estuary. The sensor was located within 100m of a wastewater treatment plant outfall and changing tidal levels would significantly affect the degree of dilution which the wastewater discharges were subject to at a given time. Fig. 4 shows that there was generally good agreement between the phosphate concentrations measured by

the sensor and by the validation method. Some discrepancies were observed, as shown in Fig. 5, and resulting in a relatively low correlation coefficient (R^2) of 0.699.

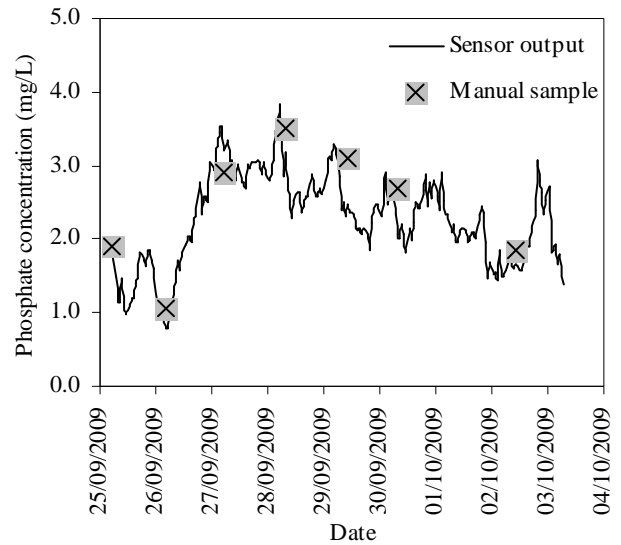


Fig. 4. Phosphate concentrations measured during the first trial period by the sensor and by manual sampling.

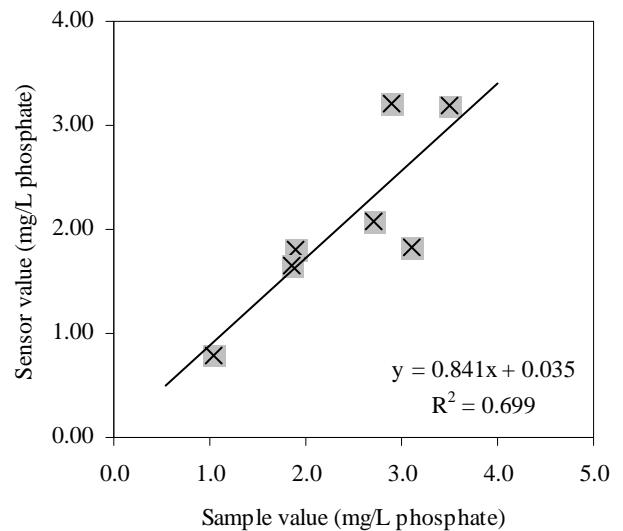


Fig. 5. Sensor output plotted as a function of phosphate concentration as measured by analysis of manually collected samples.

Figure 5 suggests that a high degree of correlation between sensor output and standard sampling-based approaches may be difficult to achieve in sensor deployments. The correlation coefficient (R^2) of 0.699 indicates that significant discrepancies existed between the two datasets, despite the synchronous collection of manual samples from as close to the sensor inlet as possible. Possible sources of error in the sensor data include the small volume (22 μL) of the sample used in the assay, which may be less representative, at a particular moment in time, of the general water body than the 250 mL sample collected manually. Possible sources of error in the manual sampling data include minor inaccuracies in the sample volume

used for analysis (25 mL), or in the volume of the reagent solutions (ammonium molybdate solution and amino acid solution, 1 mL in each case). Perhaps more significantly, differing interfering species for the two methods could also give rise to some discrepancies. The molybdenum yellow method used in the phosphate sensor is subject to interference by dissolved organic substances with light absorption in the UV region. This was unlikely to be a significant factor in this study as after filtering to remove suspended solids, all water samples were observed to have good optical clarity with no significant colour. Silica (Si), arsenate (AsO_4^{3-}) and ferrous iron (Fe^{2+}) are other potential interferants with the molybdenum yellow method [11]. Silica and arsenate, however, are mainly of concern when the reaction is heated to provide faster reaction rates, which was not the case in this study, and ferrous iron does not interfere at concentrations below 100 mg/L. Potential interferants for the amino acid method include (Na^+), nitrites (NO_2^-) and sulfide (S^{2-}) [12]. The estuarine location used in this trial is a complex and dynamic water body, affected by tidal fluctuations and a significant point source of phosphate and other nutrients (wastewater treatment plant discharge). Altering levels of these interferants over time therefore have the potential to cause discrepancies between the sensor data and manual measurements.

Discrepancies between standard, sampling-based approaches to water quality monitoring and sensor-based approaches clearly represent a barrier to wider uptake of sensor technologies. However, despite these considerations, the data shown in Fig. 4 clearly illustrates a key advantage of the deployable monitoring system. The more frequent sensor data showed regular variations in phosphate levels during the trial period which were not captured using manual sampling (even with daily manual sampling, which is not likely to be practicable in larger-scale monitoring procedures). The sensor-based monitoring is therefore more likely to identify short-term pollution events, as well as providing high-resolution data over long timeframes which can provide valuable information on the long term trends for the analyte.

After 8 days of successful operation, the initial trial was terminated due to clogging of the membrane filter by sediment which had collected around the sampling port. To prevent this problem from recurring, some alterations to the sampling port design, and the sensor was deployed close to the original site but at a location with lower susceptibility to sediment build up of on the bed of the channel. Approximately 480 measurements were carried out during this second trial period. Figure 6 shows that the performance of the system during the second trial was generally similar to that obtained in the first trial period, with phosphate levels generally varying between 2 and 6 mg/L, with lower and higher levels observed on a number of occasions. From 07/11/2009, the data became erratic, resulting in the termination of the trial. Subsequent investigations showed that these erroneous data points were caused by a particulate obstruction in the line delivering reagent to the microfluidic chip. In future this possibility will be eliminated by filtering all reagent and calibration solutions. Fig. 7 shows that the correlation between sensor output and analysis of manual samples was improved relative to the first trial period (Fig. 5) with R^2 of 0.908.

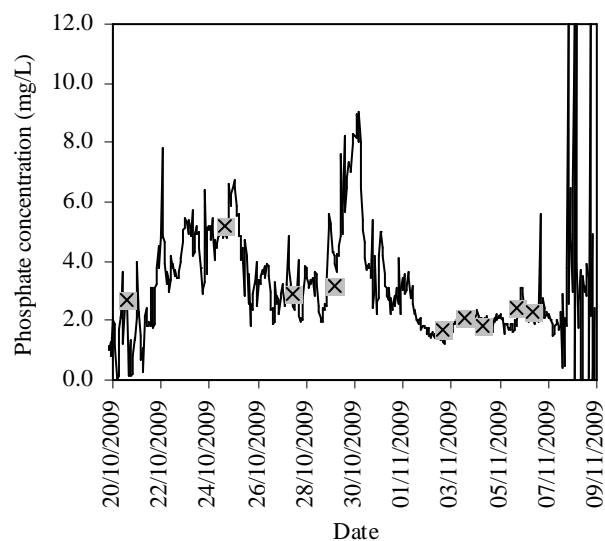


Fig. 6. Phosphate concentrations measured during the second trial period by the sensor and by manual sampling.

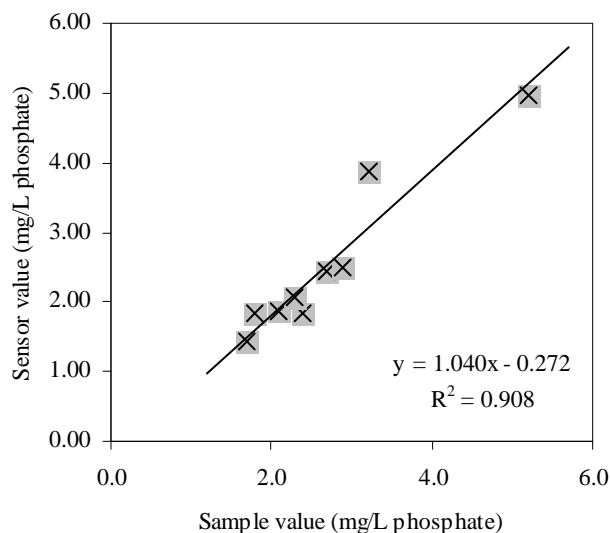


Fig. 7. Sensor output plotted as a function of phosphate concentration as measured by analysis of manually collected samples.

The results obtained in this study show that the current system represents a viable method for monitoring environmental water quality using a low-cost, autonomous sensing device. Further development of the system is ongoing in collaboration with an industrial partner, and this work is focused on optimizing system performance and reliability, and significantly reducing the fabrication cost, power consumption and size of the prototype device so as to provide a commercially viable monitoring system.

REFERENCES

- [1] S. R. Carpenter, N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith, "Nonpoint pollution of surface waters with phosphorus and nitrogen," *Ecological Applications*, 1998, vol. 8, pp. 559-568.

- [2] J. Murphy and J.P. Riley, "A single solution method for the determination of soluble phosphate in sea water," *J. Mar. Biol. Assoc. UK*, 1958, vol. 37, pp. 9-14.
- [3] P. Jordan, J. Arnscheidt, H. McGrogan, and S. McCormick. "High-resolution phosphorus transfers at the catchment scale: the hidden importance of non-storm transfers." *Hydrology and Earth System Sciences*, 2005, vol. 9, pp. 685-691.
- [4] P. Jordan, J. Arnscheidt, H. McGrogan, and S. McCormick. "Characterising phosphorus transfers in rural catchments using a continuous bank-side analyser". *Hydrology and Earth System Sciences*, 2007, vol. 11, 372-381.
- [5] "Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy." *Official Journal of the European Union*, 2000, OJ L 327.
- [6] M. Bowden, and D. Diamond. "The determination of phosphorus in a microfluidic manifold demonstrating long-term reagent lifetime and chemical stability utilizing a colorimetric method." *Sensors and Actuators B*, 2003, vol. 90, pp. 170-174.
- [7] McGraw, C. M., Stitzel, S. E., Cleary, J., Slater, C. and Diamond, D. "Autonomous microfluidic system for phosphate detection." *Talanta*, 2006, vol. 71, pp. 1180-1185.
- [8] Cleary, J., Slater, C., McGraw, C. M. and Diamond, D. "An autonomous microfluidic sensor for phosphate: on-site analysis and of treated wastewater." *IEEE Sensors Journal*, 2008, vol. 8, pp. 508-515.
- [9] C. Slater, J. Cleary, C. M. McGraw, W.S. Yerazunis, K.T. Lau, and D. Diamond, "Autonomous field-deployable device for the measurement of phosphate in natural water." *Proc. of SPIE*, 2007, vol. 6755, pp. 67550L1-67550L8.
- [10] C.W. Tsao, L. Hromada, J. Liu, P. Kumar, and D.L. DeVoe. "Low temperature bonding of PMMA and COC microfluidic substrates using UV/ozone surface treatment." *Lab on a Chip*, 2007, vol. 7, pp. 499-505.
- [11] C. Neal, M. Neal, H. Wickham, *Sci. Total Environ.* 2000, vol. 251/252, pp. 511-522.
- [12] Hach Company "DR/890 Datalogging Colorimeter Handbook", 1999, pp. 523-528.