

Analysis of Phosphate in Wastewater Using an Autonomous Microfluidics-Based Analyser

John Cleary, Conor Slater, and Dermot Diamond

Abstract—A portable sensor for the analysis of phosphate in aqueous samples has been developed. The sensor incorporates microfluidic technology, colorimetric detection, and wireless communications into a compact and rugged portable device. The detection method used is the molybdenum yellow method, in which a phosphate-containing sample is mixed with a reagent containing ammonium metavanadate and ammonium molybdate in an acidic medium. A yellow-coloured compound is generated and the absorption of this compound is measured using a light emitting diode (LED) light source and a photodiode detector. The absorption is directly proportional to the phosphate concentration in the original sample. In this paper we describe the application of this phosphate sensor to the analysis of wastewater at a municipal wastewater treatment plant in Co. Kildare, Ireland.

Keywords—Microfluidic, phosphate, sensor, wastewater.

I. INTRODUCTION

POLLUTION of natural waters by excessive inputs of nutrients is a serious issue in many countries. Elevated nutrient levels lead to excessive growth of algae and other aquatic fauna, which in turn cause oxygen depletion, leading to fish kills in severe cases. Algal blooms can result in water becoming toxic to animals and humans, impairing the use of water resources for drinking, recreation and other purposes. Phosphate is of particular significance in relation to rivers and lakes as it is typically the limiting nutrient in these freshwater ecosystems. Phosphate inputs typically come from agriculture (fertilizers and animal wastes), municipal wastewater discharges (treated and untreated) and industrial sources [1].

Due to the widely acknowledged need to protect water resources, and to ensure compliance with increasingly stringent environmental legislation, such as the European Union's Water Framework Directive (WFD), there is growing demand for improved monitoring of environmental water quality [2]. Currently most water quality monitoring is performed using manual collection of samples followed by laboratory analysis using a range of techniques. This approach provides accurate and reproducible data on the selected analytes, provided that the sampling, transport, storage and

analysis protocols are correctly observed, however it also involves significant manpower requirements and high costs. For this reason, samples are generally collected from a limited number of locations at relatively long intervals, typically monthly or quarterly. There is therefore obvious potential for short-lived pollution incidents or other significant events to be missed [3].

Autonomous monitoring systems have the potential to significantly improve our ability to monitor the quality of the aquatic environment by allowing continuous or high frequency measurements of water quality parameters at a higher temporal and geographic density than is achievable using current approaches. A key requirement of these systems is the ability to function reliably for long periods of time (months – years) without the need for frequent maintenance visits.

Microfluidic analysis systems offer the ability to perform rapid analysis of samples while minimizing the amounts of sample, reagents, waste storage, and power required [4]–[6]. In combination with low power wireless communications systems, this technology enables the development of compact, low-cost analysis systems which can provide reliable information on the state of natural waters over extended deployment periods. In this paper we describe a prototype autonomous microfluidic analyser for phosphate in water, and the application of this system to the analysis of the discharge from a municipal wastewater treatment plant (WWTP) in Co. Kildare, Ireland.

II. DESIGN AND OPERATION OF THE ANALYSER

A. Sensing Principle

The analyser is based on the molybdenum yellow method. This method involves mixing the sample with an equal volume of a reagent containing 7.143 g of ammonium molybdate and 0.357 g of ammonium in an acidic medium (95 mL of H₂SO₄) in 1 L of deionised water (DIW). Vanadomolybdophosphoric acid is formed, which is yellow in colour and absorbs strongly below 400nm. The absorbance is measured using a LED light source (370 nm) and a photodiode detector, and is directly proportional to the concentration of phosphate in the original sample. The major advantage of this detection scheme is the excellent stability of the reagent, which allows the same solution to be used for over 1 year without significant loss of activity [5], thus allowing long deployments to be carried out without the need for replacing the reagent solution.

John Cleary is with the National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland (phone: +353-1-7007926; fax: +353-1-7007995; e-mail: john.cleary@dcu.ie).

Conor Slater is with the National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland (e-mail: conor.slater@dcu.ie).

Dermot Diamond is with the National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland (e-mail: Dermot.diamond@dcu.ie).

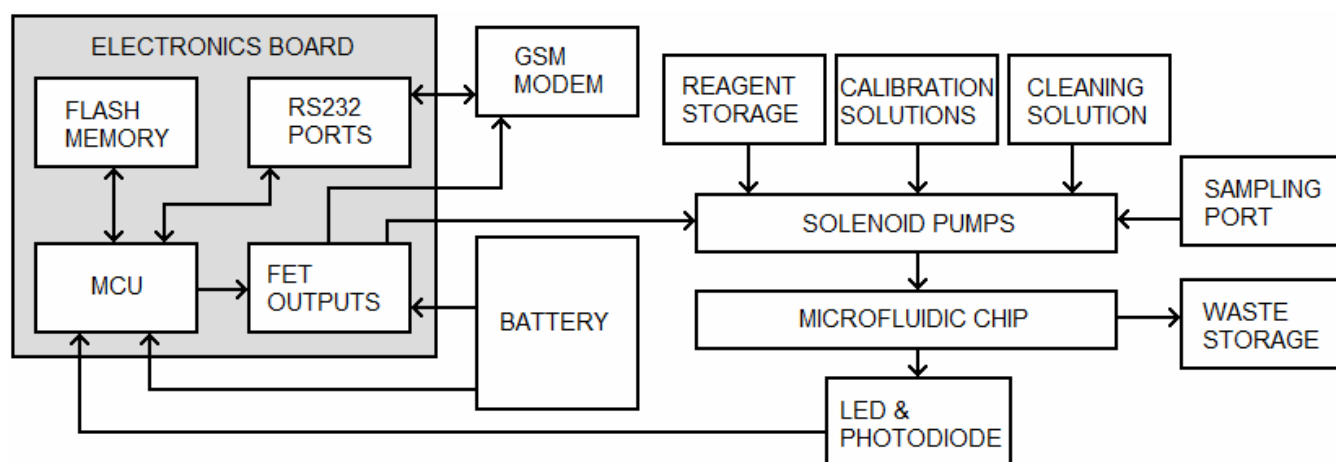


Fig. 1 Schematic representation of the analyser system, showing the various components and their relation to each other

B. Analyser Design and Operation

The design of the analyser has been described in detail elsewhere [7] and is summarized here. Fig. 1 is a schematic of the system and its mode of operation. The system contains bottles for storing the reagent, calibration solutions and cleaner, a sample port for collecting the water sample to be analysed, and an array of solenoid pumps for pumping the required liquids through the microfluidic chip. The microfluidic chip allows for the mixing of the reagent and sample. The chip also presents the reacted sample to a photodiode and LED 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 modem is used to communicate the data via the SMS protocol to a laptop computer.

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, Hertfershire, 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 air-tight when closed. The complete integrated system is shown in Fig. 2.



Fig. 2 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

C. Microfluidic Chip Design

A microfluidic chip is used as a platform for the phosphate measurement. This allows for small volumes of reagent and sample (22 μ L of each solution) to be used per measurement. The reagent and sample are mixed in a T-mixer which leads into a cylindrical optical cuvette (5 mm length \times 1 mm diameter) where the resulting solution is presented to the emitter and photo-detector for the absorbance measurement. Each side of the T-mixer has three inlet channels leading into them. On one side are the two phosphate standard solutions for a two point calibration. A third inlet channel is for the water sample. On the other side of the T-mixer there is an inlet

for the reagent and two inlets for cleaning solution. All channels are of 200µm in width and depth. The chip is manufactured from PMMA sheet (ME303011, Goodfellow, Cambridgeshire, England). The microfluidic channels are milled into the sheet using the CAT-3D-M6 micromill (DATRON Electronic, Mühlthal, Germany) using a 200µm endmill (00680029, DATRON Electronic, Mühlthal, Germany). The 35×20 mm chip layers are bonded using a pressure sensitive adhesive (ARcare® 8890, Adhesives Research, Limerick, Ireland). PEEK tubes are inserted into the inlet and outlet ports and are held in place using epoxy adhesive. The completed microfluidic chip is shown in Fig. 3.

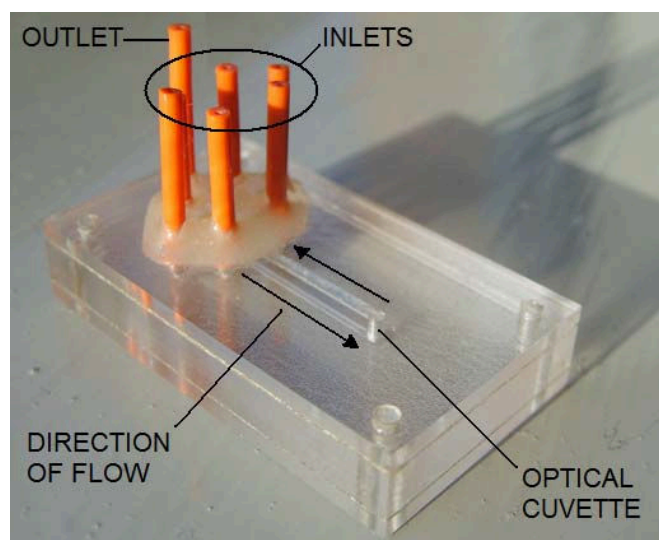


Fig. 3 Photograph of the microfluidic chip

D. Setup for sensor trial

The sensor trial was carried out over a 6-week period in July and August 2008, at Osberstown Wastewater Treatment Plant in Co. Kildare, Ireland. The analyser was situated within the monitoring building which houses the WWTP's online monitoring systems for phosphate, ammonia and nitrate. Sample is pumped from the final effluent tank adjacent to the monitoring building, and a valve was added to the sample line to deliver sample to the prototype phosphate analyser's sample inlet. The sample inlet consisted of a modified membrane filter holder (shown schematically in Fig. 4) which allowed sample to contact a membrane filter (Supor membrane filter, 0.45 µm pore size, 25 mm diameter, Sigma-Aldrich) and then exit to waste. Sample was drawn through this membrane and into the analyser itself by a solenoid pump which delivered sample to the microfluidic chip.

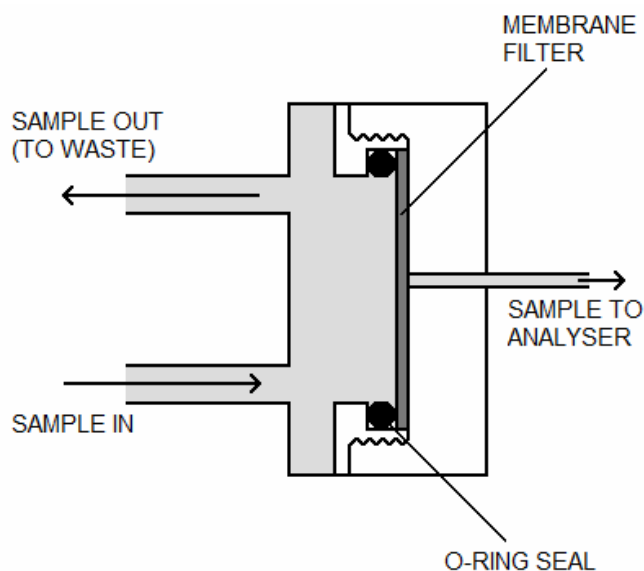


Fig. 4 Schematic cross section of the sampling port. The threaded parts of the membrane holder are screwed together so that the membrane is held in place by the O-ring seal. Sample circulates past the outer side of the membrane and the required volume is drawn into the analyser by a solenoid pump

In order to compensate for variation in the detector output due to thermal fluctuations, as well as signal drift over long time periods, a calibration procedure was automatically carried out for each sample measurement. This was performed by carrying out separate reaction and detection runs using blank (0 mgL⁻¹ phosphate) standard (10 mgL⁻¹ phosphate) solutions. The absorbance of the standard phosphate solution was calculated using the formula:

$$A = \log(I_o/I) \quad (1)$$

where A is absorbance, I_o is the measured light intensity for the blank solution, and I is the measured light intensity for the standard solution. The absorbance for the sample solution was calculated similarly, and was converted to a value for phosphate concentration using the absorbance of the standard solution (known concentration). Finally, the sample's phosphate concentration value was converted to "phosphorus as phosphate" (P-PO₄) in order to facilitate comparison with the plant's existing online phosphorus monitor (Aztec P100 instrument, Capital Controls, UK). Data from the latter system was downloaded via serial connection to a laptop computer at weekly intervals.

III. RESULTS AND DISCUSSION

The entire dataset from the 6 week trial is presented in Fig. 5. Over the period as a whole there is excellent agreement between the data from the prototype sensor and that from the online monitoring system. Between days 3 and 7 of the trial there are a number of spurious high readings in the prototype sensor data. These were caused by air bubbles accessing the external sample line and being drawn into the microfluidic

chip and interfering with the passage of light through the optical cuvette. On day 7 of the trial the valve on the sample line was adjusted which resulted in this issue being almost

entirely eliminated, with only 2 further such readings observed (days 20 and 28).

There were also a number of occasions on which the online

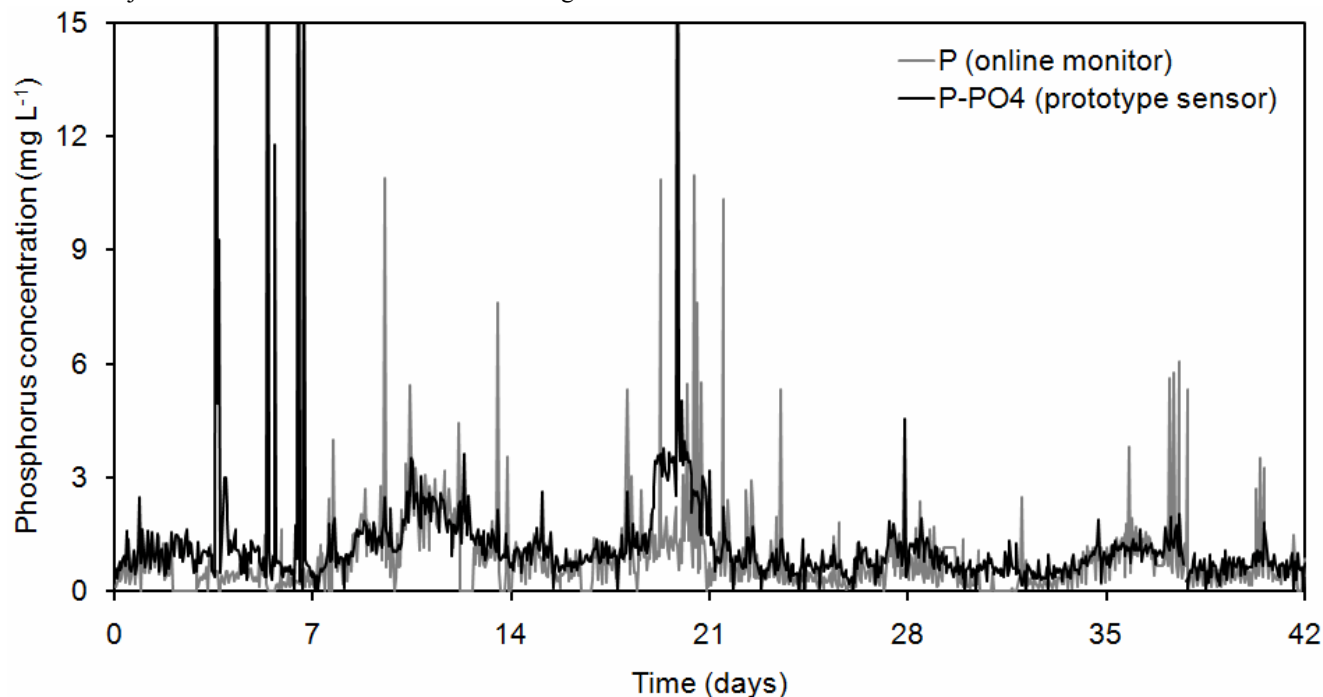


Fig. 5 Output from the prototype sensor and the plant's online phosphorus monitor during the trial period

monitor recorded elevated values which were not detected by the prototype sensor. These discrepancies were caused by solids in the sample affecting the online monitor, which has only a coarse filter at its inlet point. The online monitor uses heating and acid to digest solids in the sample, followed by analysis using the blue molybdate method for phosphate detection. Any solid material therefore has the potential to increase the measured value (acid-hydrolysable phosphorus). This is not the case with the prototype sensor, as solids are excluded from the system using the 0.45 μm filter membrane at the inlet point, in order to avoid blockage or fouling of the microfluidic channels.

Based on the trial data presented here, the prototype phosphate sensor has considerable potential to be used for monitoring of phosphate in wastewater streams. The performance of the prototype device was comparable with that of an existing commercially available phosphorus monitor with less maintenance required. The prototype device (based on current fabrication cost and estimated savings if fabricated on a larger scale) is also much less expensive. Running costs of the prototype system are much lower due to the minimal amount of reagent and other solutions required, and the power consumption is also much reduced compared to the online system. The portability and autonomous operation of the prototype device also allow it to be used for monitoring of environmental water quality. Further optimization of the detection system is required to improve the limit of the detection (currently 0.3 mgL^{-1}) in order to maximize the potential of the system for this application.

ACKNOWLEDGMENT

The authors would like to thank Fergal Cronin (Plant Manager) and Fergal Humphreys (Plant Chemist) for facilitating the sensor trial at Osberstown Wastewater Treatment Plant.

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*, Vol. 8, Aug. 1998, pp. 559-568.
- [2] M. Coquery, A. Morin, A. Bécue and B. Lepot, "Priority substances of the European Water Framework Directive: analytical challenges in monitoring water quality," *Tr. Anal. Chem.*, Vol. 24, Feb. 2005, pp. 117-127.
- [3] P. Jordan, J. Arnscheidt, H. McGrogan, S. McCormick, "High-resolution phosphorus transfers at the catchment scale: the hidden importance of non-storm transfer," *Hydrology and Earth System Sciences*, 9, Dec 2005, pp. 685-691.
- [4] M. Bowden, M. Sequeira, J.P. Krog, P. Gravesen, and D. Diamond, A prototype industrial sensing system for phosphorus based on micro system technology, *Analyst*, Jan. 2002, 127, pp. 1-4.
- [5] M. Bowden, M. Sequeira, J.P. Krog, P. Gravesen, and D. Diamond, "Analysis of river water samples utilising a prototype industrial sensing system for phosphorus based on micro-system technology," *J. Environ. Monit.*, Aug. 2002, 4, pp. 767-771.
- [6] A.G. Crevillen, M. Hervas, M.A. Lopez, M.C. Gonzalez, and A. Escarpa, "Real sample analysis on microfluidic devices," *Talanta*, 74, Oct. 2007, pp. 342-357.
- [7] 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*, Vol. 6755, Sept. 2007, 67550L1-67550L8.