Validation of a fully autonomous phosphate analyser based on a microfluidic lab-on-a-chip

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Abstract This work describes the design of a phosphate analyser that utilises a microfluidic lab-on-a-chip.The analyser contains all the required chemical storage, pumping and electronic components to carry out a complete phosphate assay. The system is self-calibrating and self-cleaning, thus capable of long-term operation. This was proven by a bench top calibration of the analyser using standard solutions and also by comparing the analyser's performance to a commercially available phosphate monitor installed at a waste water treatment plant. The output of the microfluidic lab-on-a-chip analyser was shown to have sensitivity and linear range equivalent to the commercially available monitor and also the ability to operate over an extended period of time.
Keywords Automated, Microfluidics, Lab-on-a-Chip, Phosphate, Sensor, Wireless

INTRODUCTION

Phosphate is the most common form of the essential plant and animal nutrient phosphorus and significantly contributes to eutrophication, the process of natural water becoming over enriched with nutrients. Eutrophication causes water systems to become overgrown with plants and algae, which leads to large algal blooms and also decimation of fish populations, due to the depletion of dissolved oxygen (Smith et al. 1999). Although phosphate occurs naturally there are a number of human activities that are the main source of excessive phosphate and other nutrients. These include the dumping of untreated and treated waste water from sewerage systems, agricultural sources such as fertilisers and animal waste, and waste from industrial processes (Mainstone & Parr 2002). In order to control this problem more waterways will have to be monitored (European Council 2000). Continuous in situ monitoring of phosphate has shown that 'snap shot' sampling is not adequate to assess risk or to classify a particular waterway and that high temporal resolution monitoring is needed (Jordan et al. 2005, 2007).

The most common instruments for measuring phosphate are categorised as Reagent-based optical chemical sensors (ROCS). They have many advantages over competing technologies such as electrochemical sensors. This is due to available reagents having good selectivity and simple characterisable responses to their associated target analytes. Previous work on these sensors has demonstrated that stable, calibration free, sensing systems, where the reagent is renewed for each measurement, can be created (Degrandpre et al. 1999) This allows the construction of automated systems that can achieve high temporal resolution over a long period of time (Seidel et al. 2008). However, these systems require highly stable light sources and detectors to operate.

Automated ROCS are commercially available to monitor phosphate. Examples such as the AZTEC P100 (Severn-Trent, UK) and the TresCon OP210 (WTW, Germany) are most commonly installed to monitor effluent at Waste Water Treatment Plants (WWTP). These systems provide high quality data on a continuous basis. Due to the need for mains electricity and the size of these systems, they are not portable, making it difficult for them to be deployed for in situ monitoring of a waterway. Hand held colorimeters such as the POCKET Colorimeter II (Hach-Lange, Germany) are light weight, battery powered and highly portable. This allows them to be used to measure phosphate concentrations in situ. The drawback is that the colorimeter has to be operated manually, with the

user preparing a blank solution in addition to mixing the reagent with the sample before taking the measurement.

A potential alternative to these systems is to use a Lab-on-a-Chip (LOC). This is an integration of a number of active and passive components into a single chip. The chip performs operations normally carried out in a biology or chemistry laboratory but on a miniature scale (Auroux et al. 2002). It is analogous to the electronic integrated circuit developed in the late 1950s (Thorsen et al. 2002). The micro-channels used in LOCs allow liquids to be controlled easily because all flow is laminar on the microscale (Gravsen et al. 1993). Also, it has been shown that micro-channel networks can be designed like electronic resistor networks allowing complex operations to be carried out with simple inputs (Yamada et al. 2006).

The molybdenum yellow method for phosphate determination has proven to be suitable for LOC analysers. The reagent used produces a complex product that absorbs strongly below 400nm when added to a solution of phosphate. The use of this method in a microfluidic LOC was demonstrated utilising an LED and a bench top spectrophotometer for measuring absorbance and a bench top syringe pump for pumping liquids. Further study also determined that the reagent had no significant degradation over a period of one year (Bowden et al. 2002a,b; Bowden & Diamond 2003). Other researchers have demonstrated bench top systems in conjunction with LOCs to measure nitrate and ammonia, opening the possibility of creating an integrated system that measures multiple parameters (Greenway et al. 1999; Sequeira & Diamond 2002).

Subsequent work had led to the development of an autonomous portable phosphate analyser. This analyser had all the required reagent and waste storage, fluid pumping and optical transducer components to carry out phosphate assays autonomously powered by a lead-acid battery. The key to the portability was the use of an LED-photodiode spectrophotometer and a miniature peristaltic pump. However, the analyser had to be manually calibrated and the peristaltic pump was proven to be unreliable. As a result the analyser's design was not suitable for long-term operation (McGraw et al. 2006; Cleary et al. 2008).

Here, a fully autonomous portable analyser utilising a new robust sensor design based on a microfluidic LOC is described. This paper focuses on showing the performance of the analyser in terms of quality of data and robustness. The design of the sensing component of the analyser is described. Its performance in a laboratory is demonstrated by quantifying its limit of detection and linear range. The performance of the presented analyser is quantified and compared to commercially available online phosphate monitors and a hand held phosphate analyser. To further validate the performance of the system, it is compared to a phosphate monitor at a wastewater treatment plant (WWTP). The results of a long-term trial directly validating the LOC analyser to a commercially available phosphate monitor are presented. This work shows that LOC technology can be successfully employed to create an analyser that combines the automation and reliability of commercial phosphate monitoring systems with the portability of hand held analysers, allowing it to be used to monitor fresh water rivers and lakes in situ.

METHODS

The sensor design is based on discrete injection of the reagent and sample by metering pumps into a microfluidic LOC. The design of the LOC passively controls the flow from metering pumps allowing mixing of the sample and reagent and the subsequent presentation of the reacted sample to the analyser's LED-photodiode spectrophotometer. The chemicals and components used, the layout

and fabrication method of the microfluidic LOC, and the standard operating procedure of the sensor are described here. As in previous designs (McGraw et al. 2006; Cleary et al. 2008), it contains all the components necessary to complete a phosphate assay. But in addition to this the analyser is self calibrating and self cleaning, thus overcoming the problem of sensor drift. It holds enough reagent, cleaning and calibration solutions for over 3,000 assays. A microcontroller is used to make the analyser completely autonomous. Similarly to other field deployable analysers it is also able to communicate data to a GSM modem equipped laptop via SMS.

Chemistry

The reagent and solutions were prepared following recipes published previously (McGraw et al. 2006; Cleary et al. 2008). The molybdovanadophosphoric acid method ("yellow method") of phosphate detection uses a highly stable reagent, which is thus suitable for long-term storage in a field deployable system (Bowden & Diamond 2003). Unlike the "molybdenum blue method" the reagent does not form a large amount of precipitate that would otherwise clog micro-channels and so is suitable for use in an LOC (Bowden et al. 2002a,b; Bowden & Diamond 2003). In practice the reagent is mixed at a 1:1 ratio with the water sample. The resulting solution turns yellow, which is measured as an absorbance below 400 nm. The major drawbacks of this reagent are its low molar absorptivity and that it is also a strong acid that readily corrodes stainless steel. The means that optical components have to be extremely sensitive and the materials of wetted components have to be carefully chosen.

The solutions required for the completed analyser were 250mL of reagent, 250mL of 10 mg/L phosphate standard and 750mL of a blank 0 mg/L phosphate standard. 250mL of the blank standard were used in calibration and the other 500mL were used as a cleaning solution. A further 1, 2, 5, 10, 20 and 50 mg/L phosphate standards were prepared for laboratory trials of the analyser.

Microfluidic Lab-on-a-Chip

The function of the microfluidic LOC 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 microcuvette. The micro-channel network is detailed in Figure 1 with the layout of the microcuvette where the absorbance measurement is taken. All of the microchannels have a square cross-section of 200 £ 200 mm. The microcuvette has a round cross-section of 1mm diameter and is 3mm in length.

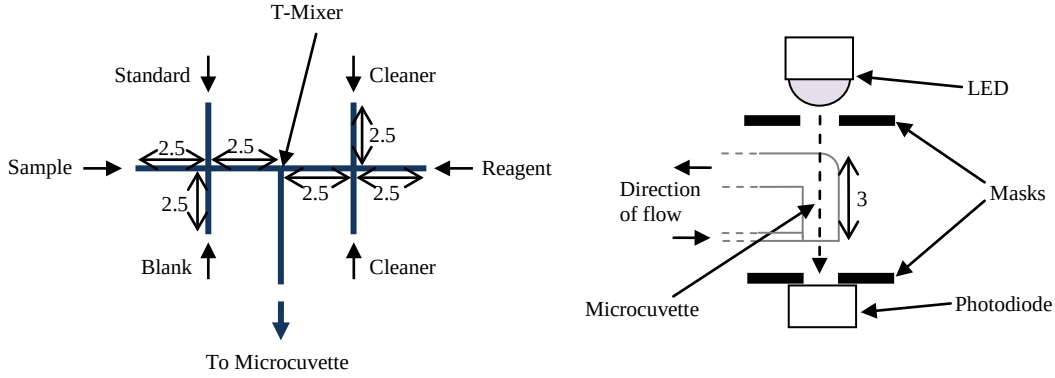

Figure 1. (Left) Microchannel network. (Right) Arrangement of microcuvette and optics. (All dimensions in mm)

All channels leading to the T-mixer are of equal length and cross-sectional area to give each of the channels an equal fluidic resistance. 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 (Takabayashi et al. 2008). The mixing and subsequent reaction of the reagent and sample takes place in under 10 min in a 200 £ 200 mm channel (Cleary et al. 2008). Flow of the sample and reagent is stopped to accommodate this long period of time and negating the need for a long mixing channel. The absorbance measurement is taken while the mixed solution is held stationary in a vertical microcuvette.

Figure 2 shows an exploded view of the LOC. The three layers are fabricated using a CNC micro-mill (CAT-3D-M6, DATRON, UK) from poly methyl-methacrylate (PMMA) sheets (Radionics, Ireland). PMMA was chosen as it has relatively simple fabrication methods associated with it and its use in previous analysers proved unproblematic (Cleary et al. 2008). The layers were then sonicated in distilled water to remove debris from the machining process. To assemble the chip the mating surfaces were irradiated with UV light at 185nm and 254 nm. This process made the surface of the normally hydrophobic PMMA layers hydrophilic, which would allow them to be bonded below the glass transition temperature of PMMA (Tsao et al. 2007). The layers were aligned and assembled using 2mm steel dowel pins placed in alignment holes in the corners. The assembled chip was put under pressure using G-clamps which were tightened by hand and heated to 858C for 2 h. 0.8mm inner diameter PEEK tubes were inserted into the inlet holes as interconnects.

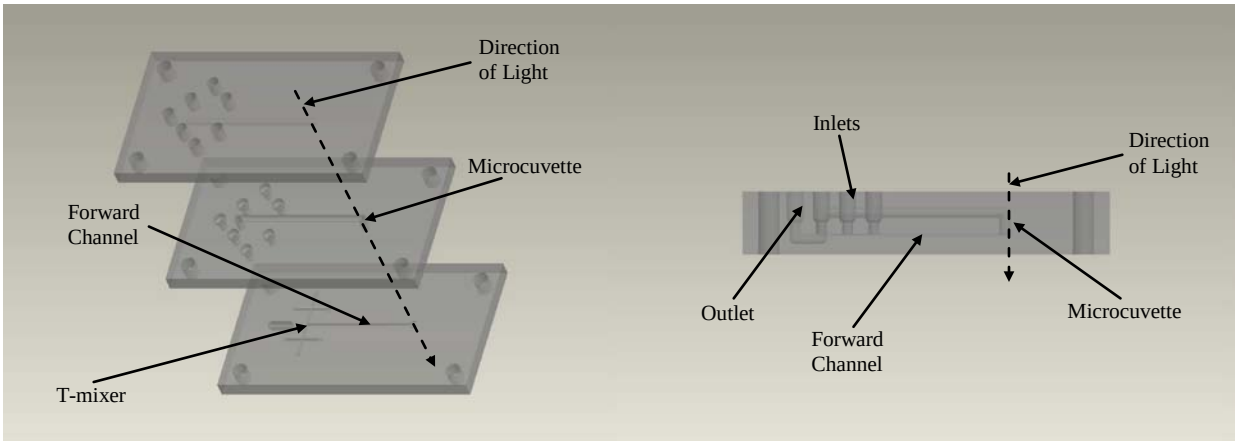

Figure 2. (Left) Exploded view of the microfluidic lab-on-a-chip. (Right) Side view of fully assembled LOC showing the micro-cuvette.

Analyser components

For the LED-Photodiode spectrophotometer a LED of peak wavelength of 375nm was chosen (NSHU590A, Nichia, Japan) to measure the absorbance at this wavelength. Also, in order to limit the effect of ambient light a photodiode with a bandwidth of 200 to 590nm was chosen (EDP- 440-0, Roithner Lasertechnik, Austria). These components were combined with a transimpedance amplifier circuit based on a TLV2772 operational amplifier (Texas Instruments, USA) to create the spectrophotometer.

An array of six solenoid actuated metering pumps (120SP, Bio-Chem Valve, USA) were used to inject each of the sample, calibration standards, reagent and cleaning solutions into the LOC. These

pumps were set to inject 20mL per stroke. The reagent, calibration solutions and cleaners are stored in five square 250mL HDPE bottles (NALGENE 2018-0250, Nalge-Nunc, USA). Two square 1L HDPE bottles were used for storing waste from the phosphate assay (NALGENE 2018-1000, Nalge-Nunc). This gave the analyser enough stored chemicals to perform 3,000 phosphate assays.

The bottle caps used (Q-Series, Bio-Chem Valve) have 14 ” 2 28UNF ports and were fitted with TEFLONw check valves, which allowed air into the bottle to replace the liquid pumped out without letting gases from the chemicals escape and corrode the systems components. As with previous work that determined the suitability of the reagent for real river water samples (Bowden et al. 2002a,b), the sample water was drawn from outside the analyser through a 0.45 mm pore diameter filter membrane (Suporw, PALL Corporation).

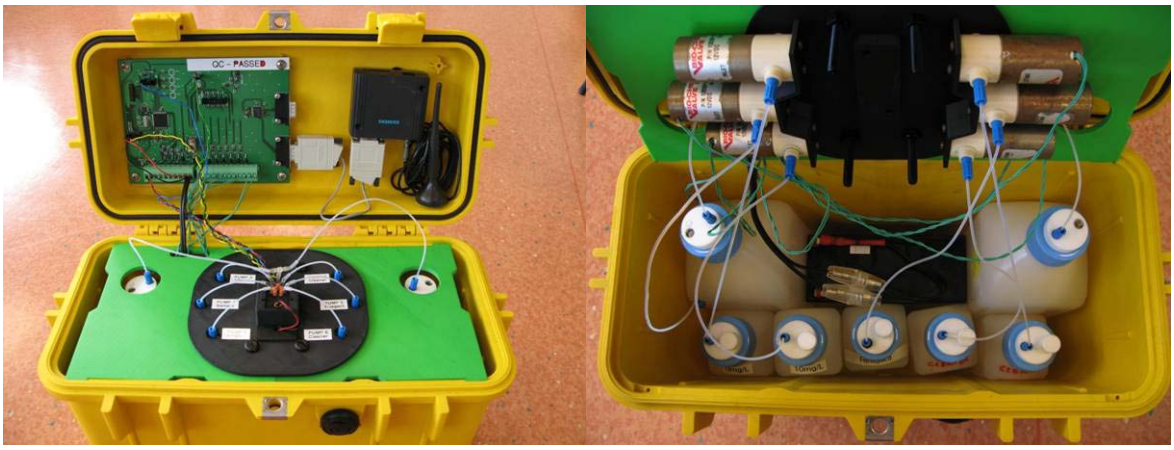

Figure 3. (Left) View of the analyser showing the location of the microfluidic lab-on-a-chip (1), the control board (2), GSM modem (3). (Right) View of the pumps (4), battery and chemical storage (5).

Figure 3 shows the fully assembled analyser and labels the location of its key components. The control board operates the pumps and acquires data from the LEDPhotodiode spectrophotometer. The GSM modem allows the data to be collected remotely, making the analyser similar to the data buoys used in the MySound project (Tedesco et al. 2003). The analyser can run off a 12V 7Ah lead acid battery that would allow the analyser to measure 3,000 samples and be on standby for an estimated 300 days. The battery is not used in these experiments and the analyser is powered from mains electricity.

Laboratory validation of system

To evaluate the performance of the analyser, a series of 4 separate calibrations were carried out where a 1, 2, 5, 10, 20 and 50 mg/L phosphate (PO_4^{3-}) standard solution were measured 4 times each. The concentration of each of the standard solutions was calculated by the analyser based on an internal two point calibration. There were three separate steps in each assay where the reagent was allowed to react for a period of 8 min with each of two standard solutions and a blank. The intensity of light passing through the microcuvette was measured by the spectrophotometer after each reaction. First, the reagent was mixed with the 10 mg/L PO_4^{3-} standard solution and measured. Then, the sample, which consisted of one of the six phosphate standards, water was drawn from the outside of the enclosure to the LOC. The sample was reacted with the reagent and the second light intensity measurement was recorded. Thirdly, the blank was mixed with the reagent and the third light intensity measurement was recorded. In between each mixing and intensity measurement the LOC was purged with a blank solution.

Using the three intensity measurements the absorbance of the sample and the absorbance standard solution is calculated. Given than the blank is known to have zero concentration and the concentration of the standard is known to be 10 mg/L PO_4^{3-} the concentration of the sample can be determined by linear interpolation. The data from the calibrations was collected and the linear range and the limit of detection were determined for the analyser.

Validation at waste water treatment plant

The performance of the analyser was compared to a commercially available phosphate analyser (Aztec P100, Severn Trent) at a WWTP over a 38 day period. It should be noted that the analysers differ slightly in their operation when measuring phosphate. The Aztec P100 incorporates an initial digestion step allowing the analyser to measure phosphate contained in solid particles. The LOC analyser draws sample water through a fine filter (450nm pore size) thus excluding larger particles. This difference in operation causes the output of a LOC analyser to deviate from analysers that incorporate a digestion step (Cleary et al. 2008).

During the validation the LOC analyser was set to a sample rate of once per hour. The Aztec P100 sampled at a rate of every 15 min; only the samples that were taken at the same time as the LOC analyser are used in the validation. All data collected by the LOC analyser were transmitted to a laptop equipped with a GSM modem. A total of 906 assays were validated against the commercial phosphate monitor. At 450 assays the sample filter on the LOC analyser was exchanged.

RESULTS AND DISCUSSION

The plot in Figure 4 shows the output of the sensor versus the actual phosphate concentrations that were measured. It can be seen that the analyser accurately returns the value of the standard solution up to 5 mg/L PO_4^{3-}. Although the plot may be a typical indication of the spectrophotometer being saturated, this cannot be the case as the path length of the LOC's microcuvette is not long enough and the molar absorbtivity of the reagent in not high enough to cause this effect.

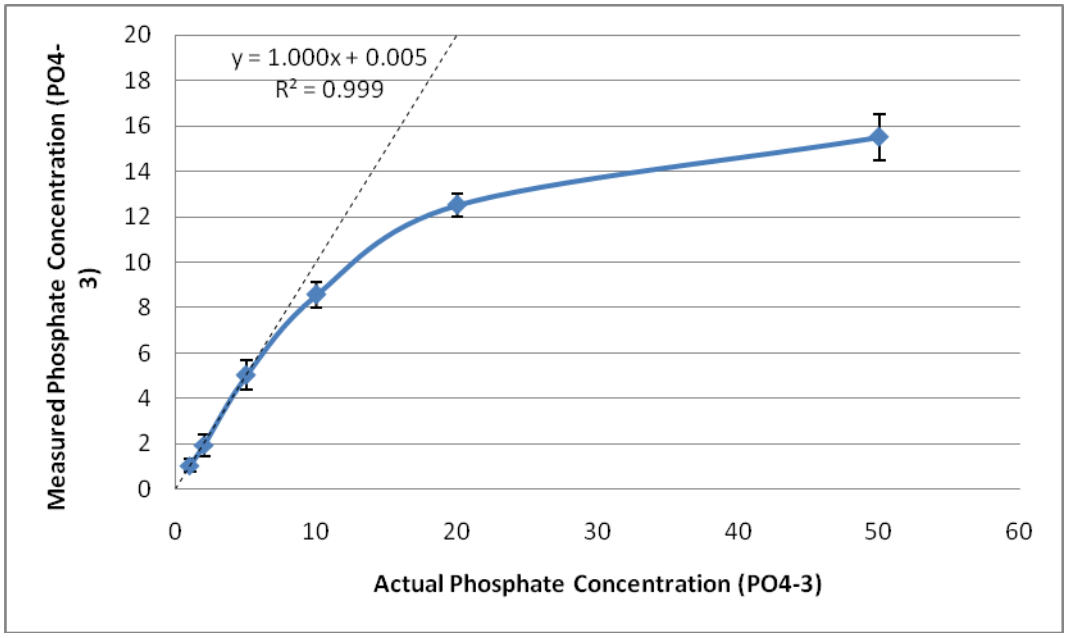

Figure 4. Results of the laboratory evaluation of the LOC analyser showing the concentration measured by the analyser versus the actual concentration of the standard solutions.

The deviation from the predicted sensor performance is due to staining of the LOC by the coloured precipitate formed after the reagent reacts with the phosphate standard solution. If the staining is too substantial to be removed in the cleaning operation it increases subsequent absorbance measurements taken in the two point calibration and the analyser's output is affected. Higher concentrations of phosphate cause more significant staining of the LOC, hence the large deviation for the 50 mg/L PO_4^{3-} standard solution. Over a large number of measurements gradual staining also occurs, but as the blank and standard solution are known concentrations these can be used to compensate for the slight drift between measurements.

The limit of detection (LOD) is determined by measuring the baseline noise of the analyser over a number of assays. A series of 10 replicates of a blank mixed with reagent were recorded and the standard deviation of these values was calculated. The LOD is defined as three times the standard deviation. For the LOC system the LOD was determined as 0.3 mg/L PO_4^{3-}.
The precision of the analyser is also determined by the baseline noise giving a precision of ±0.3 mg/L PO_4^{3-}..

To compare the LOC analyser to other commercially available phosphate analysers the values for the limit of detection and the linear range were converted from phosphate (PO_4^{3-}) to total phosphorus (P-PO_4^{3-}) giving the analyser a limit of detection of 0.1 mg/L P-PO_4^{3-}. The comparison is detailed in Table 1.

Make	Developer	LOD (mg/L P-PO_4^{3-})	Range (mg/L P-PO_4^{3-})	Attributes
Lab-on-a-Chip Analyser	Slater, et al.	0.1	0.1 - 1.6	Automated, Portable
Aztec P100	Severn-Trent	0.1	0.1 - 2.5	Automated
TresCon OP210	WTW	0.05	0.05 - 3	Automated
POCKET Colorimeter II	Hach-Lange	0.05	0.05 - 1.5	Portable

Table 1. Comparison of LOC analyser to commercially available online and handheld instruments.

Figure 5 shows a plot comparing the output of the LOC analyser to the Aztec P100 phosphate monitor. Both analysers sample the effluent from a WWTP in parallel. A total of 906 assays were validated against the commercial phosphate monitor over a 38 day period. The outputs of both analysers follow the same trend in phosphate concentration with a mean point for point deviation of 0.3 mg/L PO_4^{3-} in results within the linear range of the LOC analyser. However, there are a number of occasions where both analysers differ significantly from each other in measured concentration. A deviation of the two analysers from each other observed after 450 h was due to the filter becoming clogged with solids. After the filter was replaced correlation in the data was resumed. Spikes in the plot of the LOC analyser are due to bubbles in the microcuvette interfering with the absorbance measurements or are due to high concentrations of phosphate. Since the plant monitor does not measure high concentrations at these points it is reasonable to assume that spikes are caused by bubbles.

The Aztec P100 incorporates a digestion step into its sampling method. This allows larger phosphate containing particles, that are excluded by the LOC analyser's 0.45mm filter, to be broken down and included in the phosphate sample. The cases where the Aztec P100 measured significantly higher levels than the LOC analyser were caused when there was a significant amount of phosphate present in larger particles. The digestion of the sample by the Aztec P100 also caused the results of the two analysers to differ more significantly point for point.

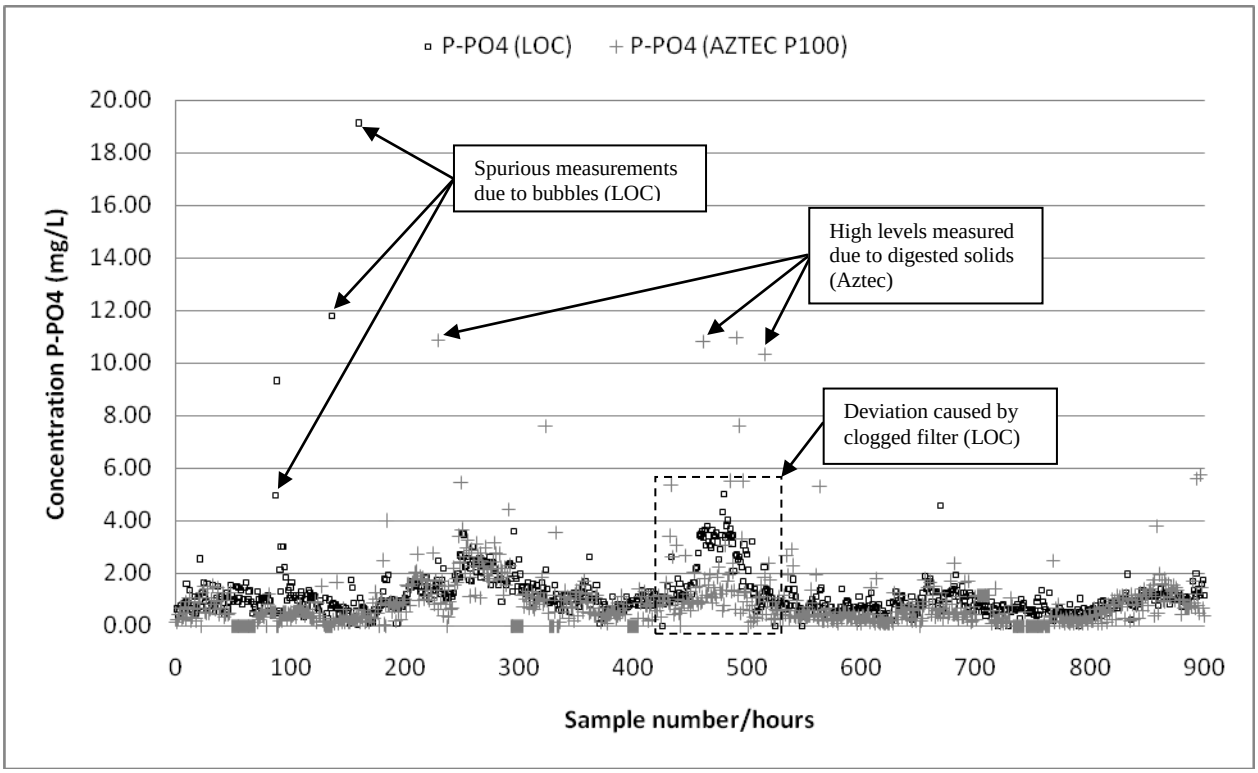

Figure 5. Plot of field trail carried out over a 38 day period comparing the output of the LOC analyser to the Aztec P100 phosphate monitor.

CONCLUSIONS

From the laboratory evaluation of the LOC analyser it was possible to directly compare its performance to commercially available analysers. The analyser was shown to be close to the capabilities of a low end phosphate monitor (Aztec P100) in terms of limit of detection and linear range. To investigate the comparability further the LOC analyser was directly validated against the Aztec P100 phosphate monitor. The validation has shown that the LOC analyser was reliable and did not suffer from drift over a long period of time.

The LOC analyser did show spurious data due to bubbles, which is a limitation of the analyser. It is envisioned that smarter software could be developed to detect these spikes and determine if they are real high phosphate concentrations by retesting the sample water. Another limitation of the LOC analyser is that it is unable to measure phosphate contained in sample with a large amount of solid particles. However, phosphate contained in particulate is not immediately available to the aquatic environment as it is not dissolved. This means the LOC analyser is well suited to measuring the total available phosphate in a given waterway.

Even considering the limitations of LOC analysers the advantage of consuming less reagent per assay is significant. The resulting small size of the analyser makes it portable and potentially low cost. There are also a number of advantages that the miniature size of LOC analysers offers that have not been utilised, such as the potential to build analysers that can carry out monitoring of multiple analytes in parallel, or the opportunity to build redundancy into the analyser allowing it to be more robust.

Future work with the LOC analyser described here will demonstrate portability by measuring phosphate concentrations in situ at a river or lake and transmitting the data in near real time to a computer database.

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