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Design, build and demonstration of a fast, reliable portable phosphate field analyser

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ABSTRACT

A fully integrated lab-on-a-disc sensor for multi-sample phosphate detection based on the molybdenum blue method for the determination of soluble reactive phosphorus in surface waters is demonstrated. An adapted and simplified molybdenum blue method which eliminates a number of steps previously associated with the detection of phosphate in environmental samples is utilised in this sensing device. The modified method facilitates the on-board storage of reagents in the microfluidic device for the determination of phosphate over a linear range of 54–600 μg/L, with a limit of detection of 16 μg/L phosphate. Validation of the fully integrated device was analysed using environmental river samples and by carrying out a spiked matric analysis. The system is comprised of a lab-on-a-disc microfluidic disc and platform that allows the disc to centrifugally spin, allowing for sample and reagent to mix, also facilitating the manipulation of fluids from one chamber to another for detection. The detector in the system is made up of a low-cost optical transducing pair (light emitting diode (LED) and a photodiode (PD). This integrated system presents a simple, cost effective and near real-time method for the direct analysis of phosphate in surface water.

1. Introduction

1.1. Need for phosphate monitoring in catchments

Catchment monitoring with an emphasis on nutrient detection is a growing area of importance in recent years [1]. Catchments are complex systems where the quality and quantity of the water is influenced by biological, chemical and physical factors [2,3]. Catchment monitoring of nutrients such as phosphorus (P) is important as it enables sustainable management of the area, it can also highlight problems or threats concerned with the area e.g. agricultural runoff, deforestation or wastewater effluent discharges. Management can be improved by increasing the temporal and spatial monitoring carried out within the catchment, using a range of techniques such as *in-situ* and remote sensing to detect problems such as algal blooms, nutrient influxes, land use and land change applications [4,5]. Nutrient pollution is an extremely difficult challenge to overcome when it comes to environmental monitoring, as it can be difficult to find a solution to repair the extensive damage it can cause [6]. Therefore, preventative measures have been taken to ensure nutrients, such as P, are regulated and monitored sufficiently. The P sources coming from human activities have had many ecological degenerative effects on water bodies across Europe [7]. This can be seen with increasing pollution discharges, alteration of water flow regimes and modification of the morphology of rivers, causing harmful effects to fish and aquatic life [7,8]. Aquatic ecosystems have significantly deteriorated due to the phenomenon of eutrophication and the pollution of water systems. Eutrophication relates to the excessive enrichment of an ecosystem by chemical nutrients, including compounds such as phosphorous, ammonia, nitrite and nitrate [2,9,10]. P can also occur naturally in water systems and are usually characterised and measured as orthophosphates. Orthophosphates are the most common form of soluble reactive phosphorous (SRP), the SRP method is widely used to determine the bioavailability of P in water [11,12]. Phosphate PO_4^{-3} ions are naturally formed when P goes through a natural process of weathering [13]. Three different forms of phosphate are available; orthophosphate, metaphosphate and organically bound phosphate [14]. To obtain more information about the levels of phosphate present in surface waters – monitoring and sampling must be as frequent as possible.

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Table 1
Comparison of handheld/portable phosphate detection devices.

Sensor	LOD (μg/L)	Linear Range (μg/L)	Analysis Time	Comments	Reference
Sea-Bird Scientific HydroCycle-PO4 Phosphate Sensor Sea-Bird Scientific	2	0–300	Real-time measurements	Commercial device, low limit of detection but high cost.	[16]
IQ SensorNet Alyza Analyser PO4 YSI xylem a brand	50	0–50	Real-time measurements	Commercial device. Vanadomolybdate (yellow) method of detection, LOD not low enough for freshwater, high cost involved.	[17]
Flow injection analysis microfluidic chip	200	0–50	3 min	High LOD, not suitable for detecting low limits of phosphate.	[18]
Smart-phone, paper-based fluorescent sensor for ultra-low inorganic phosphate	1	1–64	Real-time (4 s)	Small, portable and can detect ultra-low levels of inorganic phosphate. Single use sensor – increased cost and waste associated.	[19]

1.2. Sensors for phosphate monitoring

Commercially available sensors costly to manufacture, maintain and deploy. A typical autonomous commercial sensor can cost up to €25,000 per unit [15], due to their size, high power consumption and reagent storage chambers, but the maintenance and security of current commercial sensor can increase the cost significantly. Handheld sensor platforms are developed for rapid, robust, and reliable environmental monitoring. They can provide real/near real-time analysis of environmental waters. While existing autonomous sensors have value, the nature of phosphate occurrence in surface waters is linked to land use practices, erosion etc. which can vary along a water course. A single sensor at a single location can provide good temporal information, but additional spatial information is desirable due to the variation of phosphate levels along the water course. This would be costly to achieve with numerous existing large scale autonomous sensors.

1.3. Limitations with current monitoring technologies

The quality assurance and reliability for obtaining long-term sensing data in complex environments is scarce. Some current commercial sensors suffer from sensitivity to high background ion concentration, effecting the quality of the data being analysed [20].

The field of microfluidic based sensing systems has been widely developed in the various fields from environmental to point of care in the last decade because of the many advantages it possesses such as low sample volume, high throughput, reduced contamination, fast detection times and elimination of reagent handling and integration capabilities. A number of chemical sensors have been commercialised that are capable of detecting phosphate in natural waters (Table 1). Compared to the commercial sensors available, the use of microfluidics scales down the size of the instruments needed for *in-situ* measurements without compromising the efficiency and reliability of the results [21–23].

The aim of this work is to satisfy the need for more frequent and near real-time monitoring of phosphate in surface waters using a handheld multi-sample phosphate sampling device. This is the first demonstration of the portable field analyser for phosphate.

2. Materials and methods

2.1. Chemicals

Chemicals and reagents including potassium dihydrogen phosphate monobasic, ammonium molybdate tetrahydrate, potassium antimonyl tartrate, sodium hydrogen bisulfate and L-ascorbic acid were all purchased from Sigma Aldrich, Arklow, Ireland. Solution and standards were prepared using ultra-pure water (Elga Maxima®, 18.2 MΩ cm). A 0.032 M solution of ammonium molybdate tetrahydrate, a 0.004 M solution of potassium antimonyl tartrate and a 0.1 M solution of L-ascorbic acid were prepared. A 5 M sulphuric acid and sodium hydrogen bisulfate solutions were prepared by adding 7 mL concentrated sulphuric acid (96%) to 50 mL of deionised water and dissolving 9 g of sodium hydrogen bisulfate in 20 mL deionised water respectively. The combined molybdenum blue reagent was made freshly each day by mixing 5 mL sulphuric acid solution (control) or sodium hydrogen bisulfate solution, 0.5 mL potassium antimonyl solution, 1.5 mL ammonium molybdate solution and 3 mL ascorbic acid solution. The volume ratio of water sample to combined reagent used for all experiments was 1:0.16. A range from 0 to 400 mg/L P–PO_4^{3-} of standard solutions were prepared from dilutions of a 50 g PO_4–P mL^{-1} stock solution. The stock solution was prepared from potassium dihydrogen phosphate monobasic. The formation time for the molybdenum complex was 10 min from time of

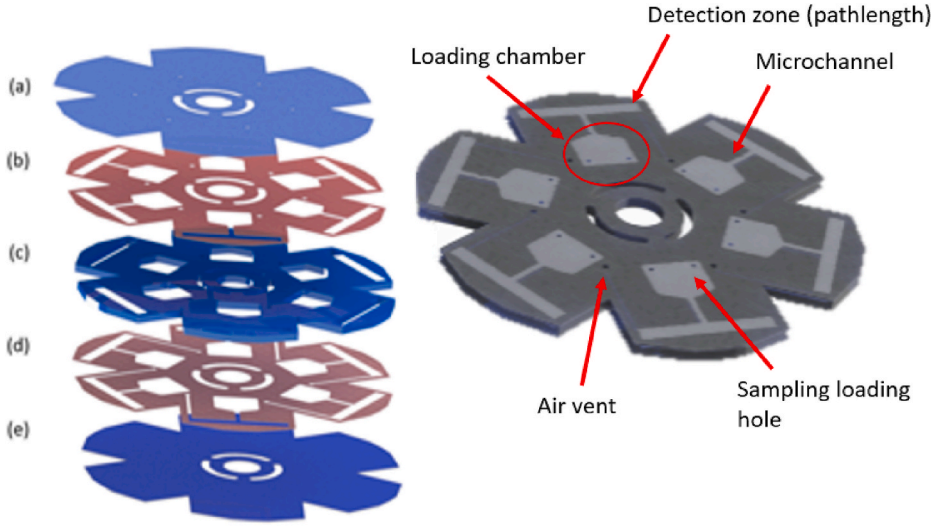


Fig. 1. Left: Rendered image showing each layer of the microfluidic disc, where the blue layers represent PMMA and red represents PSA layers. Right: Rendered image of assembled disc with features labelled; loading chamber: facilitates the storage of reagents and sampling loading, allowing dried reagents and sample to mix. Detection zone, pathlength (40 mm): detection of phosphate present in sample. Microchannel: allows liquid to flow from introduction loading chamber to detection zone for measurement. Air vent; air displacement and allows fluid to fill detection zone. Sample loading hole; enables sample to be micro pipetted into disc. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

reagent addition for the control and 5 min for the adapted dried method.

A water sample was collected from the River Tolka in Co. Dublin, Ireland. This sampling site was selected as it is known for its high levels of phosphate and ease of access for sampling. The sample was filtered through a 0.45 μm pore size filter paper in a vacuum filtration setup prior to analysis on the handheld system and spectrometer for reference.

2.2. Instrumentation

All measurements obtained on the developed handheld system were compared to the measurements taken using a Shimadzu mini 1240 spectrometer (Shimadzu Corporation, Japan) as a reference standard. Absorbance spectra were recorded using a VWR UV-1600PC UV–vis spectrophotometer (VWR, Ireland). BrandTech® cuvettes (Sigma Aldrich, Ireland) were used for optical path lengths of 1 cm. A Stratasys Objet260 Connex polyjet 3D printer (United Kingdom) was used to 3D print components such as the optics holder for the housing platform using an opaque rigid polymer. The optics used in the system included a plastic infrared light emitting diode (LED) (part number: QED223) and a Vishay silicon PIN photodiode (PD) (part number: BPW24R) purchased from Radionics Ltd., Ireland. The LCD screen was purchased from Mouser, United Kingdom. The screen was used to view options and commands the system has available to it and is also used to view readout from sample measurements. The keypad interface is used to key desired functions and commands such as, spin speed, spin duration and number of tests. The switch is used to turn on and off the operating motor.

The surface roughness of the optical windows was investigated using profilometry, Bruker Contour GT Optical Profiler.

2.3. Disc design and fabrication

In order to move to a more portable platform, centrifugal microfluidic disc technology was employed. Centrifugal microfluidics has the advantages of pre-stored reagents, and removes the need for pumps to drive fluid [24]. The disc design was realised in SOLIDWORKS™ and, where appropriate, exported to AutoCAD ™ for manufacturing purposes. The discs consisted of 5 layers in total, 3 layers of Poly-methyl methacrylate and 2 layers of pressure sensitive adhesive as shown in Fig. 1. The PMMA layers were cut using an Epilog Zing laser cutter (Epilog Corporation, CO, USA) and the PSA layers which were used to manufacture the microchannels and also seal the discs, the PSA layers were cut using a Graphtec cutter plotter (Graphtec America Inc., CA, USA). The top PMMA layer is a 0.5 mm thick sheet with a loading hole and air vent which allowed the fluid to displace the air so it could fill the detection area in the optical pathlength, the top and bottom PSA layers (46 μm thickness) were used to create the microchannel layers and air vent in the bottom PSA layer. The middle PMMA layer (3 mm thick) consists of the chambers and reservoirs essential for fluid capture and reagent storage and the base 0.5 mm PMMA layer was used to enclose the fluid and reagents within the disc and prevent leaks. Initially all discs were fabricated in house. As testing progressed manufacturing of the microfluidic disc layers were outsourced to a laser engineering company (EFJ engineering, Dublin, Ireland) and later assembled in DCU in a clean room using a hot roll laminator (ChemInstruments, OH, USA) to activate the PSA.

2.3.1. Characterisation of optical window surface roughness

The surface roughness of the optical windows was investigated using profilometry, Bruker Contour GT Optical Profiler [31]. This method was explored to characterize the surface morphology, surface roughness and step heights of the manually prepared and outsourced manufactured microfluidic discs to determine the effect on the clarity of the optical window and the degree of light scattering it might cause.

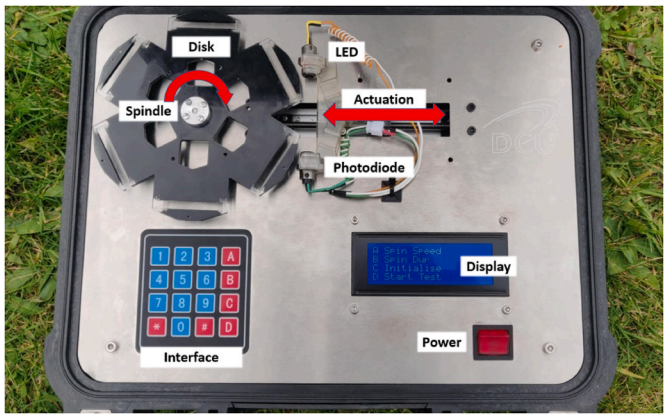


Fig. 2. Portable handheld field sensor in the field. Pictured is the peli case as a housing platform. The system incorporates a rotator for the discs that is powered by a motor, spindle to mount and secure discs, a keypad and LCD screen. The motor and electronics are stored within the peli case.

2.4. System design and operation

In parallel with the disc development, a companion instrument was developed to both, actuate fluid around and read data from the disc platform. The instrument, shown in Fig. 2, is divided into three sub systems: mechanical, electronic and firmware.

From a mechanical perspective the entire system was enclosed in a Peli-case for robustness. To rotate the disc a customised DCX 22 DC motor encoder combination (Maxon, Berkshire UK) was employed. The motor enabled rotational velocities of up to 6000 rpm and the 1024 position encoder provided both velocity measurements and angular position to within less than half of one degree. The recording of angular position was required in order to obtain readings from six disc detection zones on a single sensor disc. To facilitate ease of mounting the disk on the motor, a custom spindle was machined to fit both the motor shaft and the disc internal diameter.

The reading mechanism of the instrument was a 3D printed optical holder mounted to a NEMA 11 stepper driven actuator (IGUS, Germany). The actuator had a lead of 1 mm with a step angle of 1.8° resulting in a theoretical linear translation per step of 5 μm.

The optical holder was 3D printed using a Stratasys Objet260 Connex (Tri-Tech 3D Ltd. Trent, UK.). The part was designed using SOLIDWORKS™ and fabricated using a Stratasys RGD720 material. This material was used for high dimensional stability and surface smoothness. The optical holder was designed to hold the Light emitting diode (LED) and Photodiode (PD) in place and to ensure correct alignment.

As the pathlength dimensions were designed to facilitate 6 measurements per disc, the optics and optical alignment for the systems and discs were critical. To minimise light scatter through the main disc body and to ensure that all incident light from the LED was directed straight through the pathlength channel to the detector (PD) an optics holder with a narrow PD aperture of 1.75 mm was chosen, as shown in Fig. 3.

Both the rotational and linear actuators were mounted to a custom designed chassis manufactured from 1 mm A316 stainless steel sheets. The sheets were laser cut to enable the chassis fit easily within the Peli-case. Also fixed to the top of the chassis was the power switch, keypad for entry of experimental protocol and also a 4 × 20 character LCD screen that facilitated used interaction with the system and also provided an onsite results summary. The electronics and battery for the system was fixed to the base of the chassis.

2.5. Electronics

The fundamental circuit for reading the amount of incident light on the PD was a *trans*-impedance amplifier. The 1 MOhm feedback resistor

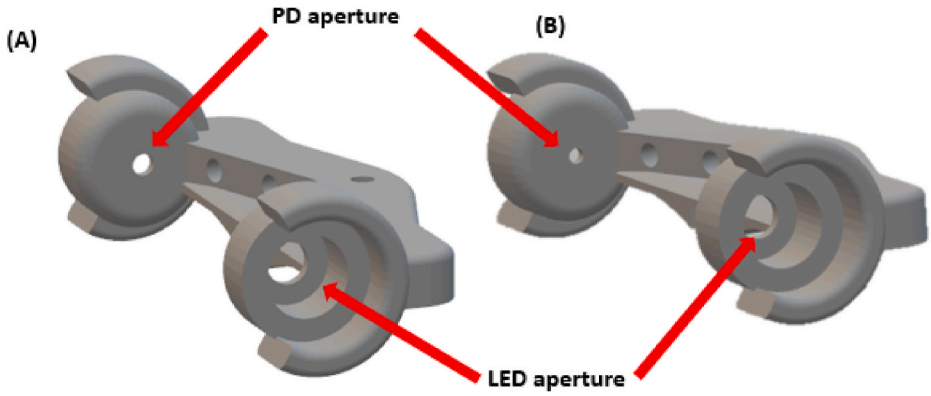


Fig. 3. Render of the 3D printer optics holder used to align the LED and PD with the disc pathlength for phosphate readings. This is attached to the actuator and is moved in and out on demand. Optics holder with (A) 2.5 mm PD aperture and (B) 1.75 mm narrower PD aperture for improved optical detection.

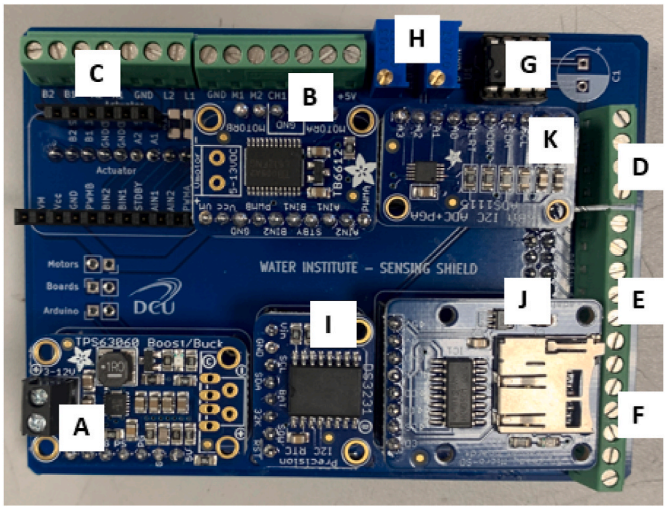


Fig. 4. Controller board used to power the system. (A) Power input, (B) Spindle driver, (C) Actuator output, (D) LED/PD inputs, (E) Keypad, (F) LCD screen LED/PD inputs, (G)) Transimpedance circuit (PD amplifier), (H) LED and Amplifier gain settings, (I) Real time clock, (J) SD card and (K) Analog to digital converter.

was adjusted such that the system blank measured just below the saturation level of the MCP601 operational amplifier. The amplifier output voltage from the MCP601 was passed to a 16-bit analog to digital (A/D) converter (ADS1115, Adafruit) to enable conversion and storage of this reading on a digital microcontroller platform (Fig. 4).

The chosen microcontroller was an ATMega 2560 embedded within an Arduino Mega development board. To interface with the Ardunio Mega a custom printed circuit board was designed and spun to allow both the transimpedance circuitry and additional functionality be incorporated onto a single board that mated directly with the Arduino Mega. The additional functionality was, in the main, enabled through the use of off-the-shelf breakout boards that enabled rapid debug and repair for on field testing.

The additional functionality included:

a) Power management to step down and isolate the 5V logic supply for the Arduino Mega 2560 from the 12V supply required for the motors;
b) Separate motor drivers to provide drive instructions to both the linear and rotary actuators;
c) SD card for onboard storage of results both for subsequent use and to produce rudimentary statical data on site during field testing;
d) Real Time clock to time stamp each experimental run;
e) A custom-printed circuit board facilitated connections to the LCD and from both the keyboard and DC motor encoder.

2.6. System user protocol

To integrate both mechanical and electronic systems and to realise a functional instrument, firmware was written to allow the user to interact with and take data from, the instrument.

The firmware functionality allowed the user to determine test parameters such as rotational velocity and duration, and the test areas of the disc measured. The system was powered using the switch (Fig. 2), on starting the test and the disc accelerated to the determined rotational velocity. The required velocity was maintained for user defined duration. Once the duration was reached the disc decelerated until the rotational velocity is zero to the first defined test location. The linear actuator then moved the optical housing the required linear distance such that the LED, detection zone and PD are aligned (measurement method discussed in Section 2.6). With the LED illuminated, a total of 50 readings were recorded. The actuator rotated the disc for another measurement position. This process continued until all measurement positions on the disc were read. On completion of the measurements, averaged and standard deviation readings were obtained.

2.7. Characterisation of disc performance

The sensor disc performance was evaluated to determine the variation between well-to-well (Fig. 1) and disc-to-disc. The performance of these discs relied on the clarity of the optical window in which the machining capabilities played a major role. This investigation was carried out to ensure that the discs were as reproduceable as possible. The outsourced and manually prepared discs were analysed using a Bruker Contour GT 3D Optical microscope, to determine surface roughness of the optical window. The hardness, elastic modulus, fracture toughness and other mechanical properties of the material used for the optical windows was also determined by a Bruker HYSITRON TI Premier nanoindentation instrument.

2.8. Assay for phosphate detection

The molybdenum blue method was chosen for the assay [25]. This method relies on the formation of a bright blue phosphomolybdenum complex in an acidic medium, which absorbs in the near infra-red region (880 nm). This method was chosen due to its ability to detect levels of phosphate in the 10 μg/L region [26]. The reagents used in the molybdenum blue method include: ammonium molybdate and potassium antimonyl tartrate which react in acidic solution (sulphuric acid or sodium bisulfate) with orthophosphate sample to form a heteropolyic acid (phosphomopydbic acid). Ascorbic acid reduced this to form a

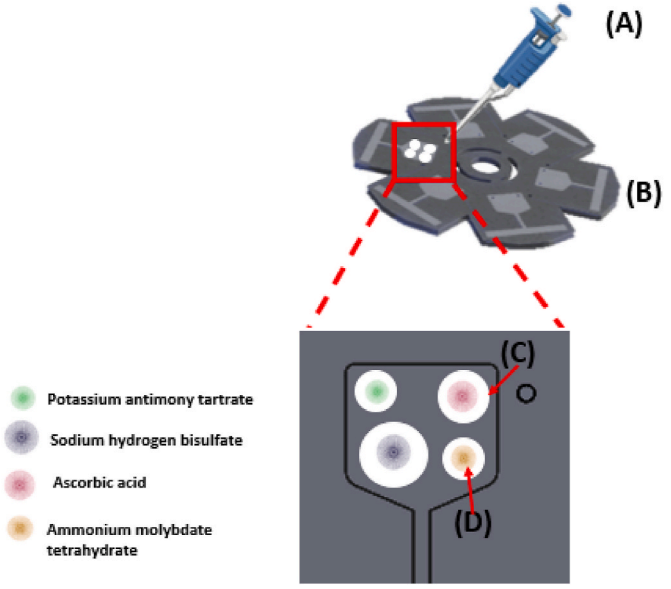


Fig. 5. Rendered image of the assembled disc with on-board reagent storage in the introduction chamber. The combined reagent is stored on the disc by separating out each of the combine reagent compounds and dried down. (A) Micropipette for reagent integration; (B) unassembled disc, top PMMA layer removed for reagent integration; (C) Graphtec cut PSA pieces for reagent integration; and (D) compounds of the combined reagent for the molybdenum blue method separated out. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

phosphomolybdenum complex. The method was adapted for the sensing disc by replacement of sulphuric acid with sodium bisulfate for improved reagent storage, reduced reagent handling and elimination of on-site contamination. For the purpose of this paper the molybdenum blue reagent with sodium bisulphate are referred to as the ***Adapted Method***.

2.9. Lifetime study of dried reagents

Each chemical component of the reagent was incorporated into the disc using Graphtec pieces of PSA. The bottom protective layer of the PSA was stuck to the base of the disc and the top layer (protective layer removed) is used to create a hydrophobic surface for the liquid chemistries to dry and adhere to see Fig. 5.

The individual reagents were dried down in liquid form using an oven at a constant temperature of 37 °C for 2 h (See Fig. 5) onto pressure sensitive adhesive (PSA) discs onto the poly methyl methacrylate (PMMA) base layer (Fig. 1.) of the microfluidic disc. To determine the performance of the dried reagents, phosphate standards were added to the chambers and allowed to react with the dried reagents for 10 min. The mixed standards and reagent were recovered using a pipette. These samples were measured on the UV/vis spectrometer at 880 nm.

To determine the lifetime of the dried reagents, a stability study was carried out using a range of phosphate standards. Each disc (in triplicate) was used to obtain a calibration curve. A range of low concentration phosphate standards (0–100 μg/L) were pipetted into the six introduction chambers of each disc and allowed to develop. The sample and reagent mixed solution were then drawn back out of the disc via a micro pipette and placed into a standard 1 cm cuvette to obtain an absorbance value of the blue complex formed in the UV/vis spectrometer and a λ of 880 nm.

2.10. Sampling site

The River Tolka flows from County Meath to Fingal and through the north of Dublin city, Ireland. The samples were obtained from Griffith Park, located 53.37047, −6.26226 were there was a footbridge and easy sampling access. The samples were collected in High Density Poly Ethylene (HDPE) wide mouth plastic bottles unfiltered. In the laboratory the samples were filtered through a 0.45 μm glass fibre filter paper. For accurate soluble reactive phosphorus (SRP) determination, the water sample was filtered immediately after collection. Samples not analysed immediately were stored in a refrigerator and analysed within 48 h. Filtration of the sample was carried out to prevent a reduction in SRP concentration over time due to sorption [30].

3. Results and discussion

3.1. Disc manufacturing method effects on optical detection

A study was carried out to determine if outsourcing the disc manufacture to a laser company would achieve a more optically clear finish on the optical windows of the discs. A more optically clear finish significantly decreases the variation in detection response from well-to-well and from disc-to-disc. Fig. 1 shows the positioning of this path length and detection zone – termed ***wells***, on a disc. Fig. 6 highlights the differences observed from well-to-well on a single disc and from disc-to-disc of both the manually prepared and outsourced discs. Both manufacturing methods and each disc were tested in triplicate. The discs were filled with water to replicate the medium of an actual sample and measured on the system. The variation from well-to-well and from disc-to-disc was dependant on the variation of the clarity of the optical windows. It can be seen that the method of manufacture had a significant effect on the clarity of the optical windows produced, with outsourced more automated manufacture leading to less variability.

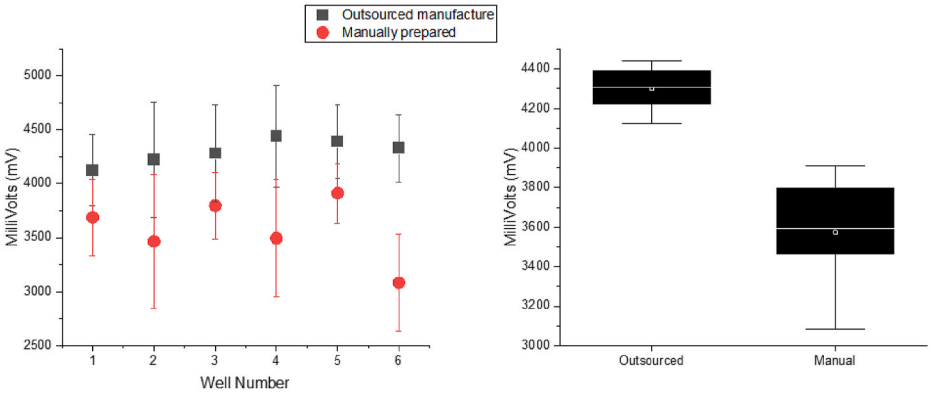


Fig. 6. Left: graph comparing variability in manual preparation and outsourced manufacture of discs, n = 3. Right: representation of the variation produced by both manufacturing methods, n = 3.

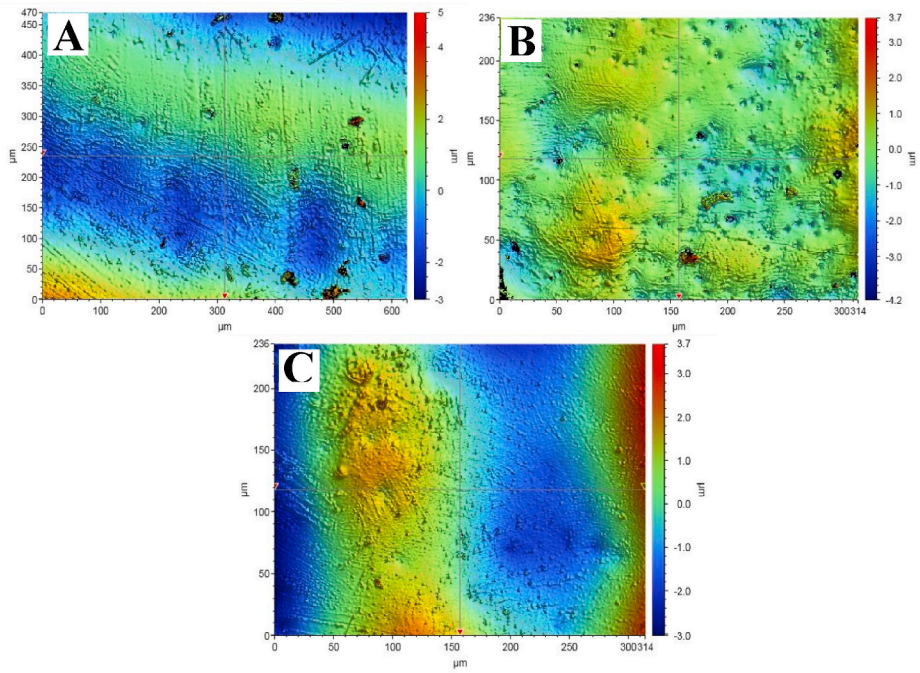


Fig. 7. Contour Profile images to investigate the variances in surface roughness of the optical window with different methods of manufacture and to determine if the differences in PMMA supplier effects the clarity of the optical window. (A) Outsourced manufacture with good optical clarify; (B) outsourced manufacture with poor optical clarity; and (C) manually prepared discs.

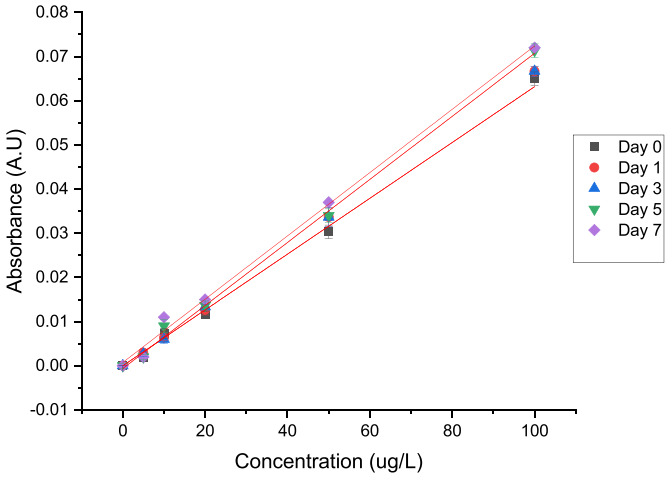


Fig. 8. Lifetime study for the onboard stored reagent for a 7 day period.

Although the signal intensity of the microfluidic discs is improved using the outsourced laser manufactured option it was found that there is still some variation between individual wells on a single disc. The low phosphate concentrations are more affected by this variation than higher concentrations. Higher phosphate concentrations are darker in the well and therefore less affected by the optical clarity. Lower concentrations, close to the limit of quantitation are impacted by any variation in optical window clarity. It was important to determine the most reproducible method of disc manufacture to produce consistently clear discs with good optical clarity to provide an overall low limit of detection (LOD) for the system. A pre-calibration of each disc was carried out to ensure all wells on that disc can be referenced to a single well. De-ionised water was used to obtained readings in the pre calibration step. This pre-calibration step enabled the determination of the well-to-well optical window variability on a single disc. The pre-calibration of the disc using water, is carried out to ensure all wells on that disc can be referenced to a single well.

Furthermore, when assessed using the contour profiler, it was found that the outsourced disc manufacture gave two finishes, one with "good optical clarity" and the other with "poor optical clarity". This suggested that there are variations in batches of the PMMA material used by the company. From Fig. 7 it is clear that the outsourced manufacture with "good optical clarity" achieved the smoothest surface roughness – making it more suitable for the application of fabricating reproducible discs with good quality optical windows.

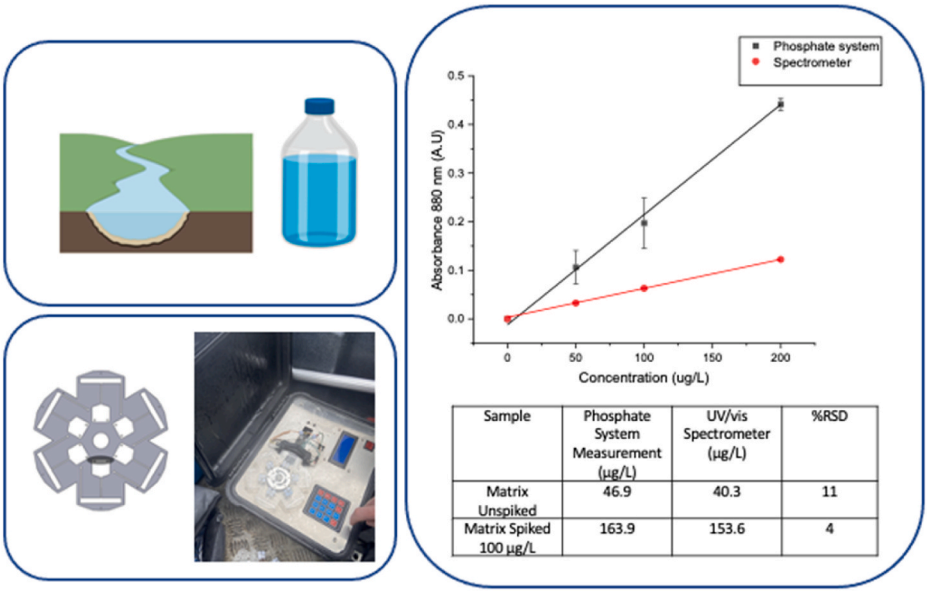
3.2. *Lifetime study of dried reagents*

To determine the lifetime of the reagents, a stability study was carried out using a range of phosphate standards. Fig. 8 shows good stability of reagents over a 7 day period, indicating the suitability of this approach for field studies where discs are prepared well in advance and easily portable.

3.3. *Integration of chemical reagents onto microfluidic discs*

This study investigated the analytical performance of drying down the reagents individually in specific ratios onto PSA material. It was necessary to prevent mixing of the different chemicals in the combined reagent so that they could be integrated separately into the microfluidic disc for onboard storage as shown in Fig. 5. The method achieved an excellent linearity and high sensitivity of R^2 0.99 and 0.0009 respectively. The LOD was found to be 2 µg/L, considerably lower than the standard ascorbic method of 10 µg/L.

A study was carried out to determine the optimum reaction and development time for the sample and reagent. The time intervals chosen included 0, 5 and 10 min. It was found that a reaction time of 10 min was optimum for reagent recovery from the dried spot and sample and reagent mixing. All solutions were allowed to react for 10 min in a well. It was found that solutions were stable for a further 10 min. The LOD of the assay method, discs and detection platform was determined by obtaining a calibration curve using low concentration phosphate standards, ranging from 5 to 100 µg/L and the standard deviation (SD) of the blank



Sample	Phosphate System Measurement (µg/L)	UV/vis Spectrometer (µg/L)	%RSD
Matrix Unspiked	46.9	40.3	11
Matrix Spiked 100 µg/L	163.9	153.6	4

Fig. 9. Phosphate measurement of river water sample obtained on handheld system (n = 3) (error bars representative of the three different discs used in the experiment) and on a spectrophotometer (n = 3), from which the concentrations of the matrix un-spiked and spiked samples were be determined. R^2 values for the phosphate system and spectrometer were 0.996 and 0.999 respectively.

baseline. The blank consisted of the reagent and a 0 µg/L phosphate standard (deionised water). The system LOD was determined 16 µg/L phosphate.

3.4. Field demonstration of the fully integrated system on environmental samples

The evaluation of the handheld system was investigated using environmental samples obtained from the River Tolka in Co. Dublin. These samples were used as a spiked matrix sample to test the performance of the sensor, demonstrating the accuracy and reliability of the system for phosphate determination in a real sample matrix. The samples were spiked with a concentration of 100 µg/L^{-1} PO^4–P. A 4-point calibration curve was carried out to determine the concentration of phosphate in the spiked and unspiked samples. This experiment was carried out in triplicate, on 3 separate discs, yielding sample to answer within 10 min (see Fig. 9). The samples were filtered prior to analysis. The sensor results were compared to a reference standard UV/vis spectrometer. The results obtained from the system showed close agreement to that demonstrated from the UV–Vis spectrometer, with a relatively low standard error displaying great accuracy for the integrated handheld system (n = 3). The percentage relative standard deviation (% RSD) for both the matrix unspiked and matrix 100 µg/L spiked sample was determined to be 11% and 4% respectively. The was determined using the average and standard deviations for both samples.

The error bars in Fig. 9 are related to the disc-to-disc variation rather than sample measurements. The portability and robustness of this sensor makes it suitable for near real-time in-situ detection of phosphate in freshwater systems. The onboard storage of reagents, mitigates any reagent handling, reduces contamination for onsite analysis and eliminates the tedious reagent preparation before sampling campaigns. The advantage of having the reagents dried down and sealed within the disc, increases the lifetime of the reagent and decreases time spent obtaining data in the field, compared to current commercially available system which do not include reagent storage [32].

The housing for the system, functions as a light weight, robust, portable, and convenient holder for in-situ measurements, a number of sensors on the market are too big to be transported from site to site and therefore are deployed in a single water body, taking spot measurements in real-time but only for one site [33]. The housing securely encloses the motor, electronics, discs, and actuator head to ensure that no damage occurs to any component of the sensor so that it can be transported around a catchment.

4. Conclusion

This is the first demonstration of a multi sample phosphate sensor capable of measuring low µgL^{-1} required under WFD legislation. The incorporation of a centrifugal microfluidic disc with integrated chemistry was crucial in the development of the handheld sensor as it facilitated on board reagent storage, mixing and sensitive detection of phosphate. The incorporation of microfluidics allowed for a low sample volume and a fast detection time. The system can measure 6 samples per disc in 10 min. The sample and reagent volume (600 µL) and low power consumption of the microfluidic device is orders of magnitude lower than for conventional laboratory systems. The housing for the system, functions as a light weight, robust, portable, and convenient holder for *in-situ* measurements. The portability and robustness of this sensor makes it suitable for near real-time *in-situ* detection of phosphate in freshwater systems with potential for application to marine and coastal waters with further testing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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